EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION
SSE
SOCIETY for the STUDY of EVOLUTION

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Journal:	*Evolution*
Manuscript ID	18-0078.R2
Manuscript Type:	Original Article
Keywords:	Arabidopsis thaliana, evolutionary experiments, Fitness, global climate change, heterogeneous selection

ORIGINAL ARTICLE

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Running title: Fitness responses to novel environments

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Abstract

The evolutionary response of organisms to global climate change is expected to be strongly conditioned by pre-existing standing genetic variation. In addition, natural selection imposed by global climate change on fitness-related traits can be heterogeneous over time. We estimated selection of life-history traits of an entire genetic lineage of the plant *A. thaliana* occurring in north-western Iberian Peninsula that were transplanted over multiple years into two environmentally contrasting field sites in southern Spain, as southern environments are expected to move progressively northwards with climate change in the Iberian Peninsula. The results indicated that natural selection on flowering time prevailed over that on recruitment. Selection favored early flowering in six of eight experiments and late flowering in the other two. Such heterogeneity of selection for flowering time might be a powerful mechanism for maintaining genetic diversity in the long run. We also found that north-western *A. thaliana* accessions from warmer environments exhibited higher fitness and higher phenotypic plasticity for flowering time in southern experimental facilities. Overall, our transplant experiments suggested that north-western Iberian *A. thaliana* has the means to cope with increasingly warmer environments in the region as predicted by trends in global climate change models.

Evaluating the evolutionary consequences of rapid environmental change represents a question of utmost importance given the unprecedented pace of global climate change currently affecting the Earth (Hoffmann and Sgrò 2011; Shaw and Etterson 2012; Alberto et al. 2013; Anderson 2016). Well-documented shifts in phenology (Peñuelas and Filella 2001; Menzel et al. 2006; Parmesan 2007; Parmesan and Hanley 2015) and distribution range (Parmesan and Yohe 2003; Thuiller et al. 2008; Jay et al. 2012; Lenoir and Svenning 2015) indicate that organisms have been responding to current global climate change in a quantifiable way. However, the ability of organisms to rapidly adapt to new environments, i.e. to maintain fitness and therefore viable populations in new environments, represents one of the keys to fully comprehend the long-term impacts of global climate change on biodiversity. However, disentangling the knotty interactions between rapid environmental change due to global climate change, demography, adaptive evolution, and phenotypic plasticity is not a straightforward task.

Experimental approaches are perhaps the most insightful tool to study fitness responses to global climate change. Indeed, transplant experiments using populations replicated in different natural settings are widely accepted methods for testing the predictions of adaptation theory (Joshi et al. 2001; Kawecki and Ebert 2004; Angert and Schemske 2005; Becker et al. 2006; Leimu and Fischer 2008; Hereford 2009; Anderson et al. 2011; Fournier-Level et al. 2011; Alberto et al. 2013; Savolainen et al. 2013; Kim and Donohue 2013; Vergeer and Kunin 2013; Anderson and Gezon 2015). To that end, experiments are often performed in a way that one of the environments is expected to mirror the climatic environments that the study organism may encounter in the near future (Anderson 2016). For example, transplant experiments across different altitudes, latitudes or sites beyond the current range of the study organism allow the assessment of how populations might respond to shifts in the environment as predicted by global climate change scenarios. Overall, these

experiments generally show that plants tend to be locally adapted to their home sites and that global climate change will imply important changes in their plant communities and probably in their distribution ranges too (De Frenne et al. 2011; Stanton-Geddes et al. 2012; Kim and Donohue 2013; Anderson and Gezon 2015; Ensslin and Fischer 2015).

All experiments invariably encompass a very small fraction of the genetic diversity of the study organism that will be affected by changing climate. This is an important shortcoming given the fundamental role that standing genetic variation may play in the ability of populations to persist in changing environments (Barrett and Schluter 2008; Jump et al. 2008; Matuszewski et al. 2015). We stress the essential role of standing genetic diversity to understand the evolutionary impact of global climate change on biodiversity (Jump et al. 2008). To this end, we propose evolutionary experiments designed for delimited geographical regions of interest, using the genetic pools occurring in these particular regions, and testing the predicted effects of global climate change for these regions on their specific genetic pools.

Based on this framework, the evolutionary approach must also take two important elements into account to better understand the impact of global climate change at a regional scale. First, the temporal variation in fitness response to environmental changes is worth considering because it quantifies the extent of temporal heterogeneity of selection, which may provide valuable clues to better assess the cumulative patterns of adaptive variation over time (Morrissey and Hadfield 2012; Siepielski et al. 2009, 2013; Porcher et al. 2004; Alberto et al. 2013; Wadgymar et al. 2017). For example, if the direction of selection reverses sign frequently over time, such temporally variable selection may contribute to the maintenance of the genetic variation within populations (Siepielski et al. 2009; Wadgymar et al. 2017), account for unappreciable changes in fitness-related traits over time, i.e. evolutionary stasis (Siepielski et al. 2009; Wadgymar et al. 2017) and/or interrupt adaptive walks predicted by the infinitesimal model of quantitative genetics (Bell 2010). Second, phenotypic plasticity is

79 also important because it may underpin the eventual response of populations to environmental
80 changes. Nonetheless, the adaptive, non-adaptive or neutral nature of phenotypic plasticity
81 has long been the subject of much debate (Charmantier et al. 2008; Nicotra et al. 2010; Merilä
82 et al. 2014; Anderson and Gezon 2015; Anderson 2016; Gibbin et al. 2017). In any case,
83 phenotypic plasticity is generally perceived as an important asset because it enables
84 populations to track rapid environmental changes. Thus, phenotypic plasticity may have the
85 potential to buffer the effects of global climate change on populations, although further
86 research is needed to quantify whether such buffer will be realized.
87 In this study, we conducted a series of transplant experiments to evaluate the spatio-
88 temporal variation in fitness and the amount of plasticity in phenotypic traits and fitness
89 components in novel environments for an entire genetic lineage of the annual plant
90 *Arabidopsis thaliana* occurring in northwest Iberian Peninsula. Mediterranean-type
91 environments, such as the Iberian Peninsula, are predicted to be affected by increasing
92 warming over this century (Klausmeyer and Shaw 2009; Gómez-Navarro et al. 2010; Jacobeit
93 et al. 2014), which means that current southern climatic conditions are expected to move
94 northwards for the decades to come in the Iberian Peninsula. Thus, we challenged multiple
95 accessions from the north-western *A. thaliana* genetic lineage to novel environments by
96 transplanting them into two experimental facilities in southern Spain differing in altitude as
97 well as in the severity of the environmental conditions during the growing and reproductive
98 seasons. We repeated the same experiments over 3-4 years in each experimental facility to
99 quantify the extent of temporal variation in fitness responses and phenotypic plasticity. It
100 must be noted that the north-western *A. thaliana* genetic lineage does not occur in southern
101 Spain, probably as a result of the demographic history of the lineage (Picó et al. 2008;
102 Méndez-Vigo et al. 2011; Brennan et al. 2014; Marcer et al. 2016).

Here, we hypothesize that north-western early-flowering accessions will generally outperform late-flowering ones in southern environments. The rationale behind this expectation is based on previous studies of phenotypic selection in *A. thaliana* indicating a general trend for higher fitness for early-flowering accessions, in spite of the geographic and environmental variation accounting for changes in the intensity and direction of selection on life-history traits detected in these studies (Fournier-Level et al. 2013; Ågren et al. 2017; Taylor et al. 2017). Specifically, we address the following questions to better understand the evolutionary and plastic response of *A. thaliana* to novel environments. First, what is the extent of the temporal variation in the form, direction and magnitude of selection on phenotypic traits? Second, what is the role of phenotypic plasticity given its potential to buffer fitness declines due to rapid environmental changes? Third, what are the contributions of recruitment and flowering time, two of the most important developmental transitions in annuals, to performance of north-western *A. thaliana* in southern environments? And forth, which are the environmental variables accounting for the observed patterns of spatio-temporal variation in life-history traits, phenotypic plasticity and fitness?

Methods

SOURCE POPULATIONS

Arabidopsis thaliana is a small annual plant native to Eurasia. The western Mediterranean Basin is the area of the species' distribution range harboring the largest genomic diversity (The 1001 Genomes Consortium 2016; Durvasula et al. 2017). In the Iberian Peninsula, the species is genetically structured including at least four clusters with distinctive geographic distributions (Picó et al. 2008; Brennan et al. 2014; Marcer et al. 2016). We used a total of 50 accessions belonging to a single genetic cluster mostly occurring in northwest Iberian Peninsula (Fig. 1A; Picó et al. 2008; Brennan et al. 2014; Marcer et al. 2016). Genetic

structure was estimated with STRUCTURE v.2.3.3 (Pritchard et al. 2000) following the protocols described elsewhere (Méndez-Vigo et al. 2011, 2013). We only used accessions whose cluster membership coefficient was higher than 0.5 for this genetic cluster (mean ± SE = 0.85 ± 0.02; range = 0.54 – 0.98), ensuring a high homogeneity in their genetic background. However, the 50 accessions were not homogenous environmentally (Fig. 1B and 1C): populations of origin are separated by a mean 202.2 km (range = 3.2 – 647.6 km) with altitudes ranging between 140 and 1234 m.a.s.l., annual mean minimum temperatures between 1.8 and 9.6 ºC, annual mean maximum temperatures between 13.6 and 21.3 ºC, and annual total precipitation between 365 and 1614 mm (meteorological data for the period 1951 – 1999; see Méndez-Vigo et al. 2011; Marcer et al. 2016). As a result, study accessions vary in fitness-related life-history traits, such as seed dormancy and flowering time (Méndez-Vigo et al. 2011; Vidigal et al. 2016), probably reflecting their adaptation to their home environments.

FIELD EXPERIMENT

Original seed was mostly collected from natural populations during surveys conducted between 2000 and 2008, as part of a long-term project pursuing a permanent collection of natural *A. thaliana* populations from western Mediterranean Basin (Spain, Portugal and North Africa) to unravel the species' evolutionary ecology and functional genetics (see Marcer et al. 2018 and references therein). After undertaking multiplication experiments on field-collected seed following the single seed descent method in a glasshouse from the Centro Nacional de Biotecnología (CNB-CSIC) of Madrid, fresh seed was stored in dry conditions in cellophane bags at room temperature in darkness. Although such storing conditions can preserve seeds for long time, seed was multiplied in 2010 and again in 2012 to use fresh seed in all experiments.

153 Field experiments using seed from north-western Iberian populations were carried out
154 in two southern Spanish experimental facilities (Fig. 1A and Fig. S1): the low altitude El
155 Castillejo Botanical Garden of Sierra de Grazalema Natural Park (GRA hereafter; 36.46ºN,
156 5.30ºW, 329 m.a.s.l.) and the high altitude La Cortijuela Botanical Garden of Sierra Nevada
157 National Park (SNE hereafter; 37.08ºN ,3.47ºW, 1,650 m.a.s.l.). The linear distance between
158 the two experimental facilities is 184.2 km. On average, original populations are separated
159 from the two experimental facilities by 590.0 km (range = 371.4 – 779.5 km; Fig. 1A).
160 *Arabidopsis thaliana* naturally occurs in the vicinity of the two experimental facilities,
161 although the known natural populations occurring there are rather small and belong to a
162 distinct genetic lineage. On top of the differences in altitude, experimental facilities also
163 differed environmentally: GRA is warmer and wetter than SNE (Fig. 1C). We used daily
164 records of temperature and precipitation obtained from the Agencia Estatal de Meteorología
165 of Spain (AEMET) from the nearest automatic meteorological stations to GRA and SNE
166 during experiments (Fig. 1D). In GRA, we used data from the local station (El Bosque). In
167 SNE, we averaged data from four stations located in the nearest villages around the
168 experimental facility (Jerez del Marquesado, El Padul, Cañar and Lugros).
169 We performed a total of nine experiments during four years (Fig. 1D). We established
170 experiments in early October (sowings between the 1st and the 5th of October) during four
171 years in a row in GRA (2010 – 2013) and three years in a row in SNE (2011 – 2013). In
172 GRA, we established two additional experiments in a row (2012 – 2013) in December
173 (sowings between the 10th and the 12th of December). In the December experiments, *A.*
174 *thaliana* was forced to complete the life cycle in a shorter period of time mimicking late
175 germination events normally occurring in Iberian natural populations (Montesinos et al. 2009;
176 Picó 2012). This is not possible in SNE as the facility is normally covered by snow by then.
177 All experiments in GRA were completed successfully for all accessions. In contrast, the first

178 experiment in SNE in 2011 exhibited very high mortality, as only seven of 6,671 rosettes
179 reached maturity (Table 1) mostly due to strong drought conditions during the course of the
180 experiment. Thus, this experiment was excluded from the analyses. The second experiment in
181 SNE in 2012 was totally successful. Finally, 42 of 50 accessions were able to complete the
182 life cycle in the third experiment in SNE in 2013, although with fewer replicates per
183 accession.

184 We used eight replicates per accession for experiments established in 2010 and 2011,
185 and six replicates for the rest of years, including 60 seeds per replicate in all cases. Seed
186 batches were prepared a few months before establishing the experiments, and stored in 1.5 ml
187 plastic tubes at room temperature in darkness until the sowing day. Seeds were sown in square
188 plastic pots ($12 \times 12 \times 12$ cm^3) filled with standard soil mixture (Abonos Naturales Cejudo
189 Baena S.L., Utrera, Spain) placed in randomized blocks, each block including one replicate
190 per accession. A 2-cm wire mesh covering the blocks protected plants from bird and rodent
191 depredation.

192 We recorded the number of rosettes per pot every 15 days from the sowing day.
193 Recruitment was estimated as the maximum proportion of seedlings observed, which was
194 obtained by dividing the maximum number of seedlings recorded per pot during the surveys
195 by 60. Maximum recruitment was always reached within the first two surveys after seed
196 sowing in all experiments. No significant germination events occurred after the germination
197 peak, as apparently indicated by our surveys and previous experiments (Méndez-Vigo et al.
198 2013; Manzano-Piedras et al. 2014). Nonetheless, we confirmed that by tagging rosettes with
199 stainless steel pins (38 mm length) in two experiments in GRA. We found that only 22 of
200 6,134 (0.36%; $N = 264$ pots; 2012 experiment) and six of 2,774 tagged rosettes (0.22%; $N =$
201 205 pots; 2013 experiment) were considered as individuals recruited after the germination
202 peak.

During the reproductive period and right after observing the first flowering individuals, experiments were surveyed between once and three times per week at both experimental facilities. The wire mesh was removed to prevent flowering stalks from being damaged. Flowering time was estimated as the number of days between the date in which we recorded the maximum number of seedlings, and flowering date. Flowering date was given at the pot level when the majority of the plants in the pot, which were full-sibs and showed homogeneous flowering behavior, had the first flower open (as in Méndez-Vigo *et al*., 2013; Manzano-Piedras *et al*., 2014). We also estimated the flowering duration for each accession and experiment as the difference between the earliest and the latest flowering dates.

We recorded the number of fruiting individuals per pot and counted the number of fruits per individual when they completely finished flowering and fruiting. Fecundity was given as the total number of seeds produced per individual. Merging data from a previous study (N = 118 individuals from natural populations; Montesinos et al. 2009) and this study (N = 142 individuals from various genotypes and experiments), we estimated the number of seeds per fruit as a function of the number of fruits per individual given as seeds/fruit = 10 × ln(fruits/individual) + 5.3 (N = 260, R^2 = 0.78; Fig. S2). Losses, due to flower abortion, fruit depredation or plant diseases, were low in our experiments: a total of 3,601 of 226,464 fruits (1.59%) and 2,346 of 36,551 individuals (6.41%) were lost and therefore excluded from the analyses. Finally, survivorship was also estimated as the proportion of individuals achieving the reproductive stage relative to the maximum number of seedlings recorded. The integrated lifetime fitness was computed as survivorship × fecundity, providing the mean number of expected seeds per individual. Overall, we sowed 174,000 seeds in 2,900 pots, which yielded 77,173 rosettes and 34,205 reproductive individuals in all nine experiments.

STATISTICAL ANALYSES

We performed linear mixed models (LMMs; Bolker et al. 2009) to analyze the fixed effects of sowing date (October and December) and experimental facility (GRA and SNE) on recruitment and flowering time by means of multi-response LMMs (MRLMMs). We focused on recruitment and flowering time because they are the two major developmental transitions in annual plants, that is, seed-to-seedling and vegetative-to-reproductive transitions. We normalized response variables by subtracting the mean and scaling the variance, in order to avoid measurement dimension effects in the joint model on recruitment and flowering time. As the 50 accessions were not genetically independent from each other, we included a random factor given by the genetic relationship matrix (Yang et al. 2011) using SNP data available for these accessions (Picó et al. 2008; Méndez-Vigo et al. 2011; Brennan et al. 2014). MRLMMs also allow the estimation of heritability of traits explained by the genetic relationship matrix (Yang et al. 2011). We fitted all models in a Bayesian framework using the *MCMCglmm* v.2.24 R package (Hadfield 2010; Wilson et al. 2010). We used uninformative priors, a Markov chain Monte Carlo (MCMC) of 50,000 iterations with a burn-in of 10%. All estimated parameters had effective sampling size (ESS) > 1000 and autocorrelation < 0.1.

Using the well-established formulation of Lande and Arnold (1983), reviewed in Kingsolver et al. (2001), we calculated for each experiment directional selection differentials ($s = \mathrm{Cov}[w,z]$), directional selection gradients, ($\beta = P^{-1}\underline{s}$), disruptive or balancing selection differentials ($C = \mathrm{Cov}[w,(z-\overline{z}\ (z-\overline{z})^{T})]$), and disruptive or balancing selection gradients, ($\gamma = P^{-1} C P^{-1}$), where w is the vector of relative fitness, z is the vector of phenotype, and P is the phenotypic variance-covariance matrix of phenotypes. Given the relevance of flowering time in this study (see below), for each accession and experiment we analyzed the correlation between flowering time and other phenological traits, such as flowering duration, and fitness components, such as survivorship, fecundity, and fitness. We used the breeder's equation to calculate the response to selection for the mean, ($\Delta z = GP^{-1}s$) and variance-covariance

matrices of phenotypes ($\Delta P = Cov[w,(z-\bar{z}\ (z-\bar{z})^T)] - ss^T$) (Lande and Arnold 1983), where *G* represents the additive genetic variance-covariance matrix. We also calculated selection differentials and gradients for grand means and variances of recruitment and flowering time across experiments. In all cases, significance was assessed by performing 1,000 bootstrap samples.

We correlated linear selection differentials of recruitment and flowering time with environmental variables recorded during the experiments (average minimum temperature, average maximum temperature and total precipitation) to detect environmental drivers of heterogeneity of selection on these traits. In addition, we computed mean fitness values across experiments for each accession and correlated them with annual mean minimum temperature, annual mean maximum temperature and total annual precipitation from source populations to detect environmental drivers of fitness response to novel environments. Given that weather records are by definition spatially autocorrelated, we performed the Dutilleul's modified *t* test that corrects the variance of the test statistic and the degrees of freedom according to the extent of spatial autocorrelation (Dutilleul et al. 1993).

Phenotypic plasticity for life-history traits was estimated by computing the relative distance plasticity index (RDPI; Valladares et al. 2006). This index ranges from 0 (no plasticity) to 1 (maximal plasticity) and it is useful for comparing differences in phenotypic values among multiple environments at the genotype level. Basically, RDPI quantifies phenotypic plasticity of traits based on phenotypic distances among genotypes grown in different environments (see Valladares et al. 2006 for further details). In our case, we used mean phenotypic values for each accession–experiment combination to compute the RDPI for recruitment, survivorship, flowering time, fecundity and fitness. We correlated RDPI values with annual mean minimum temperature, annual mean maximum temperature and total

277 annual precipitation from source populations to detect environmental drivers of phenotypic
278 plasticity. We also performed the Dutilleul's modified *t* test for the same reasons as above.
279 For each accession, we also examined the relationship between environmental
280 variables recorded during the experiments and life-history traits estimating Pearson's
281 correlation coefficients using data from all experiments. Given the relevance of flowering
282 time in this study (see below), we plotted the correlation coefficients between environmental
283 variables recorded during the experiments and life-history traits along a flowering time
284 gradient to visualize the effects of environmental differences during the experiments on life-
285 history traits as a function of flowering time.
286 Statistical analyses were conducted using SPSS v.23 statistical software (IBM,
287 Chicago, IL, USA), SAM software (Rangel et al. 2010) and scripts in R v.3.0.2 (R Core Team
288 2016).
289

290 ## *Results*

291 **ENVIRONMENTAL VARIABILITY DURING THE EXPERIMENTS**

292 The two field stations substantially differed in the environmental conditions recorded during
293 the experiments (Fig. 1D). In GRA, daily mean minimum temperature was 8.7 ± 0.5 ºC (range
294 across experiments = 8.0 – 9.3 ºC), daily mean maximum temperature was 19.2 ± 0.6 ºC
295 (range across experiments = 18.5 – 19.8 ºC), and mean total precipitation was 819.5 ± 214.5
296 mm (range across experiments = 505.6 – 986.6 mm). In SNE, the climatic conditions were
297 cooler and dryer: daily mean minimum temperature was 3.3 ± 0.5 ºC (range across
298 experiments = 2.9 – 3.6 ºC), daily mean maximum temperature was 12.3 ± 1.0 ºC (range
299 across experiments = 11.5 – 13.5 ºC), and mean total precipitation was 380.8 ± 243.9 mm
300 (range across experiments = 164.2 – 645.0 mm). The number of frost days was very low in
301 GRA (mean ± SD = 2.5 ± 3.1 days; range across experiments = 0 – 7 days) whereas in SNE

there were almost two months of frost days during the experiments (mean ± SD = 61.7 ± 8.1 days; range across experiments = 57 – 71 days).

It is worth noting the pronounced disparity in the success of the experiments at SNE. The first experiment in SNE (established in October 2011), which exhibited very high mortality and forced to exclude this experiment from the analyses (Table 1), had an extremely low total precipitation: 164.2 mm with 140 dry days during the experiment. In the case of the second experiment (established in October 2012) in which all 50 accessions successfully completed the life cycle, precipitation was quite high: 645.0 mm and 80 dry days. Finally, the third experiment (established in October 2013), which showed an intermediate performance, also recorded intermediate levels of precipitation with respect to the previous experiments: 333.2 mm and 122 dry days.

LIFE-HISTORY TRAITS, HERITABILITY VALUES AND FITNESS

Arabidopsis thaliana exhibited considerable variation in all life-history traits and fitness components among experimental facilities and over time (Table 1). The joint MRLMM quantified the differences in life history observed across experiments when comparing experiments selected by sowing time (October and December), which determined the window of time to complete the life cycle, and altitude (GRA and SNE). Overall, recruitment significantly decreased ($P < 0.01$) and flowering was significantly delayed ($P < 0.001$) in experiments established in October in comparison with those established in December (Table 1). On average, recruitment reduced 36% and flowering was delayed in 46 days in experiments established in October compared those established in December (Table 1). Differences between all experiments from the two experimental facilities were also significant for recruitment ($P < 0.001$) and flowering time ($P < 0.001$). In this case, however, recruitment

decreased 46% and flowering time was delayed in 44 days at the high altitude SNE compared to the low altitude GRA (Table 1).

Heritability values for recruitment (range = 0.037 – 0.338) were lower than those for flowering time (range = 0.319 – 0.871; Table 2). Overall, we found a negative genetic correlation between recruitment and flowering time (mean r_G among experiments = -0.24), although among-experiment variation in this correlation was considerably large ($-0.84 < r_G < 0.00$; Table 2). In addition, only two experiments (the second GRA experiment established in October 2011 and the last SNE experiment established in October 2013) showed correlation coefficients different from zero based on confidence intervals (Table 2). There were substantial differences in the relationship between recruitment and flowering time across experiments. Variation in the relationship between recruitment and flowering time was wider, albeit quite variable in shape, in experiments established in October in GRA (Fig. 2). In contrast, when the growing season was shorter (late sowings in December in GRA) or the environment was harsher (SNE), variation in the relationship between recruitment and flowering time was substantially narrower (Fig. 2). Finally, fitness variation across the space defined by recruitment and flowering time varied among experiments (Fig. 2), stressing the heterogeneity of fitness responses to environmental variation during all experiments and the complex relationship between fitness and key life-history traits in *A. thaliana*.

NATURAL SELECTION ON LIFE HISTORY

Selection differentials were rather similar to selection gradients (mean difference ± SE between β and s across experiments = 0.028 ± 0.013 and 0.026 ± 0.017 for recruitment and flowering time, respectively; Table 3), suggesting that direct selection prevailed over indirect selection through correlated traits in this set of *A. thaliana* accessions and experiments. The exception was the last experiment, i.e. the SNE experiment established in October 2013,

which is probably explained by the lower sample size and the lower number of replicates per accession in this experiment. The results also indicated that linear selection differentials and selection gradients were significant for flowering time in almost all experiments, whereas they were barely significant for recruitment (Table 3). Finally, quadratic selection was mostly non-significant for both recruitment and flowering time (Table 3), suggesting that stabilizing or disruptive selection only played a minor role in shaping quantitative variation in this set of *A. thaliana* accessions and experiments.

When significant, linear selection gradients were always negative for recruitment (range β = -0.33 – -0.29; Table 3), indicating that selection favored accessions with lower recruitment. Although this result would suggest that the average fitness per individual was lower in denser pots, we believe that that was not the case, as there were either positive correlations between survivorship and fecundity ($0.29 < r < 0.58$, $P < 0.04$ in four experiments) or no relationship at all between these two traits ($r < 0.18$, $P > 0.26$ in the other four experiments). The particularities of the two experiments in which we found such significantly negative β values would account for this result. In the GRA experiment established in October 2010, performances were far above the grand mean in terms of recruitment, survivorship and fecundity. In the SNE experiment established in October 2013, sample size was reduced and accessions were represented by fewer replicates, which might have affected the results.

In contrast, linear selection gradients for flowering time did vary in sign and magnitude (Table 3). Most of the linear selection gradients for flowering time were negative (range β = -0.37 – -0.24; Table 3), suggesting that selection favored early flowering accessions (Fig. 3A–C). However, two experiments, i.e. the GRA experiment established in October 2010 and the SNE experiment established in October 2012, exhibited positive linear selection gradients for flowering time (range β = 0.12 –0.27; Table 3), indicating that late

flowering accessions were favored by selection in these experiments (Fig. 3A–C). When significant, flowering time negatively correlated with flowering duration (Table 4), indicating that early-flowering accessions flowered for longer, except in the SNE experiment established in October 2012 that exhibited the opposite relationship. In practically all experiments, flowering time negatively correlated with survivorship, fecundity and fitness, indicating that early-flowering accessions had higher survivorship, higher fecundity, and higher fitness (Table 4). The exception was the first GRA experiment established in October 2010. In this experiment, there were positive correlations between flowering time and fecundity as well as fitness (Table 4). In contrast, the correlation was negative between flowering time and survivorship, overall indicating that early-flowering accessions had more survivorship, but lower fecundity and lower fitness (Table 4).

We also evaluated the global effects of selection for recruitment and flowering time using grand means and variances obtained from pooling data from all experiments, as well as separating the fitness contributions into its components, i.e. survivorship and fecundity (Table 5). Overall, we found consistent results with those obtained for each experiment, that is, the sign of significant selection differentials and gradients for recruitment was the opposite of those for flowering time (Table 5). On top of that, the fitness components for survivorship and fecundity along the flowering time continuum, the trait markedly under selection in this study, also exhibited an opposite relationship between these two fitness components (Fig. 3D). In particular, survivorship and fecundity made greater contributions to fitness in early and late flowering accessions, respectively (Table 5 and Fig. 3). Finally, the global selection differentials and selection gradients for variances in recruitment and flowering time, a first indicator of phenotypic plasticity for these traits, were mostly non-significant (Table 5), suggesting that selection for variance in these traits might not be important in this study.

ENVIRONMENTAL DRIVERS OF SELECTION AND PHENOTYPIC VARIATION

None of the linear selection gradients for recruitment and flowering time obtained for each experiment were significantly correlated with environmental variables recorded during the experiments ($N = 8$, $P > 0.42$ in all cases). Mean fitness across experiments was not correlated with any environmental variable from source populations ($N = 50$, $P > 0.10$ in all cases). However, when we excluded the first experiment in GRA (established in October 2010) due to its extremely high fitness value that masked the overall pattern, mean fitness showed a significant positive correlation with average annual minimum temperature ($N = 50$, $r = 0.38$, $P < 0.025$; Fig. 4A), indicating that accessions from north-western warmer environments performed better than those from cooler environments when growing in southern environments.

Phenotypic plasticity estimated by means of the relative distance plasticity index (RDPI) for recruitment ranged between 0.12 and 0.39 (mean ± SE = 0.24 ± 0.06), for survivorship between 0.20 and 0.47 (mean ± SE = 0.33 ± 0.07), for flowering time between 0.08 and 0.17 (mean ± SE = 0.12 ± 0.02), for fecundity between 0.39 and 0.71 (mean ± SE = 0.51 ± 0.06), and for fitness between 0.42 and 0.77 (mean ± SE = 0.58 ± 0.07). Hence, flowering time was the trait exhibiting the lowest phenotypic plasticity across experiments. In addition, phenotypic plasticity for flowering time was the only trait with significant correlations with weather records from source populations, in particular with average annual minimum temperature ($N = 50$, $r = 0.59$, $P < 0.001$; Fig. 4B) and to a lesser extent with average annual maximum temperature ($N = 50$, $r = 0.32$, $P = 0.049$), indicating that accessions from north-western warmer locations exhibited higher phenotypic plasticity for flowering time than those from cooler locations when growing in southern environments. The rest of traits and environmental variables did not show any significant relationship ($P > 0.12$

in all cases). Accessions with higher mean fitness also exhibited higher phenotypic plasticity for flowering time ($N = 50$, $r = 0.62$, $P < 0.001$; Fig. 4C).

Finally, we plotted the correlation coefficients between life-history traits and representative environmental variables during the experiments (average minimum temperature and total precipitation) along the mean flowering time continuum obtained across experiments. When looking only at the significant correlation coefficients between environmental variables and traits, the results showed how flowering time determined the relationship between environmental variables and life-history traits in *A. thaliana*. First, accessions with intermediate flowering time exhibited a negative relationship between average minimum temperature and recruitment, whereas accessions with the earliest and latest flowering times showed positive relationships between average minimum temperature and recruitment (Fig. 5A). The opposite picture emerged for flowering time (Fig. 5B), as a result of the negative relationship exhibited between recruitment and flowering time in these experiments. When considering fitness, most of the significant correlation coefficients were positive for accessions along the flowering time continuum, except for a few intermediate and late flowering accessions (Fig. 5C). In the case of precipitation, we also detected accessions with negative and positive correlation coefficients between precipitation and life-history traits, although the patterns were not as clear as in the case of average minimum temperature (Fig. 5D–F). The exception was recruitment in which few accessions with intermediate flowering time exhibited significant negative correlation coefficients whereas five accessions with the late flowering times showed the opposite pattern (Fig. 5D).

Discussion

Pre-existing standing genetic variation, rather than fixation of *de novo* mutations, is thought to be the most efficient primary mechanism enabling complex organisms to adapt to changing

environments (Barrett and Schluter 2008; Jump et al. 2008; Matuszewski et al. 2015). Bearing in mind such a premise, we challenged a set of *A. thaliana* accessions from north-western Iberian Peninsula to complete the life cycle in two contrasting experimental facilities in southern Spain, in terms of altitude, temperature and precipitation, over multiple years. For this particular region of the Mediterranean Basin, broad agreement exists that global climate change is going to increase warming (Klausmeyer and Shaw 2009; Gómez-Navarro *et al*. 2010; Jacobeit *et al*. 2014) in such a way that today's southern climatic environments are predicted to shift northwards. Although there is no guarantee that the particular environments observed at GRA and SNE experimental facilities will be those characterizing north-western Iberian Peninsula by the end of the century, they do represent low altitude, warm and relatively wet (GRA), and high altitude, mild and dry environments, (SNE), for most accessions from the north-western *A. thaliana* genetic lineage (Fig. 1C).

The correlation between mean fitness across experiments and environmental variables from source populations illustrated very well the response of north-western *A. thaliana* accessions in southern environments (Fig. 4A). In particular, *A. thaliana* accessions from warmer environments in north-western Iberian exhibited higher fitness than accessions from cooler environments when growing in southern environments. In addition, accessions from warmer environments also exhibited higher phenotypic plasticity for flowering time in southern environments, which clearly was the trait under stronger selection in this study. Overall, these results stress the potential of north-western Iberian *A. thaliana* to cope with increasingly warmer environments in the region. Based on these results, we predict a scenario of demographic viability and even growth of those *A. thaliana* populations occurring in north-western warmer environments as the amount of warming increases in the coming decades. In contrast, *A. thaliana* populations from north-western cooler environments might exhibit demographic shrinkage under climate change. Hence, our results support the view that global

475 climate change needs not to imply dramatic local extinction but probably a redistribution of
476 standing genetic variation of *A. thaliana* in the region.

477 Our results also allowed the assessment of the mechanism by which *A. thaliana* may
478 respond to changing environments, which is through selection on flowering time as selection
479 on recruitment was less frequent and intense (Table 3). Furthermore, heritability for flowering
480 time was higher than that for recruitment in all experiments, indicating the higher degree of
481 genetic determination for flowering time than for recruitment in *A. thaliana* (Méndez-Vigo et
482 al. 2013). We found that selection favored early flowering in six of eight experiments.
483 Interestingly, we also observed significant selection for late flowering in the other two
484 experiments. Although detecting selection for late flowering can be troublesome (Austen et al.
485 2017 and references therein), our experiments allowed the identification of two different
486 scenarios favoring late flowering in *A. thaliana* at low and high altitudes in southern Iberian
487 environments. On the one hand, the first GRA experiment established in 2010 characterized
488 by high recruitment, high survivorship and very high fecundity, where late-flowering
489 accessions had shorter flowering duration. On the other hand, the second SNE experiment
490 established in 2012 characterized by low recruitment, medium survivorship, and high
491 fecundity, where late-flowering accessions had longer flowering durations. These two distinct
492 scenarios, which revealed the enormous plasticity of the species to cope with contrasting
493 environments, took place only once over the course of the experiments.

494 The rarity of exceptional years, in which we detected selection for late flowering, does
495 not mean that their demographic and evolutionary importance should be underestimated. The
496 results of these experiments are in agreement with the behavior of natural *A. thaliana*
497 populations, which normally exhibit a huge year-to-year variation in practically all relevant
498 demographic attributes (Picó 2012) as a result of exceptional combinations of environmental
499 conditions favoring all important life -cycle transitions. Hence, rare weather events favoring

phenotypes that are normally selected against, albeit not wiped out from the population, have the chance to increase their frequency in the population by replenishing the soil seed bank in these exceptional years (Fig. S3). In the long term, it is accepted that such varying selection may enhance the persistence of genetic variation within populations across the species' range (Gillespie and Turelli 1989; Hall and Willis 2006; Fournier-Level et al. 2013; Ågren et al. 2017). In any case, further research is needed to find out how genetic diversity of natural populations may be related to the unpredictability of weather conditions occasionally favoring low-frequency phenotypes.

Despite selection for late flowering in two of eight experiments and the potential of such rare events for the long-term population dynamics, we believe that north-western *A. thaliana* will likely evolve towards earlier flowering if environmental conditions eventually become warmer and drier as predicted by climate change projections. A reason is that most of the significant correlation coefficients between average minimum temperature and fitness were significantly positive for accessions with early and intermediate mean flowering times, but not for those with the latest flowering times for which higher minimum temperatures implied a decline in fitness (Fig. 5C). However, it is worth noting that several accessions did not show any significant relationship between environmental variables and life-history traits or fitness regardless of their flowering time (hollow dots in Fig. 5), a pattern that might reveal those accessions with higher plasticity or a lower sensitivity to variation in the environmental variables recorded during the experiments. These accessions may also be very important for maintaining the genetic diversity of populations in the long run.

Another reason to believe that early flowering will become predominant in these Iberian populations in a warmer world is that Iberian *A. thaliana* populations that inhabit warm environments with mild winters and hot dry summers are characterized by early flowering and high seed dormancy (Méndez-Vigo et al. 2011; Kronholm et al. 2012; Vidigal

et al. 2016). Furthermore, in warm environments, the genetic correlation between early flowering and high seed dormancy is stronger (Vidigal et al. 2016) in a way that life cycle variation becomes constrained in southern warm regions and also in warmer coastal areas all over the Iberian Peninsula (Marcer et al. 2018). Given the tight correlation between seed dormancy and flowering time in *A. thaliana* (Debieu et al. 2013; Vidigal et al. 2016), detecting selection for early flowering might only be part of the story. Although it is not a straightforward task, future research should also focus on field experiments evaluating the extent of varying selection on both key *A. thaliana*'s life-history traits simultaneously (see Taylor et al. 2017) under contrasting environmental scenarios.

Predictive models of global climate change urgently need to incorporate demographic, genetic and evolutionary processes that will likely result in more biologically relevant predictions (Hoffmann and Sgrò 2011; Brown and Knowles 2012; Fordham et al. 2014; Gavin et al. 2014; Merow et al. 2014; Brown et al. 2016; Etterson et al. 2016). At present, there exist various modeling platforms taking demography and dispersal into account to model the spatial dynamics of species with environmental changes (Engler et al. 2012; Bocedi et al. 2014; Brown 2014), such as those mediated by global climate change, fragmentation and/or habitat loss. We believe that experimental approaches, like the one presented here providing fitness responses to novel environments and phenotypic plasticity for life-history traits using genetic pools from specific geographic regions, open great possibilities for including evolutionary processes into such existing modeling platforms. In particular, the results of this study suggest that it would be interesting to evaluate the effects of the temperature-mediated adaptive adjustment of flowering time, the phenotypic plasticity of flowering time assuming different scenarios based on the adaptive, non-adaptive and neutral nature of phenotypic plasticity, or the temporal heterogeneity of selection for flowering time, on population fitness with increasing warming.

DATA ARCHIVING

Data deposited in the Dryad repository: XXX.

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778

779 **Table 1**. Mean (SD) values for life-history traits of 50 *A. thaliana* accessions per experiment.

Facility	Sowing month	Year	Recruitment (proportion)	Survivorship (proportion)	Flowering time (days)	Duration (days)	Fecundity (seeds/individual)	Fitness (Surv. × Fec.)
GRA	October	2010 – 2011	0.54 (0.17)	0.81 (0.21)	146.01 (21.74)	13.22 (0.95) / 93	710.6 (1477.2)	488.3 (734.82)
GRA	October	2011 – 2012	0.42 (0.15)	0.40 (0.24)	142.87 (12.89)	10.06 (0.85) / 87	34.9 (37.9)	17.3 (23.4)
GRA	October	2012 – 2013	0.42 (0.14)	0.40 (0.21)	141.47 (20.64)	14.98 (1.23) / 102	39.7 (36.6)	18.1 (22.5)
GRA	October	2013 – 2014	0.55 (0.20)	0.30 (0.22)	125.33 (15.45)	11.88 (0.99) / 83	31.8 (31.6)	9.7 (11.4)
GRA	December	2012 – 2013	0.71 (0.13)	0.84 (0.31)	109.91 (11.72)	6.60 (0.43) / 50	105.6 (64.4)	94.7 (69.3)
GRA	December	2013 – 2014	0.59 (0.12)	0.51 (0.37)	96.68 (13.81)	13.06 (0.91) / 60	22.7 (21.6)	13.3 (17.4)
SNE	October	2011 – 2012	0.28 (0.10)	0.02 (0.09)	–	– / –	–	–
SNE	October	2012 – 2013	0.37 (0.15)	0.41 (0.24)	167.94 (6.75)	8.42 (0.72) / 33	141.4 (119.1)	53.7 (48.8)
SNE	October	2013 – 2014	0.21 (0.15)	0.26 (0.28)	173.28 (7.28)	7.07 (0.73) / 31	98.7 (110.6)	20.9 (25.8)

780 Entries are given for each experimental facility, sowing month and year. Data includes the maximum proportion of seeds recruited as rosettes,
781 survivorship as the proportion of rosettes becoming reproductive, flowering time (days), flowering duration per accession and for the whole
782 period (days), fecundity (mean number of seeds per reproductive individual), and fitness computed as survivorship × fecundity (mean number of
783 expected seeds per individual). The experiment established in SNE in 2011 had very low survivorship rates and was excluded from the analyses.
784 The experiment established in SNE in 2013 was based on 42 accessions and fewer replicates per accession.

785

786 **Table 2**. Heritability (95% confidence intervals) values for recruitment and flowering time, and the genetic correlation between the two traits for
787 50 *A. thaliana* accessions per experiment.

Facility	Sowing month	Year	Recruitment	Flowering time	Correlation
GRA	October	2010 – 2011	0.144 (0.063 – 0.234)	0.871 (0.778 – 0.942)	-0.216 (-0.538 – 0.097)
GRA	October	2011 – 2012	0.252 (0.146 – 0.365)	0.748 (0.661 – 0.827)	-0.385 (-0.641 – -0.115)
GRA	October	2012 – 2013	0.096 (0.014 – 0.183)	0.756 (0.652 – 0.841)	-0.062 (-0.476 – 0.344)
GRA	October	2013 – 2014	0.338 (0.210 – 0.470)	0.688 (0.589 – 0.789)	-0.209 (-0.516 – 0.094)
GRA	December	2012 – 2013	0.060 (0.000 – 0.127)	0.853 (0.797 – 0.905)	-0.096 (-0.623 – 0.399)
GRA	December	2013 – 2014	0.236 (0.127 – 0.352)	0.662 (0.549 – 0.759)	0.000 (-0.322 – 0.316)
SNE	October	2012 – 2013	0.118 (0.044 – 0.198)	0.476 (0.355 – 0.604)	-0.114 (-0.467 – 0.255)
SNE	October	2013 – 2014	0.037 (0.000 – 0.097)	0.319 (0.137 – 0.489)	-0.843 (-0.999 – -0.369)

788 Data are given for each experimental facility, sowing month and year. The experiment established in SNE in 2011 was excluded from the
789 analyses. The experiment established in SNE in 2013 was based on 42 accessions and fewer replicates per accession.
790

791 **Table 3**. Linear and quadratic selection gradients (β and γ) and selection differentials (s and C) for recruitment and flowering time for 50 *A.*

792 *thaliana* accessions per experiment.

				Linear			Quadratic		
Facility	Sowing	Year		Recruitment	Flowering time		Recruitment	Flowering time	Interaction
GRA	October	2010 – 2011	β	-0.33 (0.08) ***	0.27 (0.08) ***	γ	-0.03 (0.13) *ns*	-0.09 (0.15) *ns*	-0.13 (0.09) *ns*
			s	-0.33 (0.08) ***	0.27 (0.08) ***	C	-0.02 (0.09) *ns*	-0.13 (0.15) *ns*	-0.11 (0.08) *ns*
GRA	October	2011 – 2012	β	-0.08 (0.08) *ns*	-0.24 (0.11) *	γ	0.11 (0.13) *ns*	0.39 (0.21) **	-0.06 (0.10) *ns*
			s	-0.02 (0.09) *ns*	-0.20 (0.10) *	C	0.16 (0.09) *	0.37 (0.17) **	-0.19 (0.12) *
GRA	October	2012 – 2013	β	-0.03 (0.10) *ns*	-0.14 (0.07) *	γ	0.09 (0.16) *ns*	0.16 (0.19) *ns*	0.11 (0.08) *ns*
			s	-0.05 (0.10) *ns*	-0.14 (0.08) *	C	0.10 (0.16) *ns*	0.21 (0.21) *ns*	0.12 (0.08) *ns*
GRA	October	2013 – 2014	β	-0.09 (0.10) *ns*	-0.36 (0.09) ***	γ	0.28 (0.18) *ns*	0.15 (0.15) *ns*	0.14 (0.11) *ns*
			s	-0.10 (0.12) *ns*	-0.35 (0.09) ***	C	0.28 (0.21) *ns*	0.15 (0.11) *ns*	0.14 (0.11) *ns*
GRA	December	2012 – 2013	β	-0.02 (0.05) *ns*	-0.36 (0.05) ***	γ	0.06 (0.07) *ns*	-0.05 (0.12) *ns*	-0.10 (0.09) *ns*
			s	-0.01 (0.06) *ns*	-0.35 (0.08) ***	C	0.06 (0.06) *ns*	-0.05 (0.11) *ns*	-0.10 (0.09) *ns*
GRA	December	2013 – 2014	β	-0.03 (0.07) *ns*	-0.37 (0.09) ***	γ	0.03 (0.14) *ns*	-0.14 (0.16) *ns*	-0.02 (0.13) *ns*
			s	-0.03 (0.08) *ns*	-0.35 (0.10) ***	C	0.03 (0.12) *ns*	-0.15 (0.14) *ns*	0.01 (0.11) *ns*
SNE	October	2012 – 2013	β	0.04 (0.07) *ns*	0.12 (0.07) *	γ	0.04 (0.08) *ns*	-0.03 (0.09) *ns*	0.04 (0.06) *ns*
			s	0.02 (0.07) *ns*	0.11 (0.06) *	C	0.03 (0.07) *ns*	-0.04 (0.08) *ns*	0.04 (0.06) *ns*
SNE	October	2013 – 2014	β	-0.29 (0.17) *	-0.25 (0.12) **	γ	0.09 (0.30) *ns*	-0.28 (0.27) *ns*	-0.15 (0.19) *ns*
			s	-0.19 (0.17) *ns*	-0.12 (0.11) *ns*	C	0.16 (0.22) *ns*	-0.11 (0.17) *ns*	-0.10 (0.12) *ns*

793 Mean (SE) values per experimental facility, sowing month and year. The experiment established in SNE in 2011 was excluded from the
794 analyses. The experiment established in SNE in 2013 was based on 42 accessions and fewer replicates per accession. Significance: ***, $P <$
795 0.0001; **, $P < 0.01$; *, $P < 0.05$; *ns*, non-significant.
796

797 **Table 4**. Pearson's correlation coefficients between flowering time and life-history traits.

Facility	Sowing	Year	Duration	Survivorship	Fecundity	Fitness
GRA	October	2010 – 2011	-0.57 ***	-0.37 **	0.41 **	0.40 **
GRA	October	2011 – 2012	-0.47 **	-0.68 ***	-0.08 *ns*	-0.37 **
GRA	October	2012 – 2013	-0.42 **	-0.19 *ns*	-0.24 *ns*	-0.25 *ns*
GRA	October	2013 – 2014	-0.16 *ns*	-0.26 *ns*	-0.61 ***	-0.52 ***
GRA	December	2012 – 2013	-0.32 *	-0.55 ***	-0.74 ***	-0.74 ***
GRA	December	2013 – 2014	0.03 *ns*	-0.84 ***	0.05 *ns*	-0.53 ***
SNE	October	2012 – 2013	0.31 *	-0.05 *ns*	0.28 *ns*	0.21 *ns*
SNE	October	2013 – 2014	-0.42 **	-0.07 *ns*	-0.35 *	-0.10 *ns*

798 Life-history traits are flowering duration per accession (days), survivorship as the proportion
799 of rosettes becoming reproductive, fecundity (mean number of seeds per individual), and
800 fitness computed as survivorship × fecundity. Significance: ***, $P < 0.0001$; **, $P < 0.01$; *,
801 $P < 0.05$; *ns*, non-significant. Sample size was 50 in all experiments except in the SNE
802 experiment established in October 2013, in which sample size was 46 for duration, 44 for
803 fecundity, and 42 for survivorship and fitness.
804

805 **Table 5**. Global linear selection gradients and differentials (β and s) for means and variances
806 of recruitment and flowering time for 50 *A. thaliana* accessions.

Component		Recruitment (mean)	Recruitment (variance)	Flowering time (mean)	Flowering time (variance)
Integrated	β	-0.161 (0.069) **	-0.112 (0.059) *	0.178 (0.161) *ns*	0.033 (0.157) *ns*
	s	-0.182 (0.075) **	-0.097 (0.063) *ns*	0.142 (0.071) *	-0.097 (0.078) *ns*
Survivorship	β	0.037 (0.015) *	0.005 (0.018) *ns*	-0.043 (0.032) *ns*	0.002 (0.035) *ns*
	s	0.041 (0.017) **	-0.001 (0.018) *ns*	-0.046 (0.019) **	0.040 (0.020) *
Fecundity	β	-0.107 (0.088) *ns*	-0.070 (0.070) *ns*	0.341 (0.215) *ns*	0.146 (0.224) *ns*
	s	-0.131 (0.080) *ns*	-0.054 (0.059) *ns*	0.210 (0.079) **	-0.121 (0.087) *ns*

807 Mean (SE) values obtained by pooling all experiments. Selection gradients and selection
808 differentials were computed for each fitness component, i.e. survivorship and fecundity,
809 separately. Significance: ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; *ns*, non-significant.
810

FIGURE LEGENDS

Figure 1 (A) Map of geographic locations of the 50 *A. thaliana* populations in north-western Iberian Peninsula and the two experimental facilities (GRA and SNE) in southern Spain. (B) Distribution of latitudes and altitudes for the 50 populations and the two experimental facilities. (C) Histograms of annual mean minimum temperature, annual mean maximum temperature, and total annual precipitation for the period 1951 – 1999 obtained from the Digital Climatic Atlas of the Iberian Peninsula for the 50 *A. thaliana* populations. The same data from the two experimental facilities are also indicated. (D) Daily minimum (blue) and maximum (red) temperatures and total precipitation (green) at GRA and SNE obtained from local meteorological stations over the course of the experiments. Dashed lines indicate the duration of the experiments.

Figure 2 Scatter plots for the different combinations of flowering time and recruitment recorded per accession and experiment. Experiments are indicated by facility and sowing data (month and year). The normalized fitness for each accession and experiment is superimposed using a colour scale.

Figure 3 (A – C) Scatter plots displaying the relationship between relative fitness and flowering time for all experiments separated by experimental facility (GRA and SNE), sowing date (October and December) and year. (D) Scatter plot displaying the relationship between normalized fitness components, i.e. survivorship (hollow dots and dashed line) and fecundity (filled dots and continuous line), and flowering time using grand means per accession across experiments.

836 **Figure 4** (A) Scatter plot showing the correlation between mean fitness across experiments
837 and average annual minimum temperature from source populations. (B) Scatter plot showing
838 the correlation between phenotypic plasticity for flowering time and average annual minimum
839 temperature from source populations. (C) Scatter plot showing the correlation between mean
840 fitness across experiments and phenotypic plasticity for flowering time. All correlations were
841 significant (Dutilleul's modified *t* test).
842
843 **Figure 5** Scatter plots showing the correlation coefficients between environmental variables,
844 i.e. average minimum temperature and total precipitation recorded during the experiments,
845 and life-history traits, i.e. recruitment, flowering time and fitness. Correlation coefficients are
846 displayed along the mean flowering time continuum computed across experiments.
847 Significant and non-significant correlation coefficients are indicated by filled and hollow dots,
848 respectively. For significant correlation coefficients (only those with $P < 0.01$), we plotted the
849 best function maximizing the R^2 if any.
850

851 **SUPPORTING INFORMATION**

852

853 **Figure S1** Pictures of experimental facilities: the low altitude El Castillejo Botanical Garden
854 of Sierra de Grazalema Natural Park (GRA; 36.46ºN, 5.30ºW, 329 m.a.s.l.) and the high
855 altitude La Cortijuela Botanical Garden of Sierra Nevada National Park (SNE; 37.08ºN
856 ,3.47ºW, 1,650 m.a.s.l.).

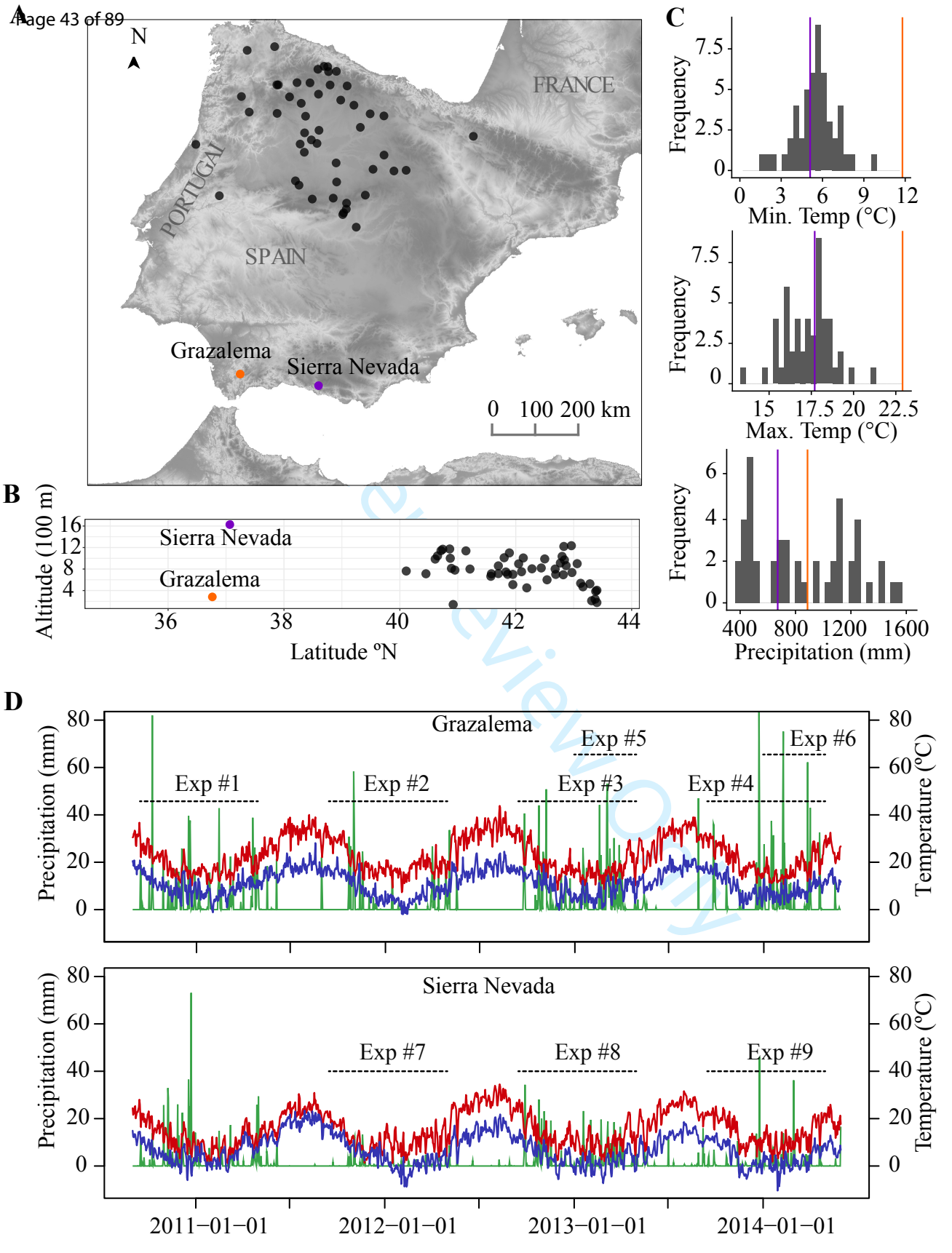
857

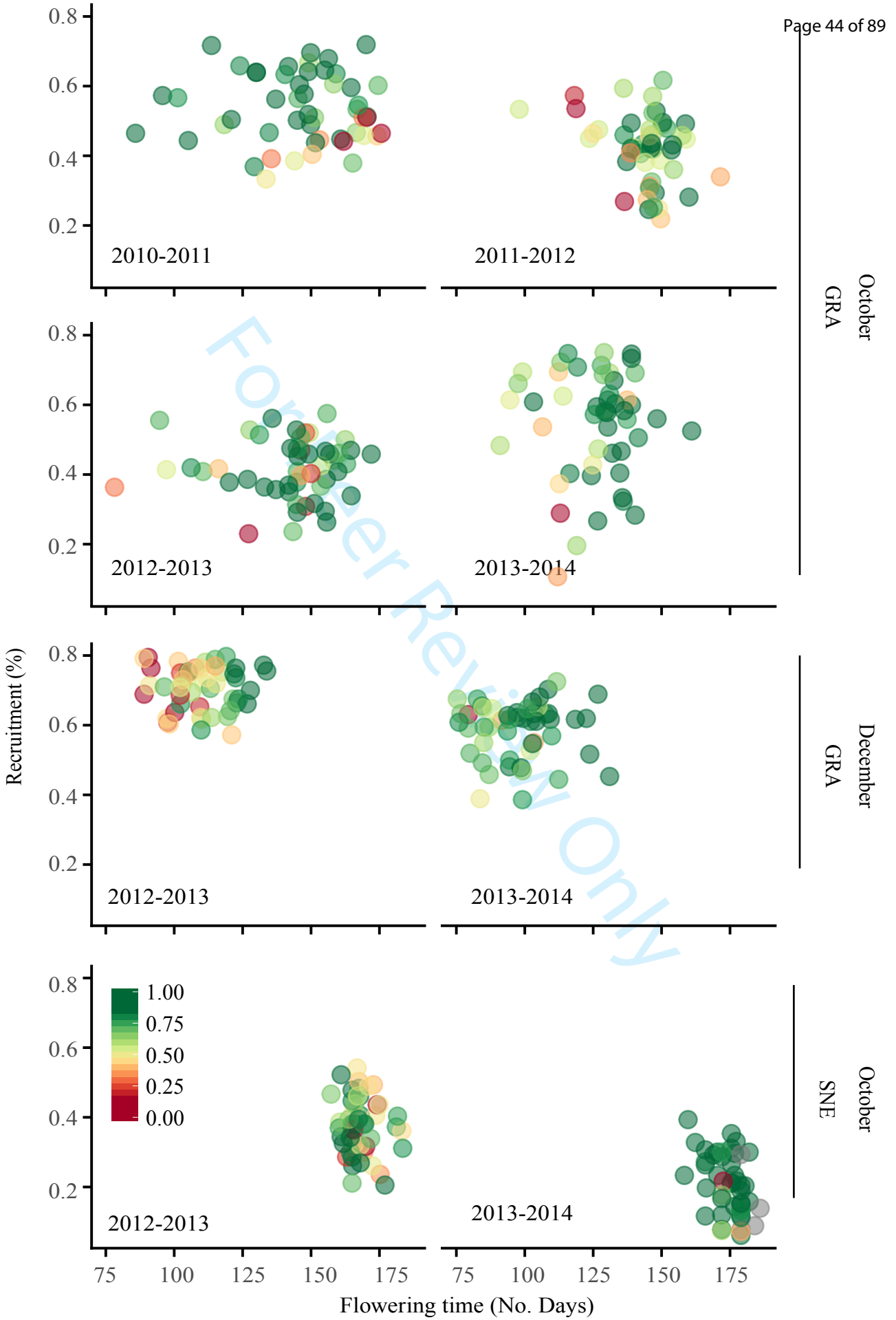
858 **Figure S2** Relationship between the number of fruits per plant and the number of seeds per
859 fruit. This relationship was used to estimate fecundity as the mean number of seeds per plant.

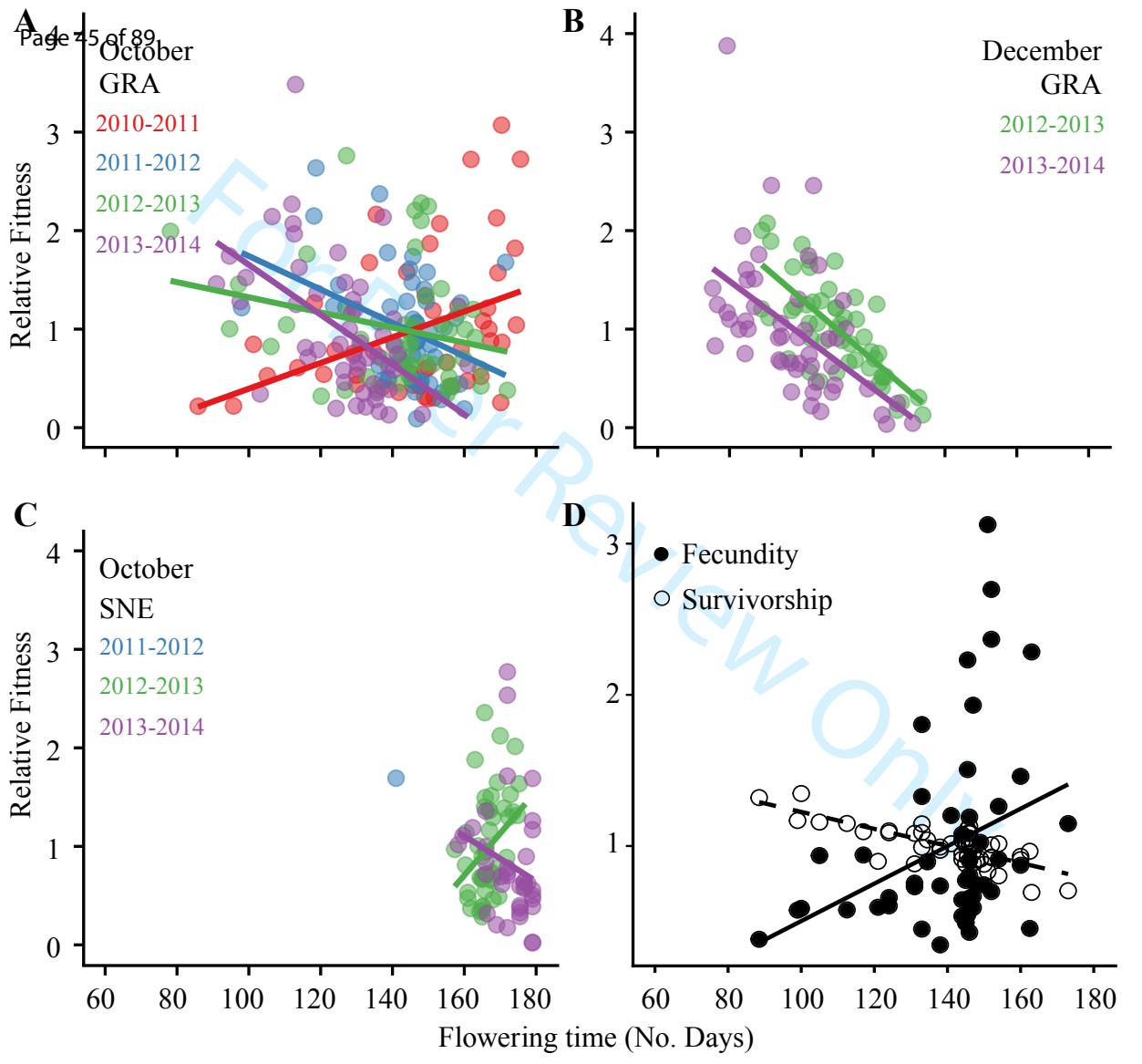
860

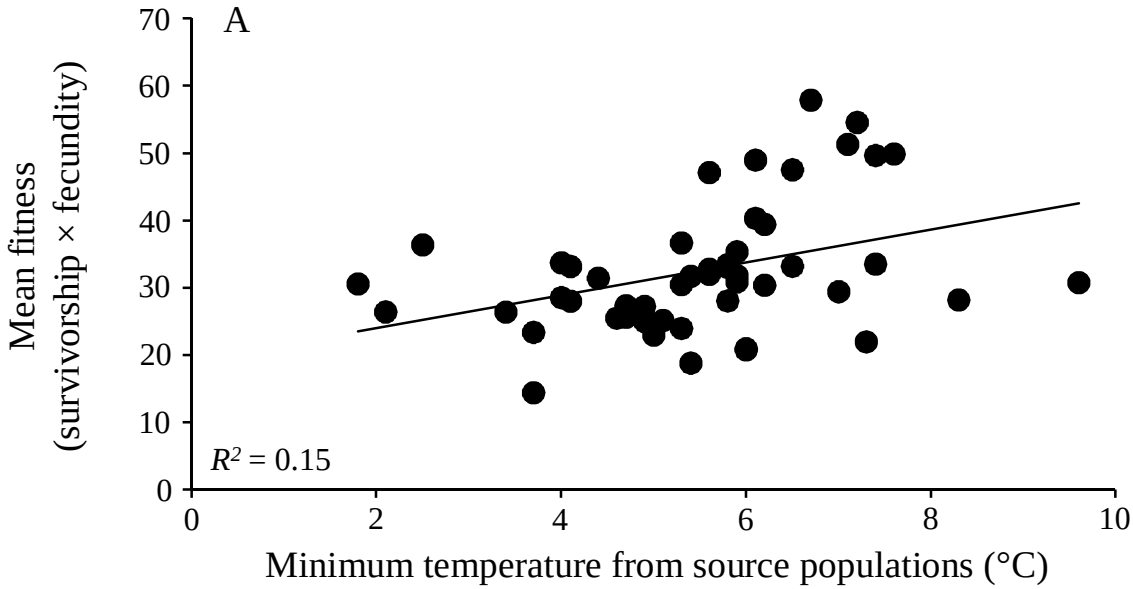
861 **Figure S3** Total number of seeds produced per experiment. The exceptional good year in
862 GRA in October 2010 illustrates the potential of *A. thaliana* to massively replenish the seed
863 bank with seed from all genotypes.

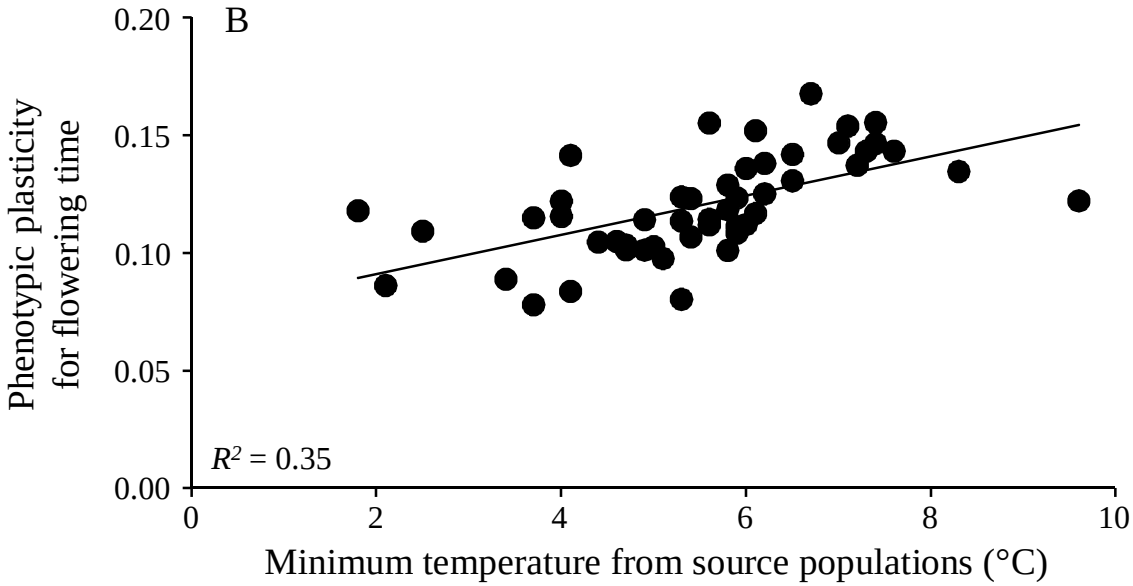
864

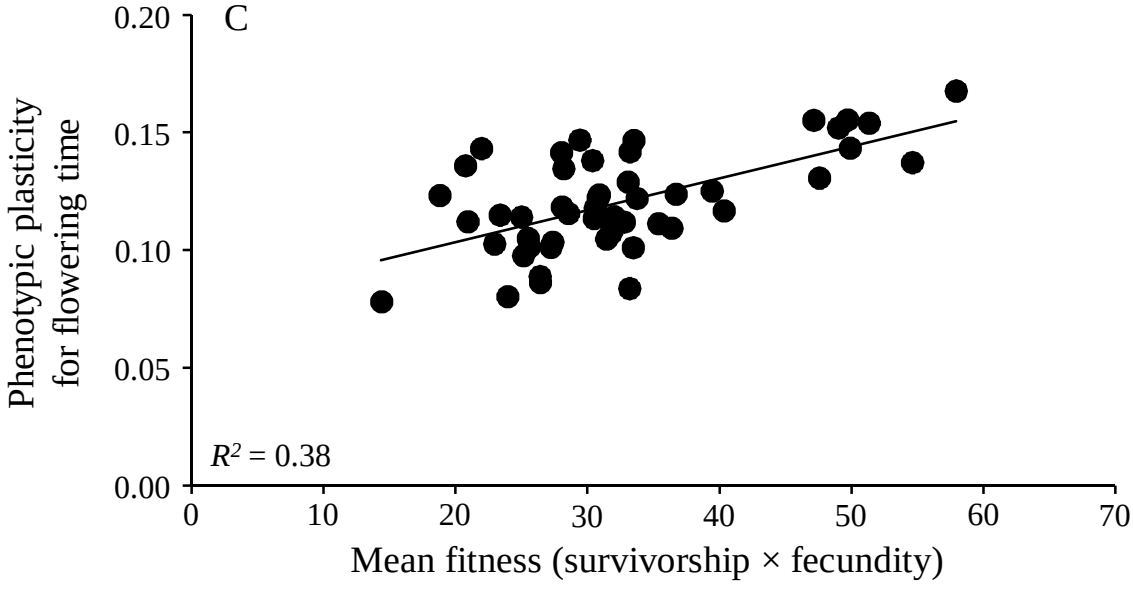


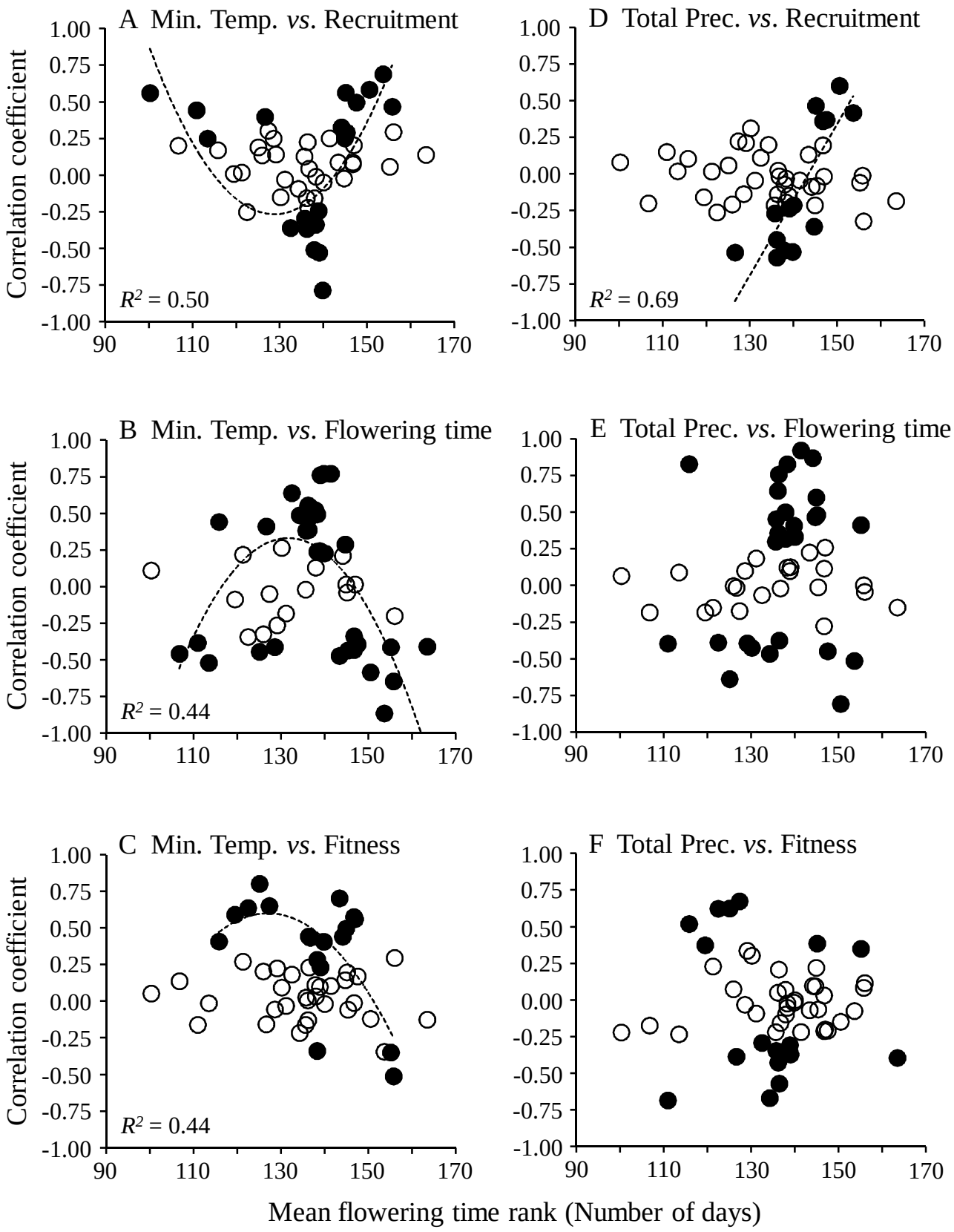





A
Mean fitness
(survivorship × fecundity)
70
60
50
40
30
20
10
0
0
2
4
6
8
10
R^2 = 0.15
Minimum temperature from source populations (°C)


B
Phenotypic plasticity
for flowering time
0.20
0.15
0.10
0.05
0.00
0
2
4
6
8
10
R^2 = 0.35
Minimum temperature from source populations (°C)


C
Phenotypic plasticity
for flowering time
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
60
70
R^2 = 0.38
Mean fitness (survivorship × fecundity)


A Min. Temp. vs. Recruitment
D Total Prec. vs. Recruitment
B Min. Temp. vs. Flowering time
E Total Prec. vs. Flowering time
C Min. Temp. vs. Fitness
F Total Prec. vs. Fitness
Correlation coefficient
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
90
110
130
150
170
R2 = 0.50
R2 = 0.69
R2 = 0.44
R2 = 0.44
Mean flowering time rank (Number of days)

ORIGINAL ARTICLE

Spatio-temporal variation in fitness responses to contrasting environments in *Arabidopsis thaliana*

Running title: Fitness responses to novel environments

Key words: *Arabidopsis thaliana*, evolutionary experiments, fitness, flowering time, global climate change, heterogeneous selection, recruitment, survivorship

Abstract

The evolutionary response of organisms to global climate change is expected to be strongly conditioned by pre-existing standing genetic variation. In addition, natural selection imposed by global climate change on fitness-related traits can be heterogeneous over time. We estimated selection of life-history traits of an entire genetic lineage of the plant *A. thaliana* occurring in north-western Iberian Peninsula that were transplanted over multiple years into two environmentally contrasting field sites in southern Spain, as southern environments are expected to move progressively northwards with climate change in the Iberian Peninsula. The results indicated that natural selection on flowering time prevailed over that on recruitment. Selection favored early flowering in six of eight experiments and late flowering in the other two. Such heterogeneity of selection for flowering time might be a powerful mechanism for maintaining genetic diversity in the long run. We also found that north-western *A. thaliana* accessions from warmer environments exhibited higher fitness and higher phenotypic plasticity for flowering time in southern experimental facilities. Overall, our transplant experiments suggested that north-western Iberian *A. thaliana* has the means to cope with increasingly warmer environments in the region as predicted by trends in global climate change models.

Evaluating the evolutionary consequences of rapid environmental change represents a question of utmost importance given the unprecedented pace of global climate change currently affecting the Earth (Hoffmann and Sgrò 2011; Shaw and Etterson 2012; Alberto et al. 2013; Anderson 2016). Well-documented shifts in phenology (Peñuelas and Filella 2001; Menzel et al. 2006; Parmesan 2007; Parmesan and Hanley 2015) and distribution range (Parmesan and Yohe 2003; Thuiller et al. 2008; Jay et al. 2012; Lenoir and Svenning 2015) indicate that organisms have been responding to current global climate change in a quantifiable way. However, the ability of organisms to rapidly adapt to new environments, i.e. to maintain fitness and therefore viable populations in new environments, represents one of the keys to fully comprehend the long-term impacts of global climate change on biodiversity. However, disentangling the knotty interactions between rapid environmental change due to global climate change, demography, adaptive evolution, and phenotypic plasticity is not a straightforward task.

Experimental approaches are perhaps the most insightful tool to study fitness responses to global climate change. Indeed, transplant experiments using populations replicated in different natural settings are widely accepted methods for testing the predictions of adaptation theory (Joshi et al. 2001; Kawecki and Ebert 2004; Angert and Schemske 2005; Becker et al. 2006; Leimu and Fischer 2008; Hereford 2009; Anderson et al. 2011; Fournier-Level et al. 2011; Alberto et al. 2013; Savolainen et al. 2013; Kim and Donohue 2013; Vergeer and Kunin 2013; Anderson and Gezon 2015). To that end, experiments are often performed in a way that one of the environments is expected to mirror the climatic environments that the study organism may encounter in the near future (Anderson 2016). For example, transplant experiments across different altitudes, latitudes or sites beyond the current range of the study organism allow the assessment of how populations might respond to shifts in the environment as predicted by global climate change scenarios. Overall, these

experiments generally show that plants tend to be locally adapted to their home sites and that global climate change will imply important changes in their plant communities and probably in their distribution ranges too (De Frenne et al. 2011; Stanton-Geddes et al. 2012; Kim and Donohue 2013; Anderson and Gezon 2015; Ensslin and Fischer 2015).

All experiments invariably encompass a very small fraction of the genetic diversity of the study organism that will be affected by changing climate. This is an important shortcoming given the fundamental role that standing genetic variation may play in the ability of populations to persist in changing environments (Barrett and Schluter 2008; Jump et al. 2008; Matuszewski et al. 2015). We stress the essential role of standing genetic diversity to understand the evolutionary impact of global climate change on biodiversity (Jump et al. 2008). To this end, we propose evolutionary experiments designed for delimited geographical regions of interest, using the genetic pools occurring in these particular regions, and testing the predicted effects of global climate change for these regions on their specific genetic pools.

Based on this framework, the evolutionary approach must also take two important elements into account to better understand the impact of global climate change at a regional scale. First, the temporal variation in fitness response to environmental changes is worth considering because it quantifies the extent of temporal heterogeneity of selection, which may provide valuable clues to better assess the cumulative patterns of adaptive variation over time (Morrissey and Hadfield 2012; Siepielski et al. 2009, 2013; Porcher et al. 2004; Alberto et al. 2013; Wadgymar et al. 2017). For example, if the direction of selection reverses sign frequently over time, such temporally variable selection may contribute to the maintenance of the genetic variation within populations (Siepielski et al. 2009; Wadgymar et al. 2017), account for unappreciable changes in fitness-related traits over time, i.e. evolutionary stasis (Siepielski et al. 2009; Wadgymar et al. 2017) and/or interrupt adaptive walks predicted by the infinitesimal model of quantitative genetics (Bell 2010). Second, phenotypic plasticity is

79 also important because it may underpin the eventual response of populations to environmental
80 changes. Nonetheless, the adaptive, non-adaptive or neutral nature of phenotypic plasticity
81 has long been the subject of much debate (Charmantier et al. 2008; Nicotra et al. 2010; Merilä
82 et al. 2014; Anderson and Gezon 2015; Anderson 2016; Gibbin et al. 2017). In any case,
83 phenotypic plasticity is generally perceived as an important asset because it enables
84 populations to track rapid environmental changes. Thus, phenotypic plasticity may have the
85 potential to buffer the effects of global climate change on populations, although further
86 research is needed to quantify whether such buffer will be realized.
87 In this study, we conducted a series of transplant experiments to evaluate the spatio-
88 temporal variation in fitness and the amount of plasticity in phenotypic traits and fitness
89 components in novel environments for an entire genetic lineage of the annual plant
90 *Arabidopsis thaliana* occurring in northwest Iberian Peninsula. Mediterranean-type
91 environments, such as the Iberian Peninsula, are predicted to be affected by increasing
92 warming over this century (Klausmeyer and Shaw 2009; Gómez-Navarro et al. 2010; Jacobeit
93 et al. 2014), which means that current southern climatic conditions are expected to move
94 northwards for the decades to come in the Iberian Peninsula. Thus, we challenged multiple
95 accessions from the north-western *A. thaliana* genetic lineage to novel environments by
96 transplanting them into two experimental facilities in southern Spain differing in altitude as
97 well as in the severity of the environmental conditions during the growing and reproductive
98 seasons. We repeated the same experiments over 3-4 years in each experimental facility to
99 quantify the extent of temporal variation in fitness responses and phenotypic plasticity. It
100 must be noted that the north-western *A. thaliana* genetic lineage does not occur in southern
101 Spain, probably as a result of the demographic history of the lineage (Picó et al. 2008;
102 Méndez-Vigo et al. 2011; Brennan et al. 2014; Marcer et al. 2016).

Here, we hypothesize that north-western early-flowering accessions will generally outperform late-flowering ones in southern environments. The rationale behind this expectation is based on previous studies of phenotypic selection in *A. thaliana* indicating a general trend for higher fitness for early-flowering accessions, in spite of the geographic and environmental variation accounting for changes in the intensity and direction of selection on life-history traits detected in these studies (Fournier-Level et al. 2013; Ågren et al. 2017; Taylor et al. 2017). Specifically, we address the following questions to better understand the evolutionary and plastic response of *A. thaliana* to novel environments. First, what is the extent of the temporal variation in the form, direction and magnitude of selection on phenotypic traits? Second, what is the role of phenotypic plasticity given its potential to buffer fitness declines due to rapid environmental changes? Third, what are the contributions of recruitment and flowering time, two of the most important developmental transitions in annuals, to performance of north-western *A. thaliana* in southern environments? And forth, which are the environmental variables accounting for the observed patterns of spatio-temporal variation in life-history traits, phenotypic plasticity and fitness?

Methods

SOURCE POPULATIONS

Arabidopsis thaliana is a small annual plant native to Eurasia. The western Mediterranean Basin is the area of the species' distribution range harboring the largest genomic diversity (The 1001 Genomes Consortium 2016; Durvasula et al. 2017). In the Iberian Peninsula, the species is genetically structured including at least four clusters with distinctive geographic distributions (Picó et al. 2008; Brennan et al. 2014; Marcer et al. 2016). We used a total of 50 accessions belonging to a single genetic cluster mostly occurring in northwest Iberian Peninsula (Fig. 1A; Picó et al. 2008; Brennan et al. 2014; Marcer et al. 2016). Genetic

structure was estimated with STRUCTURE v.2.3.3 (Pritchard et al. 2000) following the protocols described elsewhere (Méndez-Vigo et al. 2011, 2013). We only used accessions whose cluster membership coefficient was higher than 0.5 for this genetic cluster (mean ± SE = 0.85 ± 0.02; range = 0.54 – 0.98), ensuring a high homogeneity in their genetic background. However, the 50 accessions were not homogenous environmentally (Fig. 1B and 1C): populations of origin are separated by a mean 202.2 km (range = 3.2 – 647.6 km) with altitudes ranging between 140 and 1234 m.a.s.l., annual mean minimum temperatures between 1.8 and 9.6 ºC, annual mean maximum temperatures between 13.6 and 21.3 ºC, and annual total precipitation between 365 and 1614 mm (meteorological data for the period 1951 – 1999; see Méndez-Vigo et al. 2011; Marcer et al. 2016). As a result, study accessions vary in fitness-related life-history traits, such as seed dormancy and flowering time (Méndez-Vigo et al. 2011; Vidigal et al. 2016), probably reflecting their adaptation to their home environments.

FIELD EXPERIMENT

Original seed was mostly collected from natural populations during surveys conducted between 2000 and 2008, as part of a long-term project pursuing a permanent collection of natural *A. thaliana* populations from western Mediterranean Basin (Spain, Portugal and North Africa) to unravel the species' evolutionary ecology and functional genetics (see Marcer et al. 2018 and references therein). After undertaking multiplication experiments on field-collected seed following the single seed descent method in a glasshouse from the Centro Nacional de Biotecnología (CNB-CSIC) of Madrid, fresh seed was stored in dry conditions in cellophane bags at room temperature in darkness. Although such storing conditions can preserve seeds for long time, seed was multiplied in 2010 and again in 2012 to use fresh seed in all experiments.

Field experiments using seed from north-western Iberian populations were carried out in two southern Spanish experimental facilities (Fig. 1A and Fig. S1): the low altitude El Castillejo Botanical Garden of Sierra de Grazalema Natural Park (GRA hereafter; 36.46ºN, 5.30ºW, 329 m.a.s.l.) and the high altitude La Cortijuela Botanical Garden of Sierra Nevada National Park (SNE hereafter; 37.08ºN ,3.47ºW, 1,650 m.a.s.l.). The linear distance between the two experimental facilities is 184.2 km. On average, original populations are separated from the two experimental facilities by 590.0 km (range = 371.4 – 779.5 km; Fig. 1A). *Arabidopsis thaliana* naturally occurs in the vicinity of the two experimental facilities, although the known natural populations occurring there are rather small and belong to a distinct genetic lineage. On top of the differences in altitude, experimental facilities also differed environmentally: GRA is warmer and wetter than SNE (Fig. 1C). We used daily records of temperature and precipitation obtained from the Agencia Estatal de Meteorología of Spain (AEMET) from the nearest automatic meteorological stations to GRA and SNE during experiments (Fig. 1D). In GRA, we used data from the local station (El Bosque). In SNE, we averaged data from four stations located in the nearest villages around the experimental facility (Jerez del Marquesado, El Padul, Cañar and Lugros).

We performed a total of nine experiments during four years (Fig. 1D). We established experiments in early October (sowings between the 1st and the 5th of October) during four years in a row in GRA (2010 – 2013) and three years in a row in SNE (2011 – 2013). In GRA, we established two additional experiments in a row (2012 – 2013) in December (sowings between the 10th and the 12th of December). In the December experiments, *A. thaliana* was forced to complete the life cycle in a shorter period of time mimicking late germination events normally occurring in Iberian natural populations (Montesinos et al. 2009; Picó 2012). This is not possible in SNE as the facility is normally covered by snow by then. All experiments in GRA were completed successfully for all accessions. In contrast, the first

178 experiment in SNE in 2011 exhibited very high mortality, as only seven of 6,671 rosettes
179 reached maturity (Table 1) mostly due to strong drought conditions during the course of the
180 experiment. Thus, this experiment was excluded from the analyses. The second experiment in
181 SNE in 2012 was totally successful. Finally, 42 of 50 accessions were able to complete the
182 life cycle in the third experiment in SNE in 2013, although with fewer replicates per
183 accession.

184 We used eight replicates per accession for experiments established in 2010 and 2011,
185 and six replicates for the rest of years, including 60 seeds per replicate in all cases. Seed
186 batches were prepared a few months before establishing the experiments, and stored in 1.5 ml
187 plastic tubes at room temperature in darkness until the sowing day. Seeds were sown in square
188 plastic pots ($12 \times 12 \times 12$ cm^3) filled with standard soil mixture (Abonos Naturales Cejudo
189 Baena S.L., Utrera, Spain) placed in randomized blocks, each block including one replicate
190 per accession. A 2-cm wire mesh covering the blocks protected plants from bird and rodent
191 depredation.

192 We recorded the number of rosettes per pot every 15 days from the sowing day.
193 Recruitment was estimated as the maximum proportion of seedlings observed, which was
194 obtained by dividing the maximum number of seedlings recorded per pot during the surveys
195 by 60. Maximum recruitment was always reached within the first two surveys after seed
196 sowing in all experiments. No significant germination events occurred after the germination
197 peak, as apparently indicated by our surveys and previous experiments (Méndez-Vigo et al.
198 2013; Manzano-Piedras et al. 2014). Nonetheless, we confirmed that by tagging rosettes with
199 stainless steel pins (38 mm length) in two experiments in GRA. We found that only 22 of
200 6,134 (0.36%; $N = 264$ pots; 2012 experiment) and six of 2,774 tagged rosettes (0.22%; $N =$
201 205 pots; 2013 experiment) were considered as individuals recruited after the germination
202 peak.

During the reproductive period and right after observing the first flowering individuals, experiments were surveyed between once and three times per week at both experimental facilities. The wire mesh was removed to prevent flowering stalks from being damaged. Flowering time was estimated as the number of days between the date in which we recorded the maximum number of seedlings, and flowering date. Flowering date was given at the pot level when the majority of the plants in the pot, which were full-sibs and showed homogeneous flowering behavior, had the first flower open (as in Méndez-Vigo *et al*., 2013; Manzano-Piedras *et al*., 2014). We also estimated the flowering duration for each accession and experiment as the difference between the earliest and the latest flowering dates.

We recorded the number of fruiting individuals per pot and counted the number of fruits per individual when they completely finished flowering and fruiting. Fecundity was given as the total number of seeds produced per individual. Merging data from a previous study (N = 118 individuals from natural populations; Montesinos et al. 2009) and this study (N = 142 individuals from various genotypes and experiments), we estimated the number of seeds per fruit as a function of the number of fruits per individual given as seeds/fruit = 10 × ln(fruits/individual) + 5.3 (N = 260, R^2 = 0.78; Fig. S2). Losses, due to flower abortion, fruit depredation or plant diseases, were low in our experiments: a total of 3,601 of 226,464 fruits (1.59%) and 2,346 of 36,551 individuals (6.41%) were lost and therefore excluded from the analyses. Finally, survivorship was also estimated as the proportion of individuals achieving the reproductive stage relative to the maximum number of seedlings recorded. The integrated lifetime fitness was computed as survivorship × fecundity, providing the mean number of expected seeds per individual. Overall, we sowed 174,000 seeds in 2,900 pots, which yielded 77,173 rosettes and 34,205 reproductive individuals in all nine experiments.

STATISTICAL ANALYSES

We performed linear mixed models (LMMs; Bolker et al. 2009) to analyze the fixed effects of sowing date (October and December) and experimental facility (GRA and SNE) on recruitment and flowering time by means of multi-response LMMs (MRLMMs). We focused on recruitment and flowering time because they are the two major developmental transitions in annual plants, that is, seed-to-seedling and vegetative-to-reproductive transitions. We normalized response variables by subtracting the mean and scaling the variance, in order to avoid measurement dimension effects in the joint model on recruitment and flowering time. As the 50 accessions were not genetically independent from each other, we included a random factor given by the genetic relationship matrix (Yang et al. 2011) using SNP data available for these accessions (Picó et al. 2008; Méndez-Vigo et al. 2011; Brennan et al. 2014). MRLMMs also allow the estimation of heritability of traits explained by the genetic relationship matrix (Yang et al. 2011). We fitted all models in a Bayesian framework using the *MCMCglmm* v.2.24 R package (Hadfield 2010; Wilson et al. 2010). We used uninformative priors, a Markov chain Monte Carlo (MCMC) of 50,000 iterations with a burn-in of 10%. All estimated parameters had effective sampling size (ESS) > 1000 and autocorrelation < 0.1.

Using the well-established formulation of Lande and Arnold (1983), reviewed in Kingsolver et al. (2001), we calculated for each experiment directional selection differentials ($s = \mathrm{Cov}[w,z]$), directional selection gradients, ($\beta = P^{-1}\underline{s}$), disruptive or balancing selection differentials ($C = \mathrm{Cov}[w,(z-\overline{z}\ (z-\overline{z})^{T})]$), and disruptive or balancing selection gradients, ($\gamma = P^{-1} C P^{-1}$), where w is the vector of relative fitness, z is the vector of phenotype, and P is the phenotypic variance-covariance matrix of phenotypes. Given the relevance of flowering time in this study (see below), for each accession and experiment we analyzed the correlation between flowering time and other phenological traits, such as flowering duration, and fitness components, such as survivorship, fecundity, and fitness. We used the breeder's equation to calculate the response to selection for the mean, ($\Delta z = GP^{-1}s$) and variance-covariance

matrices of phenotypes ($\Delta P = Cov[w,(z-\bar{z}\ (z-\bar{z})^T)] - ss^T$) (Lande and Arnold 1983), where *G* represents the additive genetic variance-covariance matrix. We also calculated selection differentials and gradients for grand means and variances of recruitment and flowering time across experiments. In all cases, significance was assessed by performing 1,000 bootstrap samples.

We correlated linear selection differentials of recruitment and flowering time with environmental variables recorded during the experiments (average minimum temperature, average maximum temperature and total precipitation) to detect environmental drivers of heterogeneity of selection on these traits. In addition, we computed mean fitness values across experiments for each accession and correlated them with annual mean minimum temperature, annual mean maximum temperature and total annual precipitation from source populations to detect environmental drivers of fitness response to novel environments. Given that weather records are by definition spatially autocorrelated, we performed the Dutilleul's modified *t* test that corrects the variance of the test statistic and the degrees of freedom according to the extent of spatial autocorrelation (Dutilleul et al. 1993).

Phenotypic plasticity for life-history traits was estimated by computing the relative distance plasticity index (RDPI; Valladares et al. 2006). This index ranges from 0 (no plasticity) to 1 (maximal plasticity) and it is useful for comparing differences in phenotypic values among multiple environments at the genotype level. Basically, RDPI quantifies phenotypic plasticity of traits based on phenotypic distances among genotypes grown in different environments (see Valladares et al. 2006 for further details). In our case, we used mean phenotypic values for each accession–experiment combination to compute the RDPI for recruitment, survivorship, flowering time, fecundity and fitness. We correlated RDPI values with annual mean minimum temperature, annual mean maximum temperature and total

277 annual precipitation from source populations to detect environmental drivers of phenotypic
278 plasticity. We also performed the Dutilleul's modified *t* test for the same reasons as above.
279 For each accession, we also examined the relationship between environmental
280 variables recorded during the experiments and life-history traits estimating Pearson's
281 correlation coefficients using data from all experiments. Given the relevance of flowering
282 time in this study (see below), we plotted the correlation coefficients between environmental
283 variables recorded during the experiments and life-history traits along a flowering time
284 gradient to visualize the effects of environmental differences during the experiments on life-
285 history traits as a function of flowering time.
286 Statistical analyses were conducted using SPSS v.23 statistical software (IBM,
287 Chicago, IL, USA), SAM software (Rangel et al. 2010) and scripts in R v.3.0.2 (R Core Team
288 2016).
289

290 ## *Results*

291 **ENVIRONMENTAL VARIABILITY DURING THE EXPERIMENTS**

292 The two field stations substantially differed in the environmental conditions recorded during
293 the experiments (Fig. 1D). In GRA, daily mean minimum temperature was 8.7 ± 0.5 ºC (range
294 across experiments = 8.0 – 9.3 ºC), daily mean maximum temperature was 19.2 ± 0.6 ºC
295 (range across experiments = 18.5 – 19.8 ºC), and mean total precipitation was 819.5 ± 214.5
296 mm (range across experiments = 505.6 – 986.6 mm). In SNE, the climatic conditions were
297 cooler and dryer: daily mean minimum temperature was 3.3 ± 0.5 ºC (range across
298 experiments = 2.9 – 3.6 ºC), daily mean maximum temperature was 12.3 ± 1.0 ºC (range
299 across experiments = 11.5 – 13.5 ºC), and mean total precipitation was 380.8 ± 243.9 mm
300 (range across experiments = 164.2 – 645.0 mm). The number of frost days was very low in
301 GRA (mean ± SD = 2.5 ± 3.1 days; range across experiments = 0 – 7 days) whereas in SNE

there were almost two months of frost days during the experiments (mean ± SD = 61.7 ± 8.1 days; range across experiments = 57 – 71 days).

It is worth noting the pronounced disparity in the success of the experiments at SNE. The first experiment in SNE (established in October 2011), which exhibited very high mortality and forced to exclude this experiment from the analyses (Table 1), had an extremely low total precipitation: 164.2 mm with 140 dry days during the experiment. In the case of the second experiment (established in October 2012) in which all 50 accessions successfully completed the life cycle, precipitation was quite high: 645.0 mm and 80 dry days. Finally, the third experiment (established in October 2013), which showed an intermediate performance, also recorded intermediate levels of precipitation with respect to the previous experiments: 333.2 mm and 122 dry days.

LIFE-HISTORY TRAITS, HERITABILITY VALUES AND FITNESS

Arabidopsis thaliana exhibited considerable variation in all life-history traits and fitness components among experimental facilities and over time (Table 1). The joint MRLMM quantified the differences in life history observed across experiments when comparing experiments selected by sowing time (October and December), which determined the window of time to complete the life cycle, and altitude (GRA and SNE). Overall, recruitment significantly decreased ($P < 0.01$) and flowering was significantly delayed ($P < 0.001$) in experiments established in October in comparison with those established in December (Table 1). On average, recruitment reduced 36% and flowering was delayed in 46 days in experiments established in October compared those established in December (Table 1). Differences between all experiments from the two experimental facilities were also significant for recruitment ($P < 0.001$) and flowering time ($P < 0.001$). In this case, however, recruitment

decreased 46% and flowering time was delayed in 44 days at the high altitude SNE compared to the low altitude GRA (Table 1).

Heritability values for recruitment (range = 0.037 – 0.338) were lower than those for flowering time (range = 0.319 – 0.871; Table 2). Overall, we found a negative genetic correlation between recruitment and flowering time (mean r_G among experiments = -0.24), although among-experiment variation in this correlation was considerably large ($-0.84 < r_G < 0.00$; Table 2). In addition, only two experiments (the second GRA experiment established in October 2011 and the last SNE experiment established in October 2013) showed correlation coefficients different from zero based on confidence intervals (Table 2). There were substantial differences in the relationship between recruitment and flowering time across experiments. Variation in the relationship between recruitment and flowering time was wider, albeit quite variable in shape, in experiments established in October in GRA (Fig. 2). In contrast, when the growing season was shorter (late sowings in December in GRA) or the environment was harsher (SNE), variation in the relationship between recruitment and flowering time was substantially narrower (Fig. 2). Finally, fitness variation across the space defined by recruitment and flowering time varied among experiments (Fig. 2), stressing the heterogeneity of fitness responses to environmental variation during all experiments and the complex relationship between fitness and key life-history traits in *A. thaliana*.

NATURAL SELECTION ON LIFE HISTORY

Selection differentials were rather similar to selection gradients (mean difference ± SE between β and s across experiments = 0.028 ± 0.013 and 0.026 ± 0.017 for recruitment and flowering time, respectively; Table 3), suggesting that direct selection prevailed over indirect selection through correlated traits in this set of *A. thaliana* accessions and experiments. The exception was the last experiment, i.e. the SNE experiment established in October 2013,

which is probably explained by the lower sample size and the lower number of replicates per accession in this experiment. The results also indicated that linear selection differentials and selection gradients were significant for flowering time in almost all experiments, whereas they were barely significant for recruitment (Table 3). Finally, quadratic selection was mostly non-significant for both recruitment and flowering time (Table 3), suggesting that stabilizing or disruptive selection only played a minor role in shaping quantitative variation in this set of *A. thaliana* accessions and experiments.

When significant, linear selection gradients were always negative for recruitment (range β = -0.33 – -0.29; Table 3), indicating that selection favored accessions with lower recruitment. Although this result would suggest that the average fitness per individual was lower in denser pots, we believe that that was not the case, as there were either positive correlations between survivorship and fecundity ($0.29 < r < 0.58$, $P < 0.04$ in four experiments) or no relationship at all between these two traits ($r < 0.18$, $P > 0.26$ in the other four experiments). The particularities of the two experiments in which we found such significantly negative β values would account for this result. In the GRA experiment established in October 2010, performances were far above the grand mean in terms of recruitment, survivorship and fecundity. In the SNE experiment established in October 2013, sample size was reduced and accessions were represented by fewer replicates, which might have affected the results.

In contrast, linear selection gradients for flowering time did vary in sign and magnitude (Table 3). Most of the linear selection gradients for flowering time were negative (range β = -0.37 – -0.24; Table 3), suggesting that selection favored early flowering accessions (Fig. 3A–C). However, two experiments, i.e. the GRA experiment established in October 2010 and the SNE experiment established in October 2012, exhibited positive linear selection gradients for flowering time (range β = 0.12 –0.27; Table 3), indicating that late

flowering accessions were favored by selection in these experiments (Fig. 3A–C). When significant, flowering time negatively correlated with flowering duration (Table 4), indicating that early-flowering accessions flowered for longer, except in the SNE experiment established in October 2012 that exhibited the opposite relationship. In practically all experiments, flowering time negatively correlated with survivorship, fecundity and fitness, indicating that early-flowering accessions had higher survivorship, higher fecundity, and higher fitness (Table 4). The exception was the first GRA experiment established in October 2010. In this experiment, there were positive correlations between flowering time and fecundity as well as fitness (Table 4). In contrast, the correlation was negative between flowering time and survivorship, overall indicating that early-flowering accessions had more survivorship, but lower fecundity and lower fitness (Table 4).

We also evaluated the global effects of selection for recruitment and flowering time using grand means and variances obtained from pooling data from all experiments, as well as separating the fitness contributions into its components, i.e. survivorship and fecundity (Table 5). Overall, we found consistent results with those obtained for each experiment, that is, the sign of significant selection differentials and gradients for recruitment was the opposite of those for flowering time (Table 5). On top of that, the fitness components for survivorship and fecundity along the flowering time continuum, the trait markedly under selection in this study, also exhibited an opposite relationship between these two fitness components (Fig. 3D). In particular, survivorship and fecundity made greater contributions to fitness in early and late flowering accessions, respectively (Table 5 and Fig. 3). Finally, the global selection differentials and selection gradients for variances in recruitment and flowering time, a first indicator of phenotypic plasticity for these traits, were mostly non-significant (Table 5), suggesting that selection for variance in these traits might not be important in this study.

ENVIRONMENTAL DRIVERS OF SELECTION AND PHENOTYPIC VARIATION

None of the linear selection gradients for recruitment and flowering time obtained for each experiment were significantly correlated with environmental variables recorded during the experiments ($N = 8$, $P > 0.42$ in all cases). Mean fitness across experiments was not correlated with any environmental variable from source populations ($N = 50$, $P > 0.10$ in all cases). However, when we excluded the first experiment in GRA (established in October 2010) due to its extremely high fitness value that masked the overall pattern, mean fitness showed a significant positive correlation with average annual minimum temperature ($N = 50$, $r = 0.38$, $P < 0.025$; Fig. 4A), indicating that accessions from north-western warmer environments performed better than those from cooler environments when growing in southern environments.

Phenotypic plasticity estimated by means of the relative distance plasticity index (RDPI) for recruitment ranged between 0.12 and 0.39 (mean ± SE = 0.24 ± 0.06), for survivorship between 0.20 and 0.47 (mean ± SE = 0.33 ± 0.07), for flowering time between 0.08 and 0.17 (mean ± SE = 0.12 ± 0.02), for fecundity between 0.39 and 0.71 (mean ± SE = 0.51 ± 0.06), and for fitness between 0.42 and 0.77 (mean ± SE = 0.58 ± 0.07). Hence, flowering time was the trait exhibiting the lowest phenotypic plasticity across experiments. In addition, phenotypic plasticity for flowering time was the only trait with significant correlations with weather records from source populations, in particular with average annual minimum temperature ($N = 50$, $r = 0.59$, $P < 0.001$; Fig. 4B) and to a lesser extent with average annual maximum temperature ($N = 50$, $r = 0.32$, $P = 0.049$), indicating that accessions from north-western warmer locations exhibited higher phenotypic plasticity for flowering time than those from cooler locations when growing in southern environments. The rest of traits and environmental variables did not show any significant relationship ($P > 0.12$

425 in all cases). Accessions with higher mean fitness also exhibited higher phenotypic plasticity
426 for flowering time ($N = 50$, $r = 0.62$, $P < 0.001$; Fig. 4C).
427 Finally, we plotted the correlation coefficients between life-history traits and
428 representative environmental variables during the experiments (average minimum
429 temperature and total precipitation) along the mean flowering time continuum obtained across
430 experiments. When looking only at the significant correlation coefficients between
431 environmental variables and traits, the results showed how flowering time determined the
432 relationship between environmental variables and life-history traits in *A. thaliana*. First,
433 accessions with intermediate flowering time exhibited a negative relationship between
434 average minimum temperature and recruitment, whereas accessions with the earliest and latest
435 flowering times showed positive relationships between average minimum temperature and
436 recruitment (Fig. 5A). The opposite picture emerged for flowering time (Fig. 5B), as a result
437 of the negative relationship exhibited between recruitment and flowering time in these
438 experiments. When considering fitness, most of the significant correlation coefficients were
439 positive for accessions along the flowering time continuum, except for a few intermediate and
440 late flowering accessions (Fig. 5C). In the case of precipitation, we also detected accessions
441 with negative and positive correlation coefficients between precipitation and life-history
442 traits, although the patterns were not as clear as in the case of average minimum temperature
443 (Fig. 5D–F). The exception was recruitment in which few accessions with intermediate
444 flowering time exhibited significant negative correlation coefficients whereas five accessions
445 with the late flowering times showed the opposite pattern (Fig. 5D).
446

447 ## *Discussion*

448 Pre-existing standing genetic variation, rather than fixation of *de novo* mutations, is thought to
449 be the most efficient primary mechanism enabling complex organisms to adapt to changing

environments (Barrett and Schluter 2008; Jump et al. 2008; Matuszewski et al. 2015). Bearing in mind such a premise, we challenged a set of *A. thaliana* accessions from north-western Iberian Peninsula to complete the life cycle in two contrasting experimental facilities in southern Spain, in terms of altitude, temperature and precipitation, over multiple years. For this particular region of the Mediterranean Basin, broad agreement exists that global climate change is going to increase warming (Klausmeyer and Shaw 2009; Gómez-Navarro *et al*. 2010; Jacobeit *et al*. 2014) in such a way that today's southern climatic environments are predicted to shift northwards. Although there is no guarantee that the particular environments observed at GRA and SNE experimental facilities will be those characterizing north-western Iberian Peninsula by the end of the century, they do represent low altitude, warm and relatively wet (GRA), and high altitude, mild and dry environments, (SNE), for most accessions from the north-western *A. thaliana* genetic lineage (Fig. 1C).

The correlation between mean fitness across experiments and environmental variables from source populations illustrated very well the response of north-western *A. thaliana* accessions in southern environments (Fig. 4A). In particular, *A. thaliana* accessions from warmer environments in north-western Iberian exhibited higher fitness than accessions from cooler environments when growing in southern environments. In addition, accessions from warmer environments also exhibited higher phenotypic plasticity for flowering time in southern environments, which clearly was the trait under stronger selection in this study. Overall, these results stress the potential of north-western Iberian *A. thaliana* to cope with increasingly warmer environments in the region. Based on these results, we predict a scenario of demographic viability and even growth of those *A. thaliana* populations occurring in north-western warmer environments as the amount of warming increases in the coming decades. In contrast, *A. thaliana* populations from north-western cooler environments might exhibit demographic shrinkage under climate change. Hence, our results support the view that global

475 climate change needs not to imply dramatic local extinction but probably a redistribution of
476 standing genetic variation of *A. thaliana* in the region.

477 Our results also allowed the assessment of the mechanism by which *A. thaliana* may
478 respond to changing environments, which is through selection on flowering time as selection
479 on recruitment was less frequent and intense (Table 3). Furthermore, heritability for flowering
480 time was higher than that for recruitment in all experiments, indicating the higher degree of
481 genetic determination for flowering time than for recruitment in *A. thaliana* (Méndez-Vigo et
482 al. 2013). We found that selection favored early flowering in six of eight experiments.
483 Interestingly, we also observed significant selection for late flowering in the other two
484 experiments. Although detecting selection for late flowering can be troublesome (Austen et al.
485 2017 and references therein), our experiments allowed the identification of two different
486 scenarios favoring late flowering in *A. thaliana* at low and high altitudes in southern Iberian
487 environments. On the one hand, the first GRA experiment established in 2010 characterized
488 by high recruitment, high survivorship and very high fecundity, where late-flowering
489 accessions had shorter flowering duration. On the other hand, the second SNE experiment
490 established in 2012 characterized by low recruitment, medium survivorship, and high
491 fecundity, where late-flowering accessions had longer flowering durations. These two distinct
492 scenarios, which revealed the enormous plasticity of the species to cope with contrasting
493 environments, took place only once over the course of the experiments.

494 The rarity of exceptional years, in which we detected selection for late flowering, does
495 not mean that their demographic and evolutionary importance should be underestimated. The
496 results of these experiments are in agreement with the behavior of natural *A. thaliana*
497 populations, which normally exhibit a huge year-to-year variation in practically all relevant
498 demographic attributes (Picó 2012) as a result of exceptional combinations of environmental
499 conditions favoring all important life -cycle transitions. Hence, rare weather events favoring

phenotypes that are normally selected against, albeit not wiped out from the population, have the chance to increase their frequency in the population by replenishing the soil seed bank in these exceptional years (Fig. S3). In the long term, it is accepted that such varying selection may enhance the persistence of genetic variation within populations across the species' range (Gillespie and Turelli 1989; Hall and Willis 2006; Fournier-Level et al. 2013; Ågren et al. 2017). In any case, further research is needed to find out how genetic diversity of natural populations may be related to the unpredictability of weather conditions occasionally favoring low-frequency phenotypes.

Despite selection for late flowering in two of eight experiments and the potential of such rare events for the long-term population dynamics, we believe that north-western *A. thaliana* will likely evolve towards earlier flowering if environmental conditions eventually become warmer and drier as predicted by climate change projections. A reason is that most of the significant correlation coefficients between average minimum temperature and fitness were significantly positive for accessions with early and intermediate mean flowering times, but not for those with the latest flowering times for which higher minimum temperatures implied a decline in fitness (Fig. 5C). However, it is worth noting that several accessions did not show any significant relationship between environmental variables and life-history traits or fitness regardless of their flowering time (hollow dots in Fig. 5), a pattern that might reveal those accessions with higher plasticity or a lower sensitivity to variation in the environmental variables recorded during the experiments. These accessions may also be very important for maintaining the genetic diversity of populations in the long run.

Another reason to believe that early flowering will become predominant in these Iberian populations in a warmer world is that Iberian *A. thaliana* populations that inhabit warm environments with mild winters and hot dry summers are characterized by early flowering and high seed dormancy (Méndez-Vigo et al. 2011; Kronholm et al. 2012; Vidigal

et al. 2016). Furthermore, in warm environments, the genetic correlation between early flowering and high seed dormancy is stronger (Vidigal et al. 2016) in a way that life cycle variation becomes constrained in southern warm regions and also in warmer coastal areas all over the Iberian Peninsula (Marcer et al. 2018). Given the tight correlation between seed dormancy and flowering time in *A. thaliana* (Debieu et al. 2013; Vidigal et al. 2016), detecting selection for early flowering might only be part of the story. Although it is not a straightforward task, future research should also focus on field experiments evaluating the extent of varying selection on both key *A. thaliana*'s life-history traits simultaneously (see Taylor et al. 2017) under contrasting environmental scenarios.

Predictive models of global climate change urgently need to incorporate demographic, genetic and evolutionary processes that will likely result in more biologically relevant predictions (Hoffmann and Sgrò 2011; Brown and Knowles 2012; Fordham et al. 2014; Gavin et al. 2014; Merow et al. 2014; Brown et al. 2016; Etterson et al. 2016). At present, there exist various modeling platforms taking demography and dispersal into account to model the spatial dynamics of species with environmental changes (Engler et al. 2012; Bocedi et al. 2014; Brown 2014), such as those mediated by global climate change, fragmentation and/or habitat loss. We believe that experimental approaches, like the one presented here providing fitness responses to novel environments and phenotypic plasticity for life-history traits using genetic pools from specific geographic regions, open great possibilities for including evolutionary processes into such existing modeling platforms. In particular, the results of this study suggest that it would be interesting to evaluate the effects of the temperature-mediated adaptive adjustment of flowering time, the phenotypic plasticity of flowering time assuming different scenarios based on the adaptive, non-adaptive and neutral nature of phenotypic plasticity, or the temporal heterogeneity of selection for flowering time, on population fitness with increasing warming.

DATA ARCHIVING

Data deposited in the Dryad repository: XXX.

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778

779 **Table 1**. Mean (SD) values for life-history traits of 50 *A. thaliana* accessions per experiment.

Facility	Sowing month	Year	Recruitment (proportion)	Survivorship (proportion)	Flowering time (days)	Duration (days)	Fecundity (seeds/individual)	Fitness (Surv. × Fec.)
GRA	October	2010 – 2011	0.54 (0.17)	0.81 (0.21)	146.01 (21.74)	13.22 (0.95) / 93	710.6 (1477.2)	488.3 (734.82)
GRA	October	2011 – 2012	0.42 (0.15)	0.40 (0.24)	142.87 (12.89)	10.06 (0.85) / 87	34.9 (37.9)	17.3 (23.4)
GRA	October	2012 – 2013	0.42 (0.14)	0.40 (0.21)	141.47 (20.64)	14.98 (1.23) / 102	39.7 (36.6)	18.1 (22.5)
GRA	October	2013 – 2014	0.55 (0.20)	0.30 (0.22)	125.33 (15.45)	11.88 (0.99) / 83	31.8 (31.6)	9.7 (11.4)
GRA	December	2012 – 2013	0.71 (0.13)	0.84 (0.31)	109.91 (11.72)	6.60 (0.43) / 50	105.6 (64.4)	94.7 (69.3)
GRA	December	2013 – 2014	0.59 (0.12)	0.51 (0.37)	96.68 (13.81)	13.06 (0.91) / 60	22.7 (21.6)	13.3 (17.4)
SNE	October	2011 – 2012	0.28 (0.10)	0.02 (0.09)	–	– / –	–	–
SNE	October	2012 – 2013	0.37 (0.15)	0.41 (0.24)	167.94 (6.75)	8.42 (0.72) / 33	141.4 (119.1)	53.7 (48.8)
SNE	October	2013 – 2014	0.21 (0.15)	0.26 (0.28)	173.28 (7.28)	7.07 (0.73) / 31	98.7 (110.6)	20.9 (25.8)

780 Entries are given for each experimental facility, sowing month and year. Data includes the maximum proportion of seeds recruited as rosettes,
781 survivorship as the proportion of rosettes becoming reproductive, flowering time (days), flowering duration per accession and for the whole
782 period (days), fecundity (mean number of seeds per reproductive individual), and fitness computed as survivorship × fecundity (mean number of
783 expected seeds per individual). The experiment established in SNE in 2011 had very low survivorship rates and was excluded from the analyses.
784 The experiment established in SNE in 2013 was based on 42 accessions and fewer replicates per accession.

785

786 **Table 2**. Heritability (95% confidence intervals) values for recruitment and flowering time, and the genetic correlation between the two traits for

787 50 *A. thaliana* accessions per experiment.

Facility	Sowing month	Year	Recruitment	Flowering time	Correlation
GRA	October	2010 – 2011	0.144 (0.063 – 0.234)	0.871 (0.778 – 0.942)	-0.216 (-0.538 – 0.097)
GRA	October	2011 – 2012	0.252 (0.146 – 0.365)	0.748 (0.661 – 0.827)	-0.385 (-0.641 – -0.115)
GRA	October	2012 – 2013	0.096 (0.014 – 0.183)	0.756 (0.652 – 0.841)	-0.062 (-0.476 – 0.344)
GRA	October	2013 – 2014	0.338 (0.210 – 0.470)	0.688 (0.589 – 0.789)	-0.209 (-0.516 – 0.094)
GRA	December	2012 – 2013	0.060 (0.000 – 0.127)	0.853 (0.797 – 0.905)	-0.096 (-0.623 – 0.399)
GRA	December	2013 – 2014	0.236 (0.127 – 0.352)	0.662 (0.549 – 0.759)	0.000 (-0.322 – 0.316)
SNE	October	2012 – 2013	0.118 (0.044 – 0.198)	0.476 (0.355 – 0.604)	-0.114 (-0.467 – 0.255)
SNE	October	2013 – 2014	0.037 (0.000 – 0.097)	0.319 (0.137 – 0.489)	-0.843 (-0.999 – -0.369)

788 Data are given for each experimental facility, sowing month and year. The experiment established in SNE in 2011 was excluded from the

789 analyses. The experiment established in SNE in 2013 was based on 42 accessions and fewer replicates per accession.

790

791 **Table 3**. Linear and quadratic selection gradients (β and γ) and selection differentials (s and C) for recruitment and flowering time for 50 *A.*

792 *thaliana* accessions per experiment.

				Linear			Quadratic		
Facility	Sowing	Year		Recruitment	Flowering time		Recruitment	Flowering time	Interaction
GRA	October	2010 – 2011	β	-0.33 (0.08) ***	0.27 (0.08) ***	γ	-0.03 (0.13) *ns*	-0.09 (0.15) *ns*	-0.13 (0.09) *ns*
			s	-0.33 (0.08) ***	0.27 (0.08) ***	C	-0.02 (0.09) *ns*	-0.13 (0.15) *ns*	-0.11 (0.08) *ns*
GRA	October	2011 – 2012	β	-0.08 (0.08) *ns*	-0.24 (0.11) *	γ	0.11 (0.13) *ns*	0.39 (0.21) **	-0.06 (0.10) *ns*
			s	-0.02 (0.09) *ns*	-0.20 (0.10) *	C	0.16 (0.09) *	0.37 (0.17) **	-0.19 (0.12) *
GRA	October	2012 – 2013	β	-0.03 (0.10) *ns*	-0.14 (0.07) *	γ	0.09 (0.16) *ns*	0.16 (0.19) *ns*	0.11 (0.08) *ns*
			s	-0.05 (0.10) *ns*	-0.14 (0.08) *	C	0.10 (0.16) *ns*	0.21 (0.21) *ns*	0.12 (0.08) *ns*
GRA	October	2013 – 2014	β	-0.09 (0.10) *ns*	-0.36 (0.09) ***	γ	0.28 (0.18) *ns*	0.15 (0.15) *ns*	0.14 (0.11) *ns*
			s	-0.10 (0.12) *ns*	-0.35 (0.09) ***	C	0.28 (0.21) *ns*	0.15 (0.11) *ns*	0.14 (0.11) *ns*
GRA	December	2012 – 2013	β	-0.02 (0.05) *ns*	-0.36 (0.05) ***	γ	0.06 (0.07) *ns*	-0.05 (0.12) *ns*	-0.10 (0.09) *ns*
			s	-0.01 (0.06) *ns*	-0.35 (0.08) ***	C	0.06 (0.06) *ns*	-0.05 (0.11) *ns*	-0.10 (0.09) *ns*
GRA	December	2013 – 2014	β	-0.03 (0.07) *ns*	-0.37 (0.09) ***	γ	0.03 (0.14) *ns*	-0.14 (0.16) *ns*	-0.02 (0.13) *ns*
			s	-0.03 (0.08) *ns*	-0.35 (0.10) ***	C	0.03 (0.12) *ns*	-0.15 (0.14) *ns*	0.01 (0.11) *ns*
SNE	October	2012 – 2013	β	0.04 (0.07) *ns*	0.12 (0.07) *	γ	0.04 (0.08) *ns*	-0.03 (0.09) *ns*	0.04 (0.06) *ns*
			s	0.02 (0.07) *ns*	0.11 (0.06) *	C	0.03 (0.07) *ns*	-0.04 (0.08) *ns*	0.04 (0.06) *ns*
SNE	October	2013 – 2014	β	-0.29 (0.17) *	-0.25 (0.12) **	γ	0.09 (0.30) *ns*	-0.28 (0.27) *ns*	-0.15 (0.19) *ns*
			s	-0.19 (0.17) *ns*	-0.12 (0.11) *ns*	C	0.16 (0.22) *ns*	-0.11 (0.17) *ns*	-0.10 (0.12) *ns*

Mean (SE) values per experimental facility, sowing month and year. The experiment established in SNE in 2011 was excluded from the analyses. The experiment established in SNE in 2013 was based on 42 accessions and fewer replicates per accession. Significance: ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; *ns*, non-significant.

797 **Table 4**. Pearson's correlation coefficients between flowering time and life-history traits.

Facility	Sowing	Year	Duration	Survivorship	Fecundity	Fitness
GRA	October	2010 – 2011	-0.57 ***	-0.37 **	0.41 **	0.40 **
GRA	October	2011 – 2012	-0.47 **	-0.68 ***	-0.08 *ns*	-0.37 **
GRA	October	2012 – 2013	-0.42 **	-0.19 *ns*	-0.24 *ns*	-0.25 *ns*
GRA	October	2013 – 2014	-0.16 *ns*	-0.26 *ns*	-0.61 ***	-0.52 ***
GRA	December	2012 – 2013	-0.32 *	-0.55 ***	-0.74 ***	-0.74 ***
GRA	December	2013 – 2014	0.03 *ns*	-0.84 ***	0.05 *ns*	-0.53 ***
SNE	October	2012 – 2013	0.31 *	-0.05 *ns*	0.28 *ns*	0.21 *ns*
SNE	October	2013 – 2014	-0.42 **	-0.07 *ns*	-0.35 *	-0.10 *ns*

798 Life-history traits are flowering duration per accession (days), survivorship as the proportion
799 of rosettes becoming reproductive, fecundity (mean number of seeds per individual), and
800 fitness computed as survivorship × fecundity. Significance: ***, $P < 0.0001$; **, $P < 0.01$; *,
801 $P < 0.05$; *ns*, non-significant. Sample size was 50 in all experiments except in the SNE
802 experiment established in October 2013, in which sample size was 46 for duration, 44 for
803 fecundity, and 42 for survivorship and fitness.
804

805 **Table 5**. Global linear selection gradients and differentials (β and s) for means and variances
806 of recruitment and flowering time for 50 *A. thaliana* accessions.

Component		Recruitment (mean)	Recruitment (variance)	Flowering time (mean)	Flowering time (variance)
Integrated	β	-0.161 (0.069) **	-0.112 (0.059) *	0.178 (0.161) *ns*	0.033 (0.157) *ns*
	s	-0.182 (0.075) **	-0.097 (0.063) *ns*	0.142 (0.071) *	-0.097 (0.078) *ns*
Survivorship	β	0.037 (0.015) *	0.005 (0.018) *ns*	-0.043 (0.032) *ns*	0.002 (0.035) *ns*
	s	0.041 (0.017) **	-0.001 (0.018) *ns*	-0.046 (0.019) **	0.040 (0.020) *
Fecundity	β	-0.107 (0.088) *ns*	-0.070 (0.070) *ns*	0.341 (0.215) *ns*	0.146 (0.224) *ns*
	s	-0.131 (0.080) *ns*	-0.054 (0.059) *ns*	0.210 (0.079) **	-0.121 (0.087) *ns*

807 Mean (SE) values obtained by pooling all experiments. Selection gradients and selection
808 differentials were computed for each fitness component, i.e. survivorship and fecundity,
809 separately. Significance: ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; *ns*, non-significant.
810

FIGURE LEGENDS

Figure 1 (A) Map of geographic locations of the 50 *A. thaliana* populations in north-western Iberian Peninsula and the two experimental facilities (GRA and SNE) in southern Spain. (B) Distribution of latitudes and altitudes for the 50 populations and the two experimental facilities. (C) Histograms of annual mean minimum temperature, annual mean maximum temperature, and total annual precipitation for the period 1951 – 1999 obtained from the Digital Climatic Atlas of the Iberian Peninsula for the 50 *A. thaliana* populations. The same data from the two experimental facilities are also indicated. (D) Daily minimum (blue) and maximum (red) temperatures and total precipitation (green) at GRA and SNE obtained from local meteorological stations over the course of the experiments. Dashed lines indicate the duration of the experiments.

Figure 2 Scatter plots for the different combinations of flowering time and recruitment recorded per accession and experiment. Experiments are indicated by facility and sowing data (month and year). The normalized fitness for each accession and experiment is superimposed using a colour scale.

Figure 3 (A – C) Scatter plots displaying the relationship between relative fitness and flowering time for all experiments separated by experimental facility (GRA and SNE), sowing date (October and December) and year. (D) Scatter plot displaying the relationship between normalized fitness components, i.e. survivorship (hollow dots and dashed line) and fecundity (filled dots and continuous line), and flowering time using grand means per accession across experiments.

836 **Figure 4** (A) Scatter plot showing the correlation between mean fitness across experiments
837 and average annual minimum temperature from source populations. (B) Scatter plot showing
838 the correlation between phenotypic plasticity for flowering time and average annual minimum
839 temperature from source populations. (C) Scatter plot showing the correlation between mean
840 fitness across experiments and phenotypic plasticity for flowering time. All correlations were
841 significant (Dutilleul's modified *t* test).
842
843 **Figure 5** Scatter plots showing the correlation coefficients between environmental variables,
844 i.e. average minimum temperature and total precipitation recorded during the experiments,
845 and life-history traits, i.e. recruitment, flowering time and fitness. Correlation coefficients are
846 displayed along the mean flowering time continuum computed across experiments.
847 Significant and non-significant correlation coefficients are indicated by filled and hollow dots,
848 respectively. For significant correlation coefficients (only those with $P < 0.01$), we plotted the
849 best function maximizing the R^2 if any.
850

851 **SUPPORTING INFORMATION**

852

853 **Figure S1** Pictures of experimental facilities: the low altitude El Castillejo Botanical Garden
854 of Sierra de Grazalema Natural Park (GRA; 36.46ºN, 5.30ºW, 329 m.a.s.l.) and the high
855 altitude La Cortijuela Botanical Garden of Sierra Nevada National Park (SNE; 37.08ºN
856 ,3.47ºW, 1,650 m.a.s.l.).

857

858 **Figure S2** Relationship between the number of fruits per plant and the number of seeds per
859 fruit. This relationship was used to estimate fecundity as the mean number of seeds per plant.

860

861 **Figure S3** Total number of seeds produced per experiment. The exceptional good year in
862 GRA in October 2010 illustrates the potential of *A. thaliana* to massively replenish the seed
863 bank with seed from all genotypes.

864