Genetic variation in phenology of wild *Arabidopsis thaliana* plants

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Short running title: *Phenology of wild Arabidopsis*

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Author Contribution

JRL conceived of the project with TEJ, DLDM and CML assisted in experiments, VLD conducted experiments, and analyzed data, VLD and JRL led writing, all authors contributed to the interpretation and writing of the manuscript.

Data Accessibility Statement

Original data will be included as supplemental tables. Prior studies from which phenology data was sourced will be listed in a supplemental table.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Abstract

Phenology and the timing of development are often under selection. However, the relative contributions of genotype, environment, and prior developmental transitions to variance in the phenology of wild plants is largely unknown. Individual components of phenology (e.g., germination) might be loosely related with the timing of maturation due to variation in prior developmental transitions. Given widespread evidence that genetic variation in life history is adaptive, we investigated to what degree experimentally measured genetic variation in Arabidopsis phenology predicts phenology of plants in the wild. As a proxy of phenology, we obtained collection dates from nature of 227 naturally inbred *Arabidopsis thaliana* accessions from across Eurasia. We compared this phenology in nature with experimental data on the descendant inbred lines that we synthesized from two new and 155 published controlled experiments. We tested whether the genetic variation in flowering and germination timing from experiments predicted the phenology of the same lines in nature. We found that genetic variation in phenology from controlled experiments significantly predicts day of collection from wild individuals, as a proxy for date of flowering, across Eurasia. However, local variation in collection dates within a region was not explained by genetic variance in phenology in experiments, suggesting high plasticity across small-scale environmental gradients or complex interactions between the timing of different developmental transitions. This apparent low heritability in phenology within regions contrasts with the well-known genetic clines in phenology, suggesting that strong selection or many generations are required for adaptation and the emergence of phenological clines.

Introduction

The predictability of trait variation from genetic variation, *i.e.* trait heritability, is a key quantity determining the rate of trait evolution and the ability of populations to adapt to changes in selection (Lush 1937; Gomulkiewicz and Houle 2009). Plant biologists have long relied on common garden experiments to estimate breeding values and heritability for a range of traits (Mazer and LeBuhn 1999). However, natural populations often occur in highly heterogeneous environments to which organismal traits may respond plastically. As a result, heritability in natural populations may be much lower than those in common gardens. Furthermore, even field common gardens might fail to accurately recreate natural selective pressures if the direction and magnitude of selection varies in time (Karrenberg & Widmer 2008). Alternatively, heritability can be estimated using pedigrees or genomic data from wild individuals (Gienapp et al. 2017), but these models rely on assumptions of certain genetic architectures (Mitchell-Olds and Rutledge 1986) and the resulting heritability estimates are not often compared with common garden estimates of trait variation (but see (Weigensberg and Roff 1996)).

Phenology, or the timing of the developmental transitions between an organism's life stages, directly and indirectly influences plant fitness and selection by determining the traits that are expressed at any point throughout the year (Donohue 2005). In seasonally variable environments, traits such as flowering time and dormancy can ameliorate harsh abiotic conditions by timing dormant periods to coincide with unfavorable seasons (e.g. drought escape Ludlow 1989; Lawrence-Paul and Lasky 2024). Flowering, vegetative phase change, and germination timing often show geographic clines, suggesting their importance in adaptation to local environments (Baughman et al. 2019; Martínez-Berdeja et al. 2020; Lawrence-Paul et al. 2023, 2025). Common garden studies often find that genetic variation in individual components of phenology are under selection and have further demonstrated variable strength and direction of selection across field sites (Hall and Willis 2006; Fournier-Level et al. 2013; Ågren et al. 2017; Gamba et al. 2024b). However, the duration of individual developmental transitions (e.g. flowering time) might be loosely related to phenology in nature due to variation in prior developmental transitions (e.g. germination) (Burghardt et al. 2015). While phenology is determined by both genetic factors and plastic responses to environment (Amasino 2004; Andrés and Coupland 2012; Auge et al. 2018), the relative importance of these two sources of

phenotypic variance in the timing of individual developmental transitions determining phenology in nature is unclear, even in model systems.

Much of our understanding of the genetic basis of plant phenology comes from studies in the model *Arabidopsis thaliana* (hereafter Arabidopsis). Common garden trials in both lab and field settings have uncovered genetic loci contributing to much of the observed variation in dormancy and flowering time (Juenger et al., 2005; Brachi et al., 2010; Fournier-Level et al., 2013; Ågren et al., 2017). These traits are also plastic and show genotype by environment interactions (Juenger et al. 2005; Zhou et al. 2005; Wilczek et al. 2009; Lorts and Lasky 2020): germination responds to temperature, photoperiod, moisture, and nutrient availability (Huang et al. 2010, 2018; Penfield and Springthorpe 2012; Footitt et al. 2013; Kenney et al. 2014), while flowering responds to multiple cues such as temperature and daylength (Thomas and Vince-Prue 1997; Lempe et al. 2005; Balasubramanian et al. 2006).

The broad phenological variation described in controlled environment experiments is observed in wild populations as well (Ratcliffe 1976; Simpson and Dean 2002; DeLeo et al. 2020). Arabidopsis can exhibit the life history of a winter annual, germinating in the fall, overwintering as a rosette, and flowering in the spring (Figure 1). However, Arabidopsis can also germinate and flower in a single growing season. These shorter-lived plants tend to be facultative — flowering in spring, summer, or fall — and in some regions this rapid life cycle enables multiple generations within a year. This variation in life histories occurs in many annual plants (Baskin and Baskin 1988) and is made possible, in part, by a range of flowering times and germination traits that can vary independently from each other (Marcer et al. 2018; Martínez-Berdeja et al. 2020). In Arabidopsis, geographic clines in breeding values for phenology, clines in allele frequencies of phenology QTL, and evidence of selection on phenology QTL support the conclusion that phenology is adapted to local environmental conditions in the wild (Caicedo et al. 2004; Stinchcombe et al. 2004; Samis et al. 2012; Fournier-Level et al. 2013; Ågren et al. 2017; Debieu et al. 2018; Exposito-Alonso et al. 2018; Lasky et al. 2018, 2024; Gamba et al. 2024a) (Figure 1). Yet, even within geographic proximity one can find genotypes with substantial genetic differences in flowering time (Jones 1971; Alonso-Blanco et al. 2016; Méndez-Vigo et al. 2022).

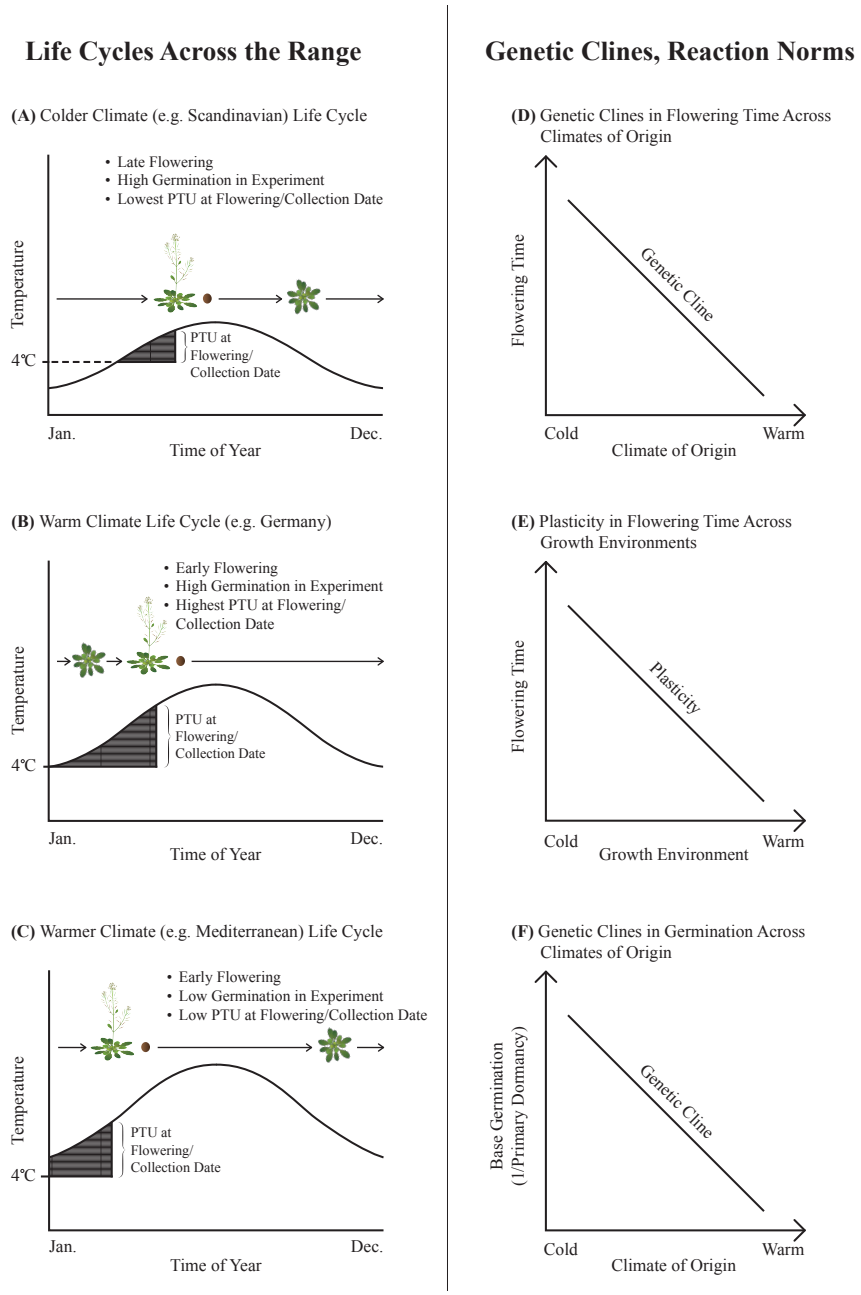


Figure 1. *Genotype and environment likely influence phenology of individual Arabidopsis plants across its geographic range the wild. Genetics and environmental cues may determine how long a plant remains in vegetative growth (green) or how long a plant remains dormant as a seed (brown circles), with three representative life cycles shown from Scandinavia to the Mediterranean (A–C), with shaded areas showing accumulation of photothermal units (PTUs) in the growing season (e.g. temperature >4ºC). Existing knowledge of clines in flowering time and germination combined with plastic acceleration of flowering in warmer temperatures (D–F).*

There are several mechanisms that may explain why phenology observed in controlled experiments shows poor correlation with phenology observed in nature (Wilczek et al. 2009). While genotype likely influences the phenology of an individual plant in the wild, the dependence of sequential developmental transitions on each other, as well as plasticity in their expression, may limit the translation of genetic values of single stage to natural phenology. First, there are interactions between germination and flowering time, both because of a shared genetic basis and because the timing of early life transitions influences environmental exposures in later life stages (Chiang et al. 2009; Huang et al. 2010; Springthorpe and Penfield 2015; Huo et al. 2016). Secondly, there is stochasticity in individual germination and flowering time (Jimenez-Gomez et al. 2011; Abley et al. 2020). Thirdly, spatial environmental variation is extensive in nature and genotype-environment interactions have major impacts on phenology (Sasaki et al. 2015). Finally, maternal effects on phenological transitions may be strong, as reported extensively for seed dormancy (Boyd et al. 2007; Chiang et al. 2013; Burghardt et al. 2016; Huang et al. 2018). As a result, flowering time loci identified in common garden lab or field experiments might have loose relationships with variation among natural individuals (Chiang et al. 2013); genotypes differing in phenology in one set of conditions may express the same phenology in another set of conditions (Wilczek et al. 2009; Burghardt et al. 2016).

Here, we compare phenological observations of wild individual plants from natural history collections (herbaria and seed stock centers) (Miller-Rushing et al. 2006; MacGillivray et al. 2010; Davis et al. 2015; Ramirez-Parada et al. 2022) with plants in controlled environment experiments. Arabidopsis are naturally inbred (Jones 1971), thus we can compare collection dates of wild individuals with phenology of nearly genetically identical descendants in controlled experiments. This comparison provides a window into how genetic variation shapes phenology in natural populations against the forces of plasticity and GxE. Natural history collections can be used to characterize the climate conditions during development preceding collection (Bontrager et al. 2025b). We estimated phenology as collection date, and using the climate conditions preceding collection, we also estimated phenology as accumulated photothermal units (PTUs) at collection (Burghardt et al. 2015). PTUs allow us to account for weather effects on development and effectively estimate how far into growing seasons plants were collected.

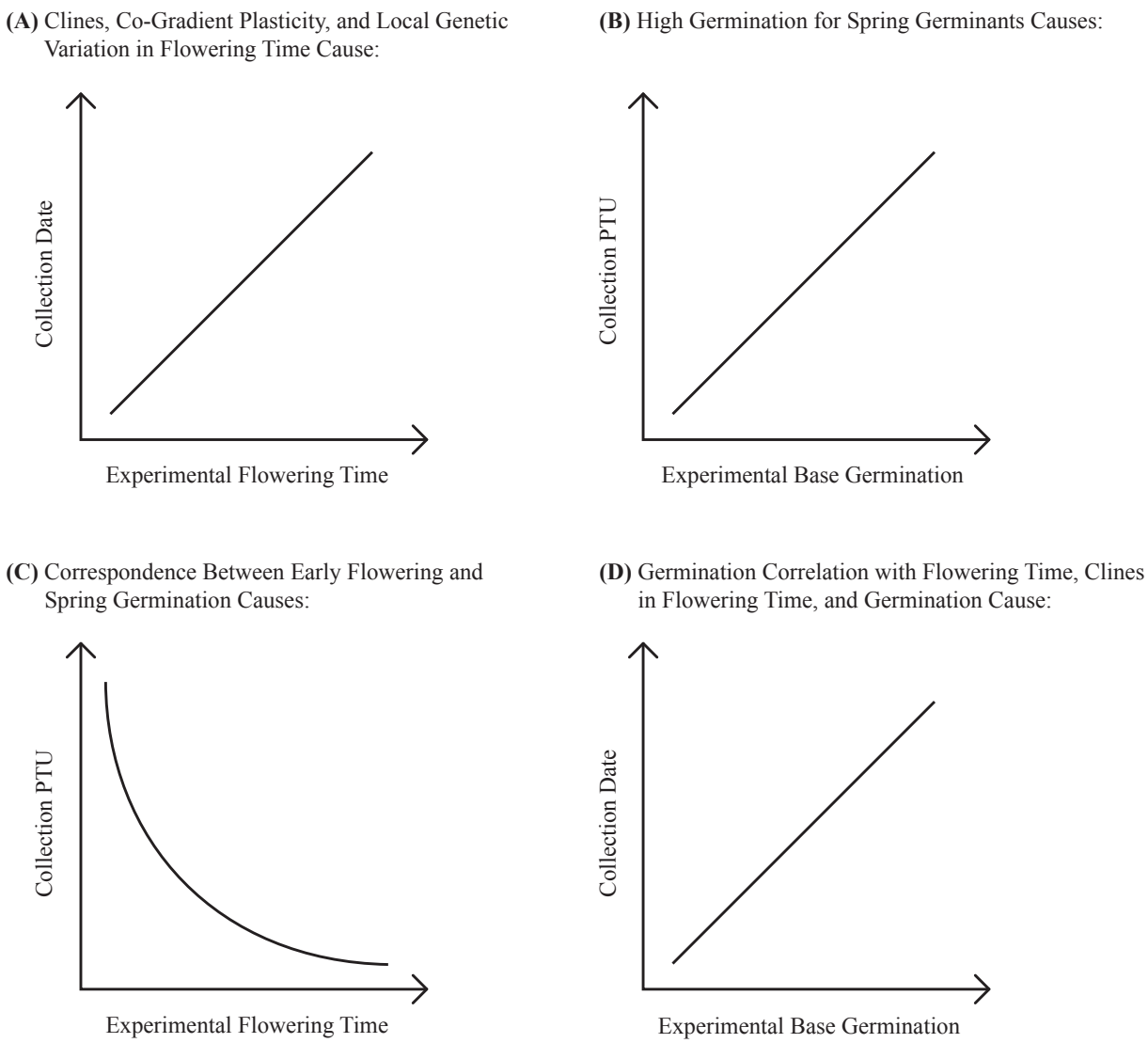


Figure 2*: Existing knowledge of clines in flowering time and germination combined with plastic acceleration of flowering in warmer temperatures (see Figure 1) lead to our predictions (A–D here) of how flowering time and base germination rates in controlled experiments will correspond to phenology in natural wild plants.*

We developed three hypotheses for how the phenology of individual plants in nature may relate to genetic variation in phenological traits, illustrated in Figure 2:

H1. We predict that Arabidopsis plants collected earlier in a natural growing season will have early flowering and low germination breeding values in controlled environments. Specifically, we predict that genetic clines in flowering time (here, early flowering in warmer climates) and germination rate, combined with plastic acceleration of flowering in warmer conditions (Figure 1), leads to range-wide positive relationships between breeding values for flowering time, germination rate, and collection date (as a proxy for flowering date in the wild; Figure 2).

H2. For cumulative photothermal units (PTU) at collection, we predict that spring annuals with high germination rates and early flowering times will have been collected later in growing seasons (high PTU) than winter annuals with later flowering breeding values (Figure 2).

H3. Locally, within populations, environmentally induced phenological variation (i.e., plasticity associated with different timing of spring warming) is likely lower than that seen across the species range. Thus we predict that flowering time breeding values and germination rates would be positively related to collection dates largely due to genetic effects (Figure S1).

Materials and Methods

Natural genotypes

We first conducted an experiment on a set of 101 naturally inbred genotypes ("ecotypes") selected based on known collection dates either from the herbarium specimen label or recorded in the Arabidopsis Biological Resource Center https://abrc.osu.edu/ database. 47 of these ecotypes had not been studied in previous flowering time experiments and 75 had not been included in previous germination experiments. Seed was ordered from ABRC (94 accessions) or was germinated from herbarium sheets (7 accessions from Real Jardín Botánico Madrid, Komarov Botanical Institute, and Oslo Natural History Museum).

Seeds from herbarium specimens were cold-stratified in tap water at pH 7 and placed at 4°C for 7 d. Seeds were then directly sown into damp Fafard germination mix and grown in Conviron growth chambers under 10/14hr, 18/22°C days/nights. Seven herbarium accessions germinated using this protocol. In the second round of germination, we surface sterilized seeds

159 using standard protocols and then cold-stratified, as above. We plated seeds onto
160 MS+Gamborg's vitamins + agar plates containing 1% sucrose and 10uM GA4 and then placed
161 them in growth chambers, as above. Two more herbarium accessions flowered using this
162 protocol; these were transplanted to Fafard Germination mix. To induce flowering for seed
163 collection, plants were exposed to 30d of 4°C with 10/18hr day/night cycles.
164
165 *Flowering and germination experiments*
166 Ecotypes were grown in common conditions prior to the flowering time experiment, and
167 flowering time replicates were descended from a single mother plant. For each ecotype, three
168 replicates were grown in separate pots. Seeds were stratified at 4°C for 5 days before sowing in
169 pots. Each pot was thinned to a single individual after the emergence of the second set of true
170 leaves. Plants were grown at 22°C under 16h days of fluorescent light in a walk-in Conviron
171 growth chamber (model MTPS144). Day of bolting and day petals appeared were both recorded
172 as measures of flowering time.
173 Seeds from each replicate maternal plant in the flowering time experiment were collected
174 and stored separately in dry conditions until the germination trial. For each treatment, forty seeds
175 from each parent plant (or as many seeds as were available for replicates with low fecundity)
176 evenly divided across 2 plates were sown on filter paper in petri dishes and germinated at
177 23/18°C during day/night with constant 16h daylength in a Conviron growth chamber. In total,
178 1,752 plates and >50k seeds were assayed.
179 Seeds were subjected to cold stratification at 4°C in the dark for 3 treatment lengths: 2
180 weeks, 3 days, and 0 days. Cold stratification can break primary dormancy, however 2 weeks of
181 chilling can prompt secondary dormancy in the seedbank (Penfield and Springthorpe 2012). The
182 difference in germination rate between 3 days and 0 days of stratification can therefore indicate
183 primary dormancy while the difference in germination rate between 3 days and 2 weeks of
184 stratification may indicate secondary dormancy. As a caveat, lower germination after 2 weeks of
185 stratification could be due to other, unmeasured negative effects on germination, such as
186 bacterial infection. We staggered the planting so that all the plates came out of stratification on
187 the same day. The number of seeds that had germinated in each plate was recorded on days 1, 3,
188 5, 10, 14, 21, and 28. Seeds were considered germinated if the radicle was visible. Because
189 maternal plants flowered at different times, germination rates for this experiment may be

influenced by the length of the time between flowering and planting and any after-ripening that may have occurred. We also tested for these maternal effects among individuals of the same inbred line (Supplemental Material).

Phenology from published experiments

We searched the literature for experiments on Arabidopsis that measured flowering time or germination traits across different natural inbred lines (commonly referred to as Arabidopsis "ecotypes"). The minimum number of ecotypes in any single experiment was 17. Our final set used data from 38 previous studies (31 included some measure of flowering time, 15 included germination) that, combined with our new experiments described above, included over 3,000 ecotypes for 86 flowering time experimental conditions and 66 germination experimental conditions, although all ecotypes were used in only a subset of the trials and only 291 ecotypes had a reliable date of original collection from the wild.

We used this dataset of phenology measurements from the literature to create an estimate of genetic variation in flowering time and dormancy among ecotypes. We sought to gain statistical power by combining data from different experiments. To make phenotypes comparable, we standardized across treatments and experiments, transforming each flowering time experiment such that the earliest flowering accession had a value of 0 and the latest flowering accession had a value of 1.

We averaged standardized experimental flowering times across experiments, keeping vernalized, non-vernalized, and field experiments separate. Here, we use 'vernalization' to describe extended cold treatments applied to rosettes. Non-vernalized growing conditions uncover genetic variation in flowering time due to vernalization requirements that is masked under vernalized or fall-sown field conditions and may be important for determining phenology and life history in the wild (Wilczek et al. 2009). However, Arabidopsis plants growing in many natural settings are expected to experience changes in temperature and photoperiod that would be more similar to vernalized and field experiments (Li et al. 2010). By keeping the three treatments separate, we could test whether non-vernalized flowering time or vernalized flowering time was more predictive of wild phenology. Field flowering time was aggregated across all seasons, which may lead to less coherent estimates of field genetic flowering time if seasonal differences lead to meaningful differences in flowering time, and also broken down further into spring (4

221 treatments over 2 experiments), summer (4 treatments, 1 experiment), and fall plantings (23
222 treatments, 9 experiments). To control for regional bias, this aggregation was performed both for
223 all experiments and without experiments that tested only ecotypes collected from a single
224 country.

225 Data collection approaches for estimating germination rate and dormancy were more
226 heterogeneous than for flowering time. Depending on the experiment, dormancy was reported as
227 the number of days after planting until a set percentage of germination was reached, the
228 percentage of seed germinated a given number of days after planting, the number of days of
229 storage until a set percentage of germination, or the germination rate after a given number of
230 days of storage. Because of the difference in metrics across experiments, dormancy values were
231 standardized by rank within each experiment with zero indicating low to no dormancy and one
232 indicating high dormancy. Measures that reported a percentage germinated were ranked in the
233 opposite direction from measures that reported number of days until germination or the number
234 of days of storage before 50% germination. We found that standardized rank-based metrics that
235 increase with dormancy (such as days to 50% germination) were correlated with rank based on
236 metrics that decrease with dormancy (such as percentage of seeds germinated after a set number
237 of days, Pearson's $r = 0.485$).

238 These experiments captured variation in primary dormancy, or recalcitrance to germinate
239 immediately after harvest. Secondary dormancy, or dormancy induced when a seed experiences
240 conditions unfavorable to germination, has been studied in experiments that measured an
241 increase in dormancy during storage. Primary and secondary dormancy could lead to different
242 phenological and ecological outcomes (Martinez-Berdeja et al. 2020), so we averaged across
243 experiments that measured primary or secondary dormancy separately to estimate each of these
244 two traits. Secondary dormancy was not included in the Generalized Additive Models described
245 below. Maternal conditions may be an important source of phenological plasticity; thus we also
246 used our dormancy experiment to examine how variation in flowering time among maternal
247 plants influences germination (Supplemental Material).
248

249 *Wild phenology*

250 The date of collection (for ecotypes maintained by ABRC) or collection date (for ecotypes grown
251 from a known herbarium record) was used as an estimate of reproductive phenology of

individuals in the wild (Primack et al. 2004; Davis et al. 2015). We excluded records from regions where Arabidopsis has been recently introduced, like North America and Japan. Accessions from outside the native range or collected after the 320th day of the year (which we deemed likely errors based on location, sometimes due to intentional plantings, and were greater than 2 standard deviations from the mean) were removed. Our geographic limits also excluded island accessions to the south of the Mediterranean, e.g. Cape Verde Island.

We hypothesized that genetic differences in wild phenology may be more apparent if we account for spatiotemporal environmental fluctuations causing plasticity. Therefore, we calculated photothermal units (PTUs) for each accession from the day of collection using monthly climate time series data from CRU (Harris et al. 2014), beginning with January 1 of each year, following the methods of (DeLeo et al. 2020) and (Burghardt et al. 2015). PTUs integrate the temperature and light experienced by a plant at a given location through the growing season and therefore may capture environmental cues relevant to phenology and better describe genetic variation in development (Wilczek et al. 2009; Brachi et al. 2010). We only sought to use PTUs to estimate how far into growing seasons plants were collected; we did not attempt to estimate PTUs since germination since germination timing was unknown. The models described below use the square root of PTU because the resulting distribution was closer to normal than the log transformation.

Statistical comparison of collection date in the wild versus phenology in experiments

H1: Range-wide comparison of phenology breeding values vs. collection date

We tested if genetic variation in normalized phenology measured on naturally inbred lines explained variation in the wild phenology of the parent of the line (Figure 2), using linear regression between the normalized phenology breeding values (flowering time and dormancy rank) and collection day. Vernalized, non-vernalized, and field flowering times were modelled separately, because the genetic variation in phenology uncovered by each treatment could relate to wild phenology in different ways. Under the hypothesis that vernalization and field conditions better recreate environments that a plant would experience at their geographic origin, these genetic flowering time measures should be more positively related to wild flowering time. Likewise, non-vernalized flowering times may better recreate the original temporal niches of summer annuals and thus be positively related to flowering time in these ecotypes. However, it is

283 also possible that long non-vernalized flowering times indicate obligate winter annuals. In these
284 plants, non-vernalized flowering times would be negatively related to wild phenology since later
285 flowering times in non-vernalized experiments would indicate plants that overwinter and are
286 collected early the next year (Figure 2).

287 We also investigated how range-wide differences in wild phenology related to regional
288 differences in breeding values using a larger sample size, taken from a broader collection of
289 ecotypes than just those with documented collection dates. This comparison included stock
290 center ecotypes with experimental phenology data and known location-of-origin, but no recorded
291 date of wild collection, and herbarium specimens for which we had not germinated seed and
292 grown plants to measure traits in experiments. To do so, we first estimated wild flowering time
293 for each stock center ecotype in experiments using herbarium records near the ecotype collection
294 location. These wild flowering times were estimated from a previously published generalized
295 additive model (GAM) with spatially-varying intercepts of herbaria collection dates from 2,655
296 Eurasian Arabidopsis records used in (DeLeo et al. 2020), which included year of collection as a
297 nuisance variable, as collection date has changed over time across the range of Arabidopsis
298 (DeLeo et al. 2020). Values of the smooth intercept surface were extracted at the coordinates of
299 stock center ecotypes having an experimentally measured flowering time. These estimated wild
300 flowering times were regressed against breeding values for flowering times under vernalized,
301 non-vernalized, and field experiments and rank primary dormancy. In addition, for these same
302 data we performed Spearman rank correlation between estimated wild flowering times and
303 phenology breeding values.
304

305 *H2: Range-wide comparison of phenology breeding values vs. PTU*

306 To account for plasticity in phenology due to differences among locations in the timing and
307 progression of growing seasons, we also tested the relationship between phenology breeding
308 values and the estimated PTUs at the time of collection from the wild. We conducted the same
309 analyses across the range of Arabidopsis as described in H1, except that the PTU at collection
310 was used instead of collection date. Specifically, we conducted linear regression between
311 normalized breeding values with PTU at collection and we also conducted linear regression
312 between breeding values and the PTUs at collection of nearby herbarium specimens.
313

H3: Within population variation

Next, we tested for the effects of genetic variation in flowering time and dormancy on phenological variation *within* populations in the wild. By first accounting for geographic variation in mean flowering time, we aimed to isolate local within-population variation (Figure S1). This analysis, in essence, asks whether genetic variation in individual phenological stages explains natural phenological variation within populations. Generalized Additive Models (GAMs) allow for model parameters to vary smoothly across space and thus can capture spatially varying patterns, e.g. in local average collection date (Yee and Mackenzie 2002; Yee and Mitchell 2006). We built a GAM for the dependent variable of collection date (Y) at location j of individual i which included covariates of experimental flowering time (β_1), dormancy (β_2), elevation (β_3) and a spatially varying intercept (μ_j):

Equation 1.

$$Y_{ij} = \mu_j + \beta_1 \text{Flowering Time}_{ij} + \beta_2 \text{Primary Dormancy}_{ij} + \beta_3 \text{Elevation}_j + \varepsilon_{ij}$$

The smooth spatially varying intercept term μ_j estimates spatial variation in mean collection date (e.g., due to environmental gradients). The upper limit on degrees of freedom for the spatially varying intercept was increased to 45 following the recommendations of Wood (2006). In models that allowed for higher degrees of freedom, the effective degrees of freedom did not meaningfully increase. Elevation was included because of its known importance to flowering times and spring onset (Vidigal et al. 2016; Gamba et al. 2023) and the high resolution of elevation data compared to smooth variation in GAM parameter surfaces. We fit GAMs using the 'gam' function in the 'mgcv' package in R (Wood 2006) using restricted maximum likelihood (REML), although Generalized Cross Validation returned similar estimates. Model fitting allowed for penalization of smooth terms to 0 so that uninformative covariates could be removed from the equation. Residuals were plotted using the 'gam.check' function in 'mgcv' (Wood 2006), and one ecotype (Nok-10) was removed from our analyses that was an extreme outlier based on its residuals. Spatial variation in β coefficients for flowering time and dormancy covariates (Eq 1) were not significant, so a model using a constant coefficient was used in our analyses. Field, vernalized, and non-vernalized flowering times were tested in separate versions of the model and tested different hypotheses with regards to wild phenology. To compare among

345 the three measures of flowering time, a version of Equation 1 was fit on a subset of ecotypes that
346 had all three flowering time measures, and Akaike's Information Criterion (Akaike 1974) was
347 compared.
348 In the wild, both flowering time and dormancy contribute to phenology. Thus, we also
349 tested whether including interactions between flowering time and primary dormancy (β_4)
350 improved the model:
351
352 Equation 2.
353 $Y_{ij} = \mu_j + \beta_1 \text{Flowering Time}_{ij} + \beta_2 \text{Primary Dormancy}_{ij} + \beta_3 \text{Elevation}_j + \beta_4 \text{Flowering Time}_{ij} *$
354 $\text{Primary Dormancy}_{ij} + \varepsilon_{ij}$
355
356 Finally, given the importance of plasticity in response to temperature in the timing of
357 germination and flowering time, PTU might better capture genetic variation in phenology in the
358 wild (Figure 2 "Predictions"). Therefore, we also tested the models above using PTU in place of
359 collection day to correct for climate.
360
361 **Results**

362 *Genetic correlation among phenology traits in controlled experiments*

363 Across all compiled experiments (both previously published and our new experiments) mean
364 flowering times of ecotypes measured in vernalized and non-vernalized experiments were
365 strongly positively correlated (Pearson's $r = 0.90$, $p < 0.001$). Although fall-sown field trials
366 might be expected to expose plants to cold seasonal temperatures that give vernalization cues,
367 standardized flowering time breeding values in both non-vernalized and vernalized experiments
368 were similarly correlated to published field experiment values ($r_{\text{non-vernalized}} = 0.77$, $r_{\text{vernalized}} =$
369 0.72, $p < 0.001$ for both). Primary and secondary dormancy were negatively correlated, but not
370 significantly so ($r = -0.15$, $p = 0.11$). Despite known interactions between flowering time and
371 dormancy due to seed maturation environment and pleiotropy of causal loci, only non-vernalized
372 flowering time was significantly correlated with primary dormancy ($r = -0.17$, $p = 0.02$, Figure 3;
373 for a heatplot of all phenotypes, see Figure S2). These correlations were qualitatively unchanged
374 when using an alternate standardization method across experiments (Figure S3).

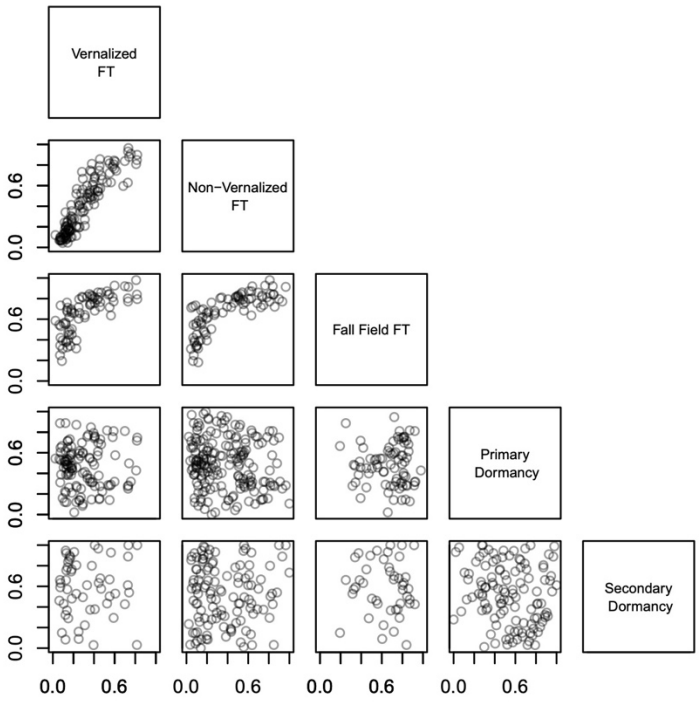


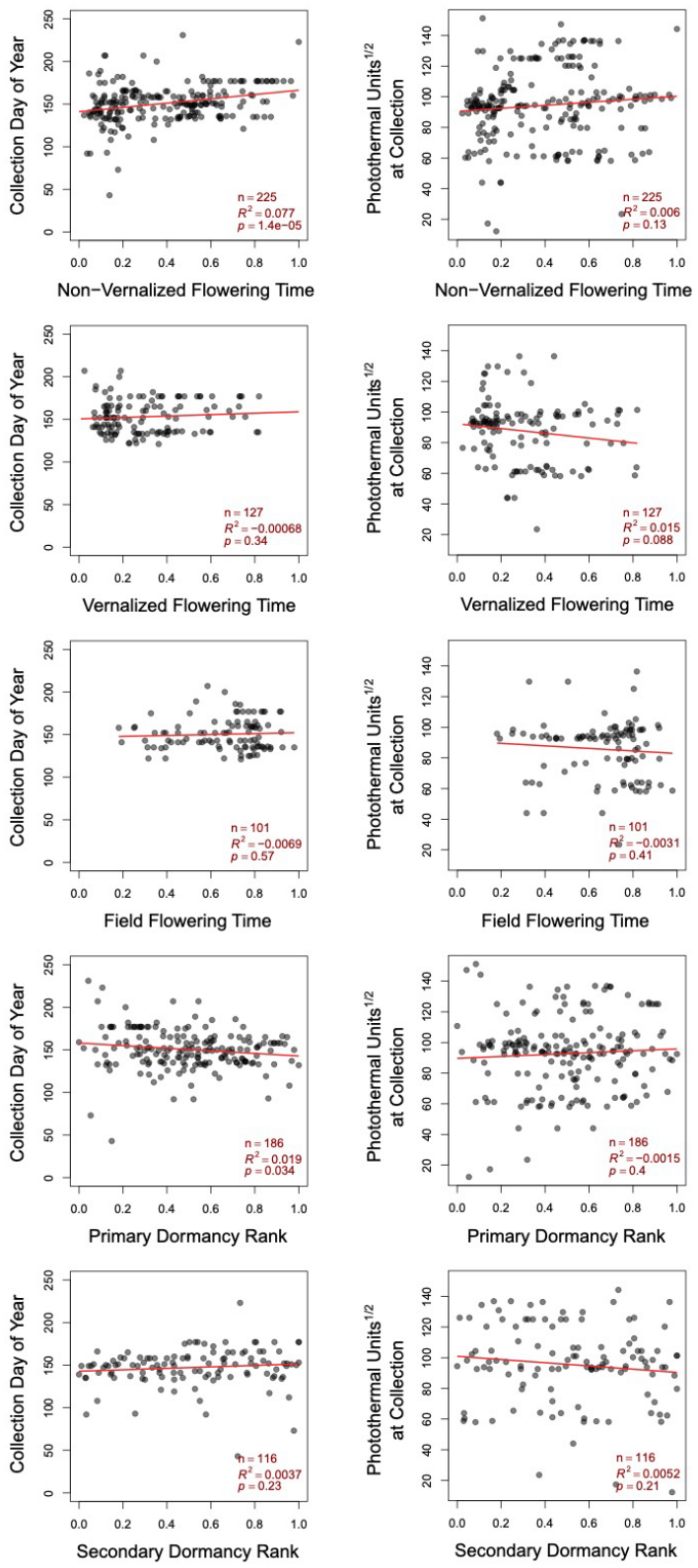
Figure 3. Standardized phenology traits combined across multiple published controlled experiments combined with our new experiments. Standardized flowering times were correlated across all three types of treatments (vernalized, non-vernalized, field), but they were weakly correlated with dormancy.

H1: Comparing wild phenology with breeding values in experiments across the range

Experimental breeding values for phenology traits were modest predictors of collection day in simple linear models, with the only statistically significant predictors being non-vernalized flowering time (estimated slope = 27.7 days/standardized flowering time, $r^2 = 0.09$, $p < 0.001$) and primary dormancy (-17.1 days/standardized rank dormancy, $r^2 = 0.02$, $p = 0.02$). Flowering time breeding values under vernalized or field conditions did not significantly predict day of collection with a linear model ($p > 0.05$) (Figure 4). Flowering time and germination measured within individual experiments did not predict wild collection day better than our averaged

389 breeding values, suggesting some power was gained by combining individual experiments into
390 standardized values (Figure S7).
391

Figure 4. Date of collection (left panels) and PTUs (photothermal units) at collection (right panels) of wild plants compared to standardized breeding values for phenological traits from experiments. Collection day of wild plants was positively related to non-vernalized flowering time in experiments. Collection day was negatively related to primary dormancy. The values shown for field flowering time experiments were calculated from fall-sown experiments only.



Flowering time and dormancy breeding values predict regional variation in collection date of nearby herbarium specimens

Our model of collection date, above, was limited to the 227 ecotypes with both a known collection date and experimental phenology data. To expand our predictions across more of the Arabidopsis range, we tested whether genetic variation in flowering time in experiments predicts local mean collection dates of herbarium specimens across the landscape. First, we found that local mean collection dates across a landscape from >2500 herbarium collections were positively correlated with actual collection days of individual stock center ecotypes (which were not used in fitting the model, $\rho = 0.51$, $p < 0.001$ Figure S8), validating these local predictions of phenology in the wild based on herbarium collections.

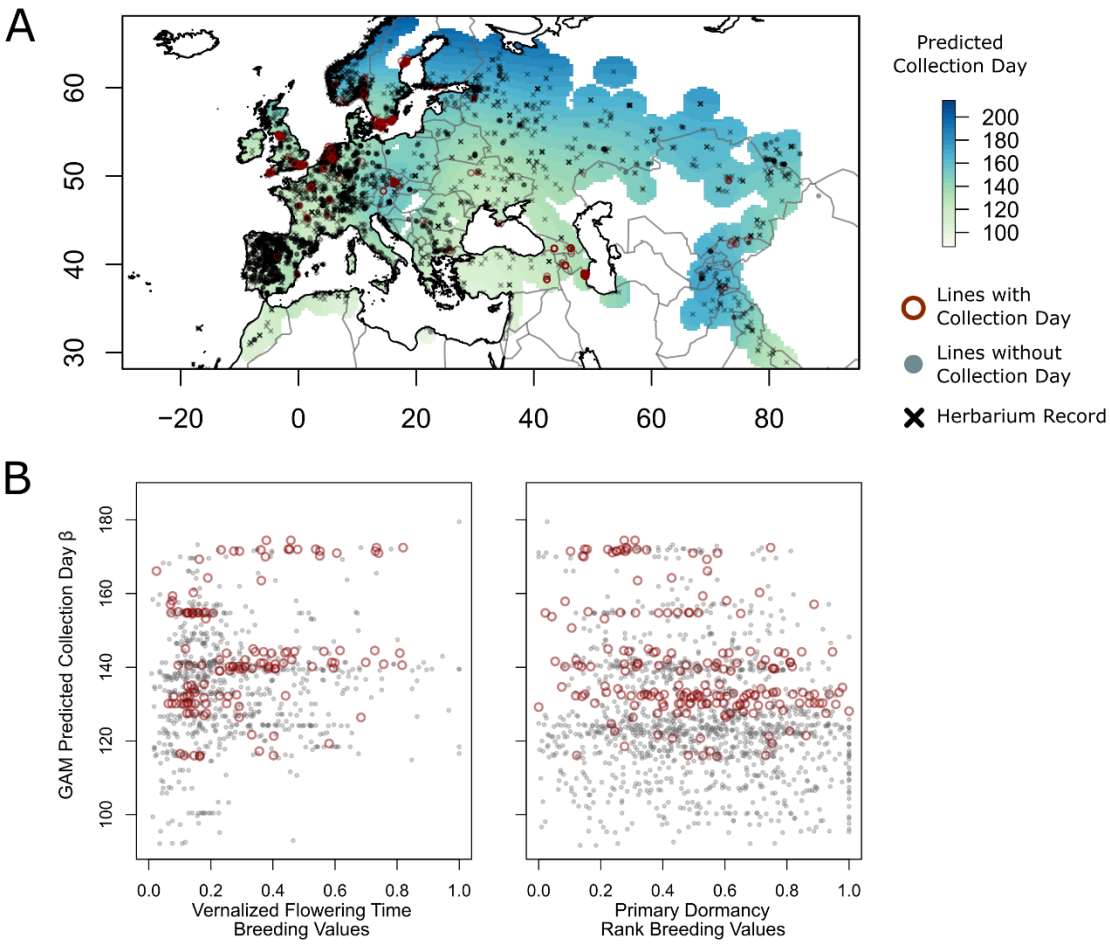


Figure 5. Genetic variation in flowering time experiments predicts collection dates of nearby

herbarium specimens. A) There is geographic variation in collection dates as shown by estimated mean date from GAMs with spatially varying means, with earlier collections (light green) around the Mediterranean (DeLeo et al. 2020). *B) Local mean collection dates were positively correlated with genetic variation in vernalized flowering time experiments ($p < 0.01$), and all ecotypes from locations with earlier mean collection dates in the wild (largely Mediterranean) had rapid flowering breeding values. Primary dormancy breeding values were negatively correlated with local mean collection dates ($p < 0.001$).*

As with the collection dates of the original maternal sources of stock center lines, breeding values for non-vernalized flowering times significantly predicted local mean herbarium collection dates ($r^2 = 0.10$, $p < 0.001$). For comparison, latitude was a better predictor of mean herbarium collection date ($r^2 = 0.37$, $p < 0.001$). These patterns were similar for vernalized, non-vernalized, and field measurements, although not significant for field trials when fall plantings were grouped with spring and summer plantings ($r^2 = 0.07$, $p = 0.002$, $r^2 = 0.10$, $p < 0.001$, and $r^2 = 0.02$, $p = 0.13$, respectively, Figure 5). These results show that range-wide genetic variation in phenology measured in controlled environments corresponds to actual phenology of plants in nature. However, because plants in nature from different regions experience different environments, these patterns cannot determine the degree to which genetic variation causes the variation in natural phenology as opposed to genetic variation being correlated with environmental variation that causes phenological plasticity in nature (i.e. co-gradient variation, Figure 1).

H2: Range-wide comparison of phenology breeding values vs. Photothermal Units

Because environmental differences among locations can influence phenology (Fournier-Level et al. 2013), we also calculated PTU at collection and compared to these values to traits from experiments. However, PTU at collection was not significantly predicted by any phenology trait breeding values (Figure 4). Thus, while ecotypes with later flowering time breeding values tend to be collected later in the year, these later flowering ecotypes are not collected at higher PTU, i.e. later in the local growing season. The fact that phenology breeding values are correlated with

453 date, but not PTU at collection, is consistent with the hypothesis that geographic climate
454 variation maintains clines in breeding values due to local adaptation and clines in collection date
455 partly due to plasticity (co-gradient variation), generating a breeding value-collection date
456 correlation. These correlations were qualitatively unchanged when using an alternate
457 standardization across experiments and when excluding herbarium specimens (Figure S4–S6).

458

459 *H3: Variation in phenology breeding values does not predict local, within-region variation in*
460 *wild collection dates*

461 We next examined whether genetic values could explain variation in natural phenology *within*
462 regions (Figure S1). We used GAMs that included spatially varying intercepts and an elevation
463 covariate to account for the among population variation in environmental effects on collection
464 dates, leaving local variation to be potentially explained by genetic variation. However, we found
465 that normalized flowering time (slope of non-vernalized flowering time = -8.2, $p = 0.098$) and
466 dormancy (slope of dormancy = 2.2, $p = 0.51$) were not significantly related to day of collection
467 in this GAM (Equation 1), although elevation effects were significant (slope of elevation =
468 0.012, $p = 0.049$, Figure 6, Figure S9, Table S1). In comparing models using vernalized, non-
469 vernalized, and field experiments, we found that standardized non-vernalized flowering times led
470 to lower AIC compared to other flowering time measures but higher AIC than a model without
471 flowering time at all, although the difference was slight (difference < 2 for an AIC of 530). We
472 used non-vernalized flowering times in the final models, in part because there were more
473 ecotypes with non-vernalized flowering times (184 for non-vernalized vs 113 for vernalized or
474 78 for field).

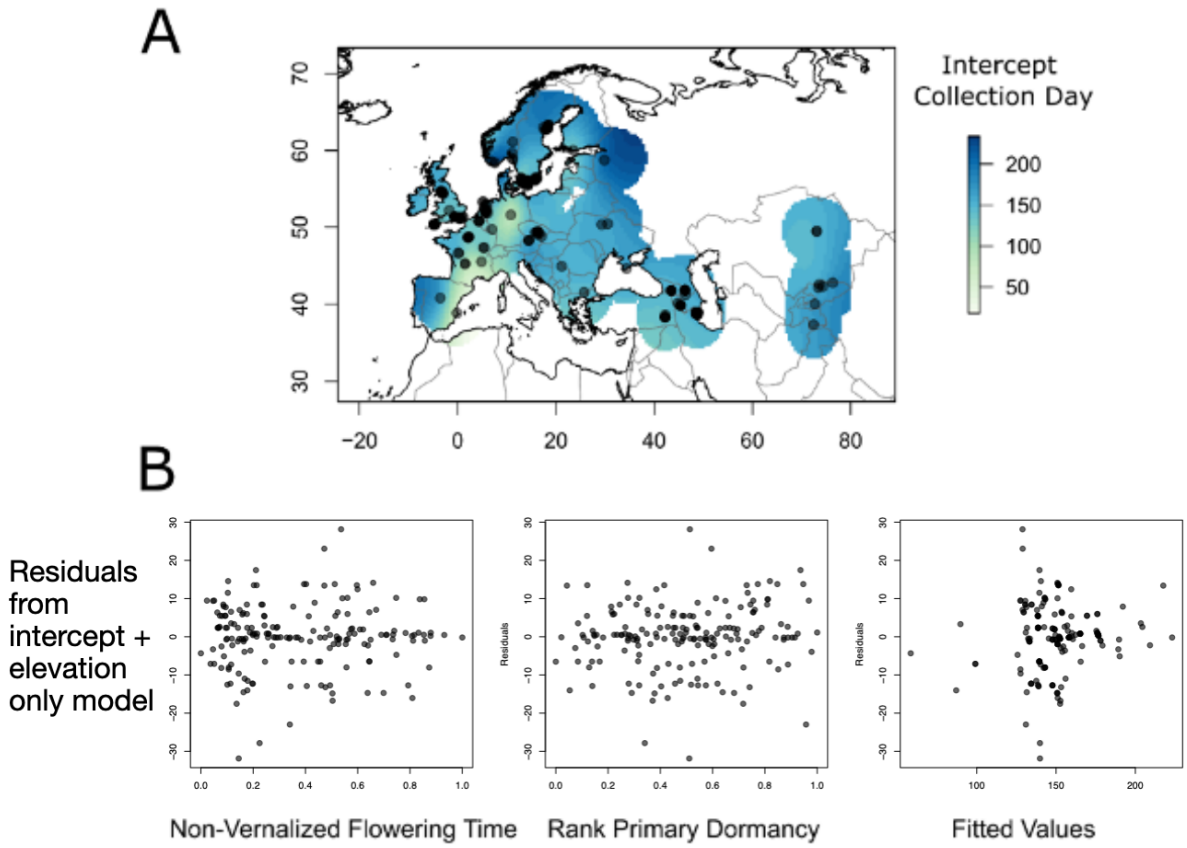


Figure 6. Top: Spatial variation in date of collection for 227 ecotypes with known collection dates. A) Color represents the intercept value for collection day at a location after fitting a linear (i.e. non-spatially varying) relationship with elevation, experimental primary dormancy and non-vernalized flowering time. Much of the variation in collection date was explained by the spatially varying intercept rather than being explained by experimental flowering time. B) Residuals of a GAM predicting collection date using only intercept and elevation parameters, compared to non-vernalized flowering time, primary dormancy, or fitted collection dates.

Although we found that flowering time, dormancy, and the interaction between the two were not significantly related to collection day, including phenology breeding values did lower the AIC and slightly increase the deviance explained by the model. This suggests that dormancy and flowering time could be related to variation in phenology within regions. However, most of the variation was described by the spatially varying intercept –a model with only a spatially

489 varying intercept had a deviance explained of 80.1% – which could account for geographic
490 genetic clines in phenology and plastic responses to geographic climate gradients (Figure S8).

491 As with the linear models, we also tested whether genetic variation in phenology in
492 experiments could explain local variation PTU of collection date in nature. Again, we found that
493 flowering time and dormancy were not significantly related to wild PTU at collection in GAMs
494 with spatially varying intercepts. Elevation was negatively related to PTU, indicating that higher
495 elevation plants were collected earlier in growing seasons, reflecting a potential winter annual
496 strategy (Figure 1, slope: -0.018 $\sqrt{PTU}$/m, $p < 0.01$). Additionally, elevation was positively
497 correlated with date of collection (slope: 0.012 days/m, $p < 0.05$).

498

499 **Discussion**

500 Plant phenology comprises multiple traits that contribute to fitness. In Arabidopsis, flowering
501 time and germination can vary independently to create a landscape of possible life histories
502 across environments (Debieu et al. 2013; Marcer et al. 2018; Martínez-Berdeja et al. 2020). This
503 variation in phenology is likely partly maintained by selection, given that the traits vary
504 geographically in association with climate (Fournier-Level et al. 2011; Vidigal et al. 2016;
505 Exposito-Alonso 2020) and biotic pressures (Lyons et al. 2015; Davila Olivas et al. 2017), that
506 QTL show evidence of local adaptation (Gamba et al. 2023; Lasky et al. 2024), and that the
507 timing of individual phenological transitions is often correlated with fitness in experiments
508 (Korves et al. 2007; Stock et al. 2015). Standing genetic variation is necessary for the timing of
509 developmental transitions to evolve in response to selection by local environments, but little is
510 known about the heritability of phenology in natural, wild individuals. Furthermore, because
511 multiple developmental transitions influence phenology, the effects of variation in the timing of
512 one stage might be compensated by (or, in under-parameterized experiments, confounded by)
513 variation in the timing of other stages. Given widespread evidence that genetic variation in life
514 history is adaptive, we investigated to what degree experimentally measured genetic variation in
515 Arabidopsis phenology predicts phenology of plants in the wild, based on collection dates of
516 natural history records.

517 The influence of genotype and environment on flowering time and dormancy have been
518 well studied experimentally in Arabidopsis. Yet, common garden and controlled environment
519 experiments must be designed thoughtfully to highlight genetic differences between ecotypes
520 that are relevant to selection in natural environments (Karrenberg and Widmer 2008). While
521 experimental design has increasingly recognized the importance of field conditions to acquire a
522 measure of phenology that is more representative of nature (Wilczek et al. 2009; Anderson et al.
523 2012; Brachi et al. 2013; Poorter et al. 2016), there has been little comparison of controlled
524 experiments, field or otherwise, to phenology in wild, naturally cycling individual plants. We
525 found that flowering time and dormancy breeding values are related to date of collection and
526 capture variation in phenology primarily *among* populations in the wild, but explain little
527 variation *within* regions/populations.

528

529 *Genetic variation in flowering time and dormancy predicts variation in wild phenology among*
530 *populations across the species range*

531 Across the species range, both primary dormancy and non-vernalized flowering time
532 significantly predicted collection dates. These phenological transitions are not independent of
533 each other. As seen in individual experiments (Martínez-Berdeja et al. 2020), we found that the
534 average breeding values for primary dormancy and non-vernalized flowering time were
535 negatively correlated (Figure 3). Furthermore, dormancy is strongly affected by environmental
536 conditions during seed maturation (Penfield and Springthorpe 2012; Huang et al. 2015;
537 Burghardt et al. 2016). Thus, interaction between flowering time and dormancy in the wild could
538 stem from both genetic correlations and environmentally induced interactions. Despite our
539 expectation for interactions between germination and flowering traits, models of collection day
540 that included flowering time by dormancy interactions had a higher AIC and did not explain
541 more deviance than a model with both traits separately. Including interactions between flowering
542 time and dormancy in our models did not help to explain collection date in the wild.

543 We found that predicted wild collection date was positively related to flowering time
544 breeding values when we extended our analysis to predict wild flowering times from a smoothed
545 surface of collection dates fit to herbarium records. In our linear models, average flowering time
546 breeding value was more closely related to this estimated day of collection than was the actual

547 date of collection of ecotypes in experiments. However, a large portion of phenological variation
548 within populations remained unexplained even with phenological breeding values. We attempted
549 to avoid records that were unusually young or old by only using specimens that had both flowers
550 and fruits; moreover, both our herbarium and seed collections spanned nearly the entire year
551 (Julian days 5–350 for herbarium records, 43–346 for seed collections). Still, herbarium records
552 are known to skew slightly towards earlier in seasons (though removing herbarium specimens
553 from our analyses did not change results) (Daru et al. 2018), while seed collections must be
554 collected long enough after the initiation of flowering to allow for the development of some
555 mature seeds. A single observation during reproduction, common for natural history collection
556 vouchers, cannot resolve the uncertainty around when plants begin their vegetative growth in the
557 wild, making it difficult to describe phenology of the full life cycle. With better information and
558 in climates where growing conditions are clearly defined, it may be possible to estimate
559 germination dates and full life cycle phenology from herbarium specimens (Bontrager et al.
560 2025a). Nevertheless, natural history collections could perhaps provide large numbers of
561 observations useful for fine tuning models of landscape phenology.

562 Spring onset differs among locations, and so a calendar date at a higher latitude or
563 elevation may be functionally earlier in the season than a lower latitude or elevation. PTUs
564 represent how much of a growing season has passed by a given date by recording temperature
565 and daylight above a threshold. Thus, plants collected at lower PTUs may be early flowering
566 (developmentally) despite a later collection date. Higher PTU may indicate plants growing as
567 summer or fall annuals that germinated later in the year. In that case, ecotypes with higher PTUs
568 would likely be fast cycling, earlier flowering plants (Figure 2). While we did find a negative
569 correlation between flowering time breeding values in field and vernalized experiments versus
570 PTU, we see the opposite in non-vernalized experiments. This last observation is somewhat
571 surprising, since we expected plants that flower later in the absence of vernalization to be more
572 likely to grow as winter annuals in the wild and flower early in the spring (Figure 1). Higher
573 PTUs in these presumptive winter annuals may suggest that plants in these locations regulate
574 their life histories to flower later in the year than expected by temperature and daylight alone.
575 There is some evidence that later flowering ecotypes could in fact be more flexible in their
576 flowering time relative to germination than early flowering ecotypes (Miryeganeh et al. 2018),
577 which could hide the signal of early spring flowering we expected in late flowering Arabidopsis.

578

579 *Within regions, breeding values do not predict phenology in the wild*

580 Because Arabidopsis exhibits substantial local within-population variation in phenology (Jones
581 1971; Brachi et al. 2013; Alonso-Blanco et al. 2016; DeLeo et al. 2020), we asked how genetic
582 variation in phenology was related to phenological variation within populations. This within-
583 population phenological variation can have fitness consequences. Variation contributes to
584 population persistence in variable environments, as when different germination behavior
585 provides bet hedging in the seedbank (Cohen 1967; Gremer and Venable 2014).

586 We found that neither dormancy nor flowering time breeding values from experiments
587 were significantly related to collection date when we accounted for geographic variation in local
588 mean collections dates in a GAM. However, flowering time and dormancy did improve GAMs
589 for collection date over a model that included only geographic location and elevation, suggesting
590 that genetic variation in flowering time does explain a minor fraction of the phenological
591 variation within regions. Non-vernalized flowering time was negatively, non-significantly related
592 to local variation in collection date, perhaps because in many locations, genotypes flowering
593 later in experiments without vernalization are more likely to behave as winter annuals, flowering
594 early in the spring before spring annuals.

595 In our models, there was very little difference between non-vernalized, vernalized, and
596 field-measured flowering times in predicting collection dates, despite the expectation that field
597 experiments recreate conditions similar to nature. However, data from field experimental trials
598 were available for a smaller set of ecotypes than non-vernalized and vernalized indoor trials, and
599 we lacked Iberian ecotypes with field experimental data and date of collection, limiting the
600 usefulness of this measure across the range. Our model provided weak evidence that the breeding
601 values for flowering time and dormancy explained within-population variation in wild collection
602 day. However, our set of ecotypes with known collection day numbered 258, potentially still too
603 few to detect strong statistical significance given the noise arising from plasticity in responses to
604 local environmental gradients.

605

606 *Conclusion*

We demonstrated that experimental measures of genetic variation in the timing of individual developmental transitions in Arabidopsis are significantly, but modestly, correlated with phenological variation in wild plants. On one hand, the existence of a relationship suggests there is some relevance of phenotypes measured in experiments to those exhibited in nature. However, the weakness of the relationships (especially locally, within regions) suggests the existence of either a) complex interactions between developmental stages to determine phenology, (b) low heritability in nature, or (c) extensive rank changing genotype-environment interactions across microsites. Genetic differences in Arabidopsis phenology across the species range are often cited as evidence of the adaptive importance of phenology (Stinchcombe et al. 2004; Fournier-Level et al. 2011; Samis et al. 2012; Brachi et al. 2013; Exposito-Alonso 2020). Incorporating information on between-population and within-population phenology diversity helps to clarify how selection may be acting on phenology across the landscape and how influential plasticity is in the phenology of this annual herb. Yet, genetic flowering times among populations across the species range are associated with phenological variation in natural history collections, due to some combination of genotypic and environmental effects (and their possible co-gradient variation) (Jones et al. 2024). While phenological plasticity is often of large magnitude, it may not be enough to maintain fitness under environmental change (Zettlemoyer et al. 2024). Ultimately, controlled experiments on many plants have suggested phenology is under selection in nature, but understanding more subtle environmental differences and stochasticity may help to clarify the evolution of phenology and translate genetic values into reliable predictions with and between populations.

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867 **Supplemental Material**

868

869 *Plasticity due to maternal effects*

870 Maternal conditions may be an important source of phenological plasticity; thus we used our
871 dormancy experiment to examine how variation in flowering time among maternal plants
872 influences germination. Variation in phenology may not be adaptive in regions where there is
873 harsh seasonality or environmental conditions are less variable year to year (Cohen 1967).
874 Similarly, observations of phenology in the wild could be temporally variable because of year-to-
875 year environmental differences (Walker et al. 1995; Hu et al. 2017; Postma and Ågren 2018) or
876 genetically diverse individuals germinating from the seedbank (Ratcliffe 1976). Among replicate
877 maternal individuals in the same controlled experiment, variation in phenology is likely due to
878 responses to very subtle environmental differences and some developmental stochasticity.

879 For each ecotype and for each maternal replicate, we used R packages 'drc' (Ritz et al.
880 2015) and 'drcSeedGerm' (Onofri et al. 2018) to fit a logistic function to germination counts to
881 estimate three parameters: maximum germination proportion, time to 50% germination, and
882 slope of the germination curve. A Generalized Linear Mixed Model was fit using the lmer
883 package in R (Bates et al. 2015) to estimate the influence of relative flowering time of maternal
884 replicates of each ecotype on germination traits. Because plasticity or responsiveness to
885 environmental cues may confer greater fitness in some environments and not others (Alpert and
886 Simms 2002; Baythavong 2011), germination traits and variation were regressed against
887 maternal flowering time and location of origin. We performed hierarchical clustering of a
888 distance matrix of phenotypic variation among ecotypes to group ecotypes that reacted similarly
889 to different stratification treatments.

890 We found that some ecotypes had more consistent phenology across maternal
891 lines. For example, DAM1 maternal replicates clustered together in germination across cold
892 treatments, whereas Yeg-7 replicates did not (Figure S10A). Cold stratification altered
893 expression of variance among maternal sources. In Yeg-7, for example, maternal lines P vs A
894 had similar total germination following 3 days or 2 weeks of stratification but different
895 germination for 0 days stratification. Thus, stratification (or lack of stratification) could
896 contribute to diverse phenology for similar genetic backgrounds under natural conditions. The
897 first and second principal components in an analysis of flowering and germination traits

898 explained 24.55% and 17.85% of the variation respectively, but again maternal replicates of
899 ecotypes did not always cluster in PC space (Figure S10B). The variance in phenology for an
900 ecotype was not related to collection date or geographic origin (latitude and longitude, Figure
901 S10C), suggesting that clinal variation in maternal plasticity effects do not confound our earlier
902 large-scale analyses comparing breeding values with phenology in nature. Nevertheless, these
903 effects may further obscure genetic contributions to phenology in nature.

904 We expected to see more variation among ecotypes from regions with favorable growing
905 conditions year-round. However, the lack of a geographic association between variance in
906 germination traits and flowering time among replicate individuals of an ecotype may indicate
907 geographically consistent selection on the canalization of phenology.

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Figure S1. An illustration of the importance of accounting for local mean flowering dates when testing for correlations between breeding value and observed flowering dates in the presence of co-gradient variation (correlated plasticity and genetic clines). In both A and B there is co-gradient variation, but in A the breeding values predict variation in actual natural phenology (flowering date) while in B the breeding values do not predict local phenology independent of plasticity and environmental differences among sites.

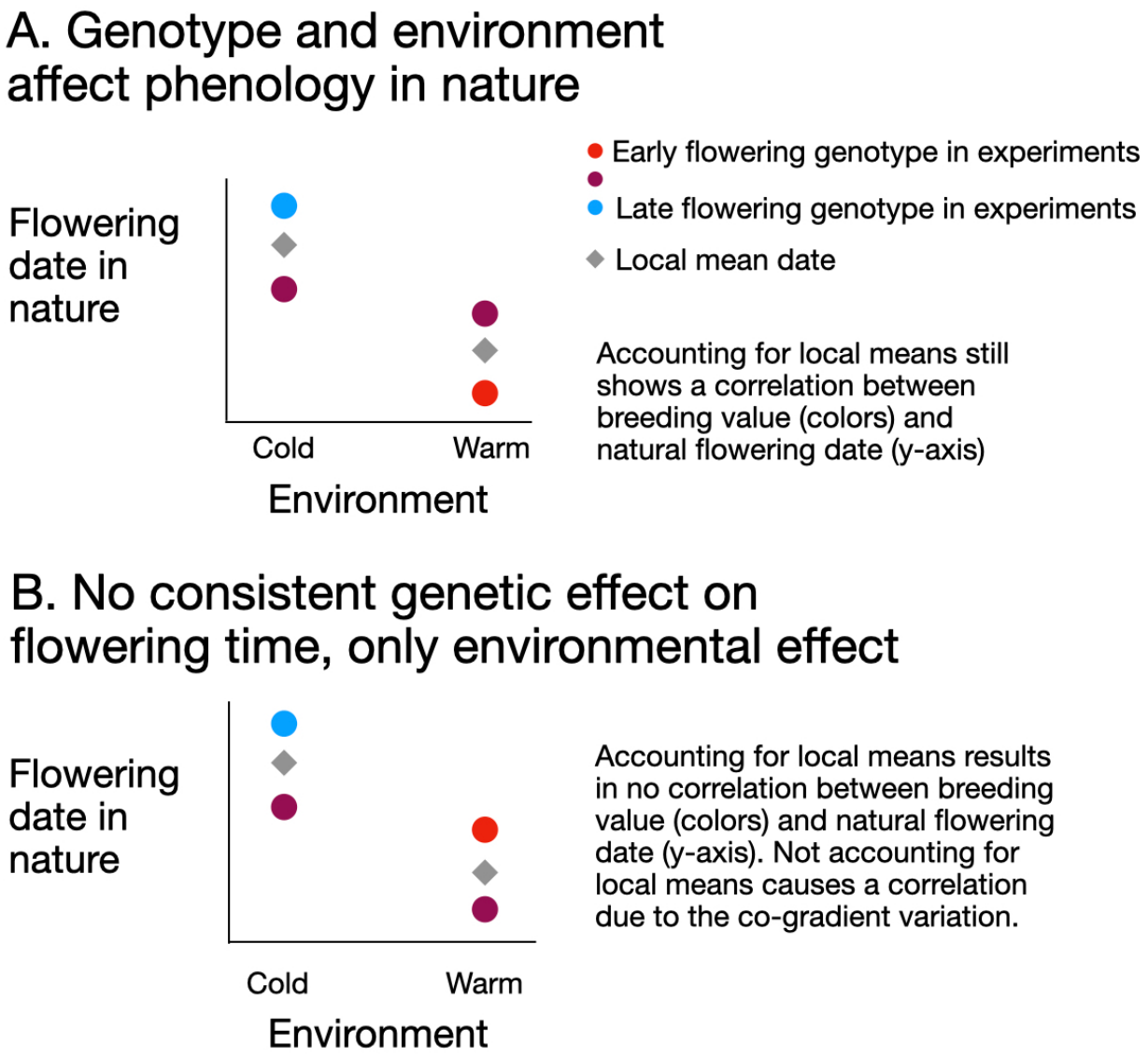


Figure S2. 42 ecotypes have measures of vernalized, non-vernalized, and field flowering times as well as primary and secondary dormancy traits (standardized trait units are shown). The latest flowering accessions tend to be late flowering across all treatments, but primary and secondary dormancy vary independently from flowering time.

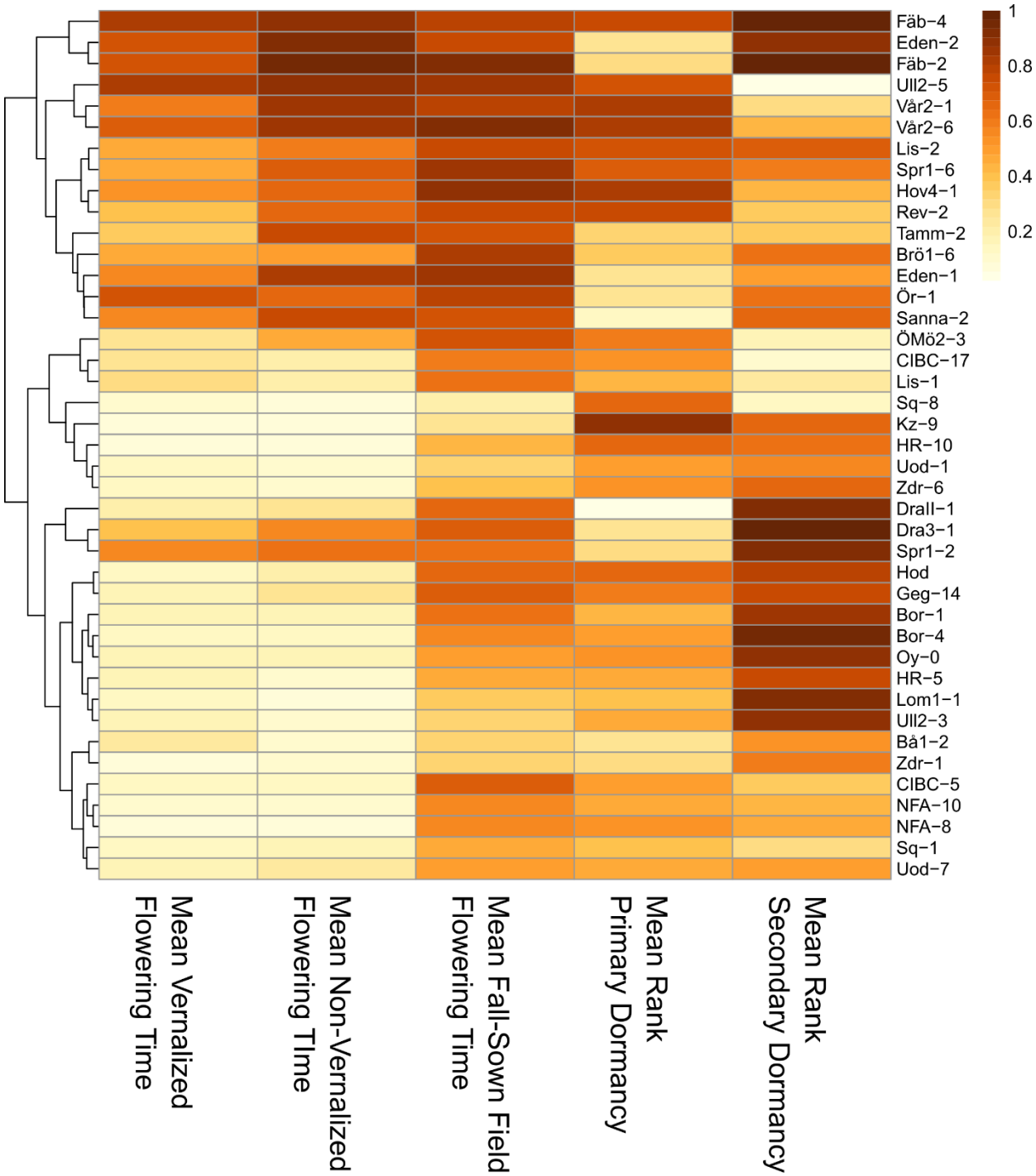


Figure S3. Standardized phenology traits combined across multiple published controlled experiments combined with our new experiments, using an alternate standardization (compare with Figure 3). Here, each experiment's values were standardized to mean zero unit standard deviation.

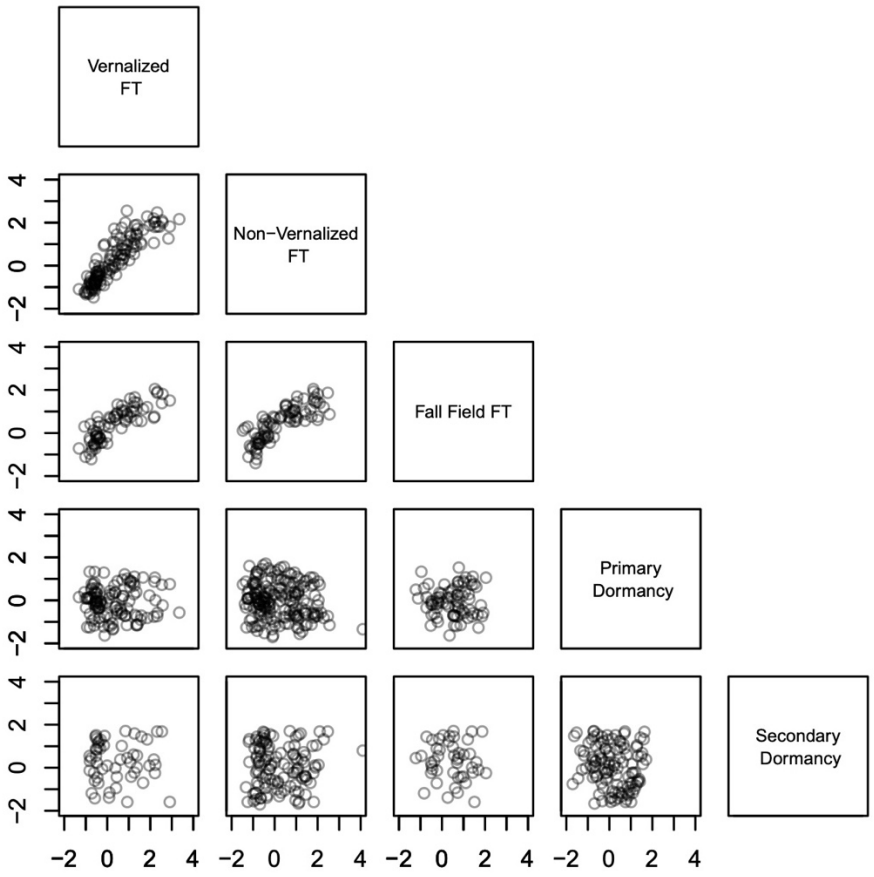


Figure S4. Date of collection (left panels) and PTUs (photothermal units) at collection (right panels) of wild plants compared to standardized breeding values for phenological traits from experiments, here using an alternate standardization (compare to Figure 4). Here, each experiment's values were standardized to mean zero unit standard deviation. The values shown for field flowering time experiments were calculated from fall-sown experiments only.

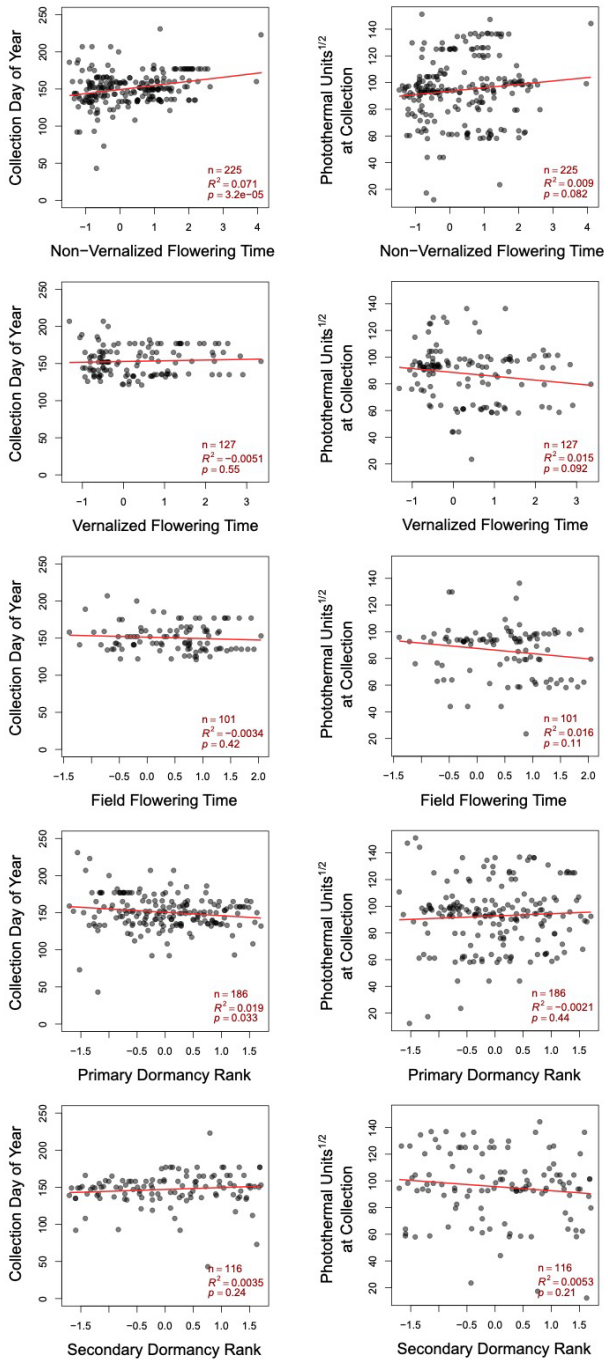


Figure S5. Date of collection (left panels) and PTUs (photothermal units) at collection (right panels) of wild plants compared to standardized breeding values for phenological traits from experiments, here using an alternate standardization and excluding herbarium specimens (compare to Figure 4). Here, each experiment's values were standardized to mean zero unit standard deviation. The values shown for field flowering time experiments were calculated from fall-sown experiments only.

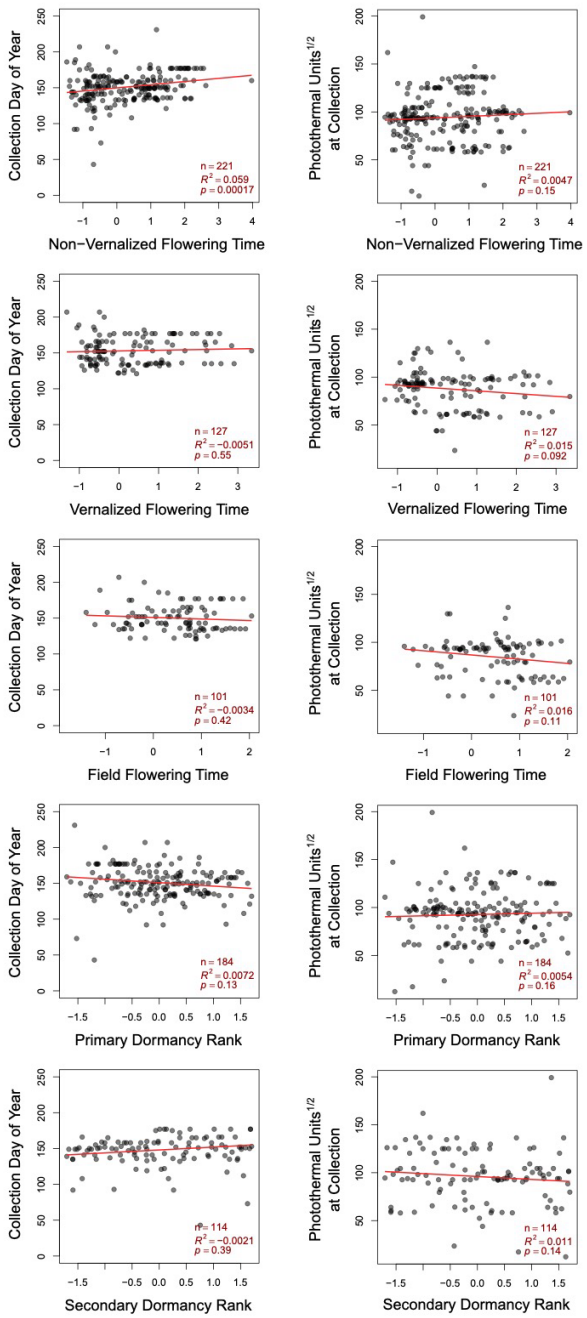


Figure S6. Date of collection (left panels) and PTUs (photothermal units) at collection (right panels) of wild plants compared to standardized breeding values for phenological traits from experiments, excluding herbarium specimens (compare to Figure 4). The values shown for field flowering time experiments were calculated from fall-sown experiments only.

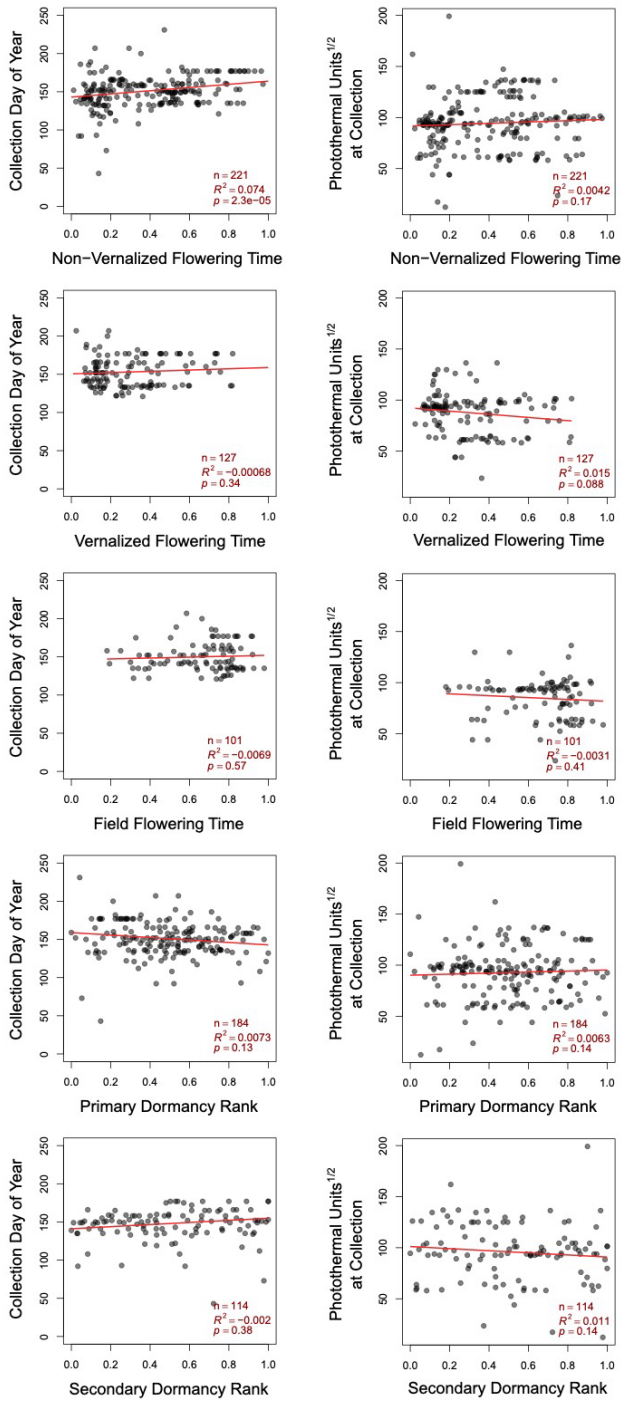


Figure S7. Flowering times and germination rates within experiments do not predict wild phenology better than average values across multiple experiments. Left, non-vernalized flowering times at 16°C (using the same criteria to remove collection day and geographical outliers as in the full set of accessions) Alonso-Blanco et al. (2016) did not significantly predict collection day ($p > 0.2$, $n = 173$). Right, germination rates after 4 days of stratification at 4°C Martínez-Berdeja et al. (2020) did not significantly predict collection day ($p > 0.1$, $n = 84$).

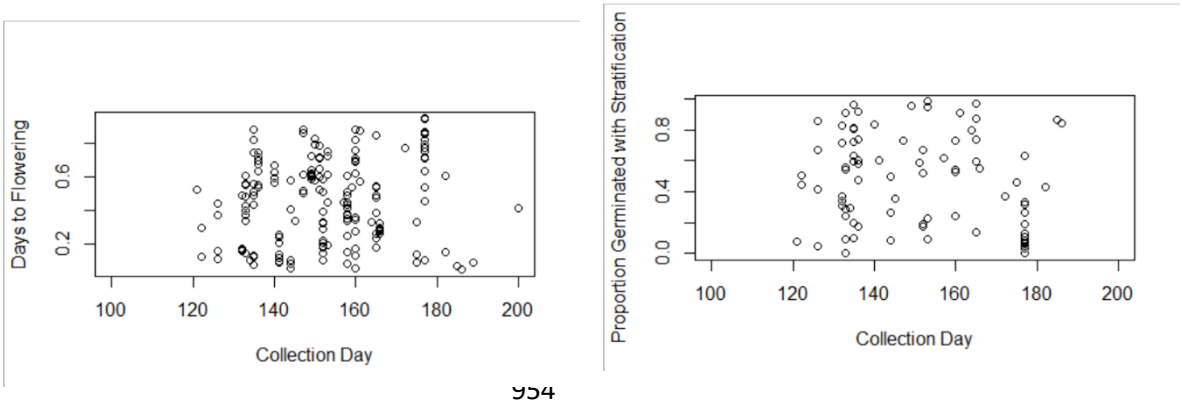


Figure S8: Predicted collection day vs. actual collection day. A) Difference between collection day and predicted collection day in ecotype accessions is designated by the size of the black circles. Accessions from Turkey were collected earlier than expected in our model, while expectations for Western Europe were closer to observed values. Herbarium records on which the model was built are shown as gray circles. B) Density plots of predicted collection days (black) and actual collection days (gray). Collections tended to be later than model predictions. C) Relationship between non-vernalized flowering time and collection day. Both predicted (black) and observed (gray) collection days are positively related to non-vernalized flowering times, but the observed collection days have greater range and a lower slope. Model-predicted flowering times underestimate the variation in collection days, which might be due to actual collection days picking up plants on the tails of the flowering distribution.

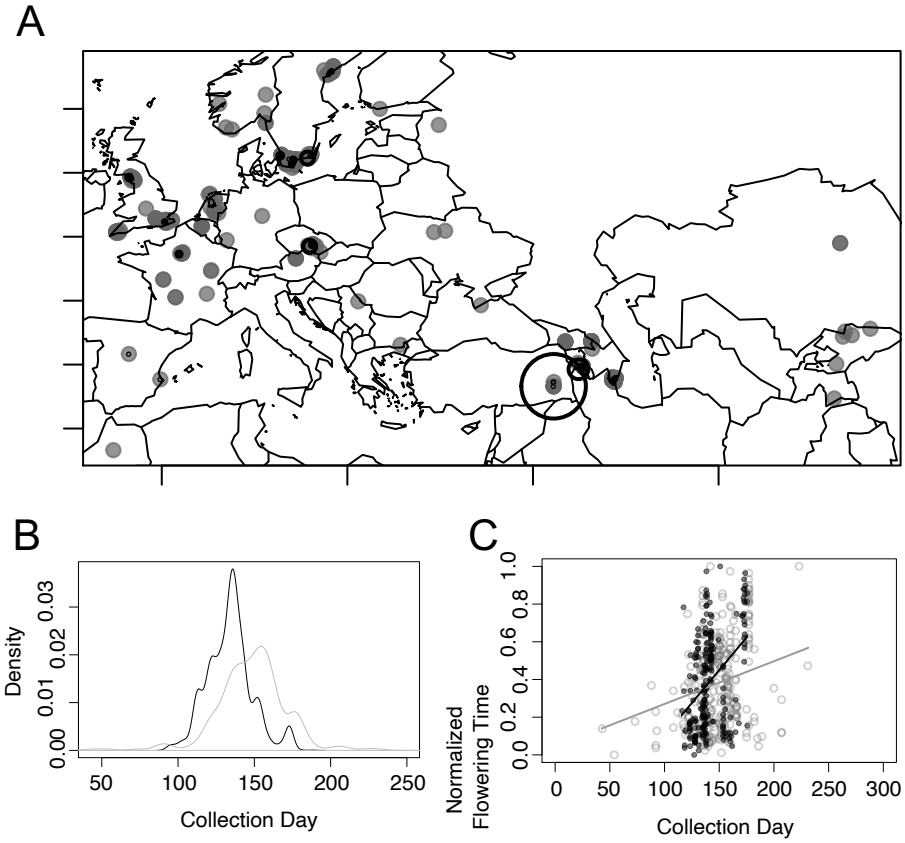


Figure S9. Spatially varying intercept of models of within-population phenological variation. The spatially varying intercept absorbed most of the phenological variation in collection date and photothermal units. Red, dashed lines represent areas where collection day was earlier while green, dotted lines represent areas where collection day was later. Collections are represented as circles. The model attributes a great deal of variation to geographic location, despite including phenological breeding values. Elevation was more important in predicting photothermal units than collection date.

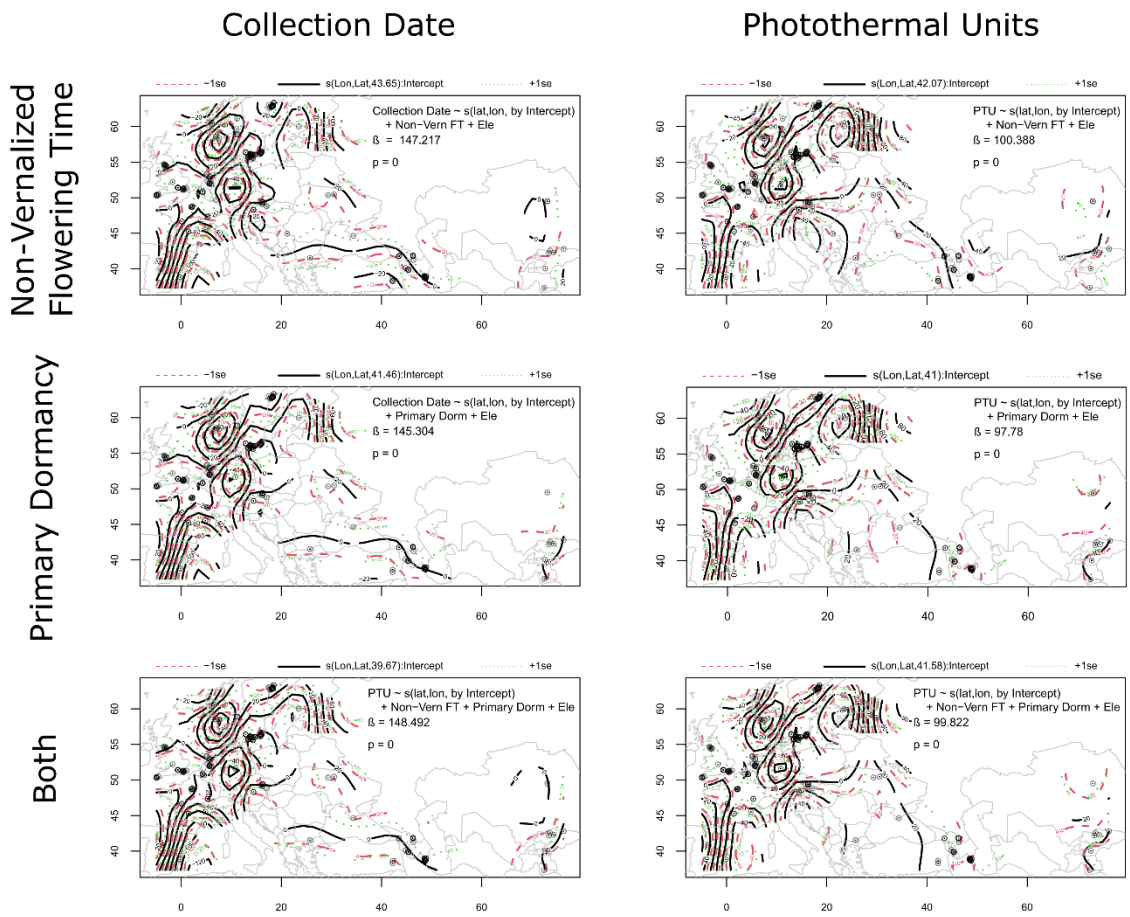
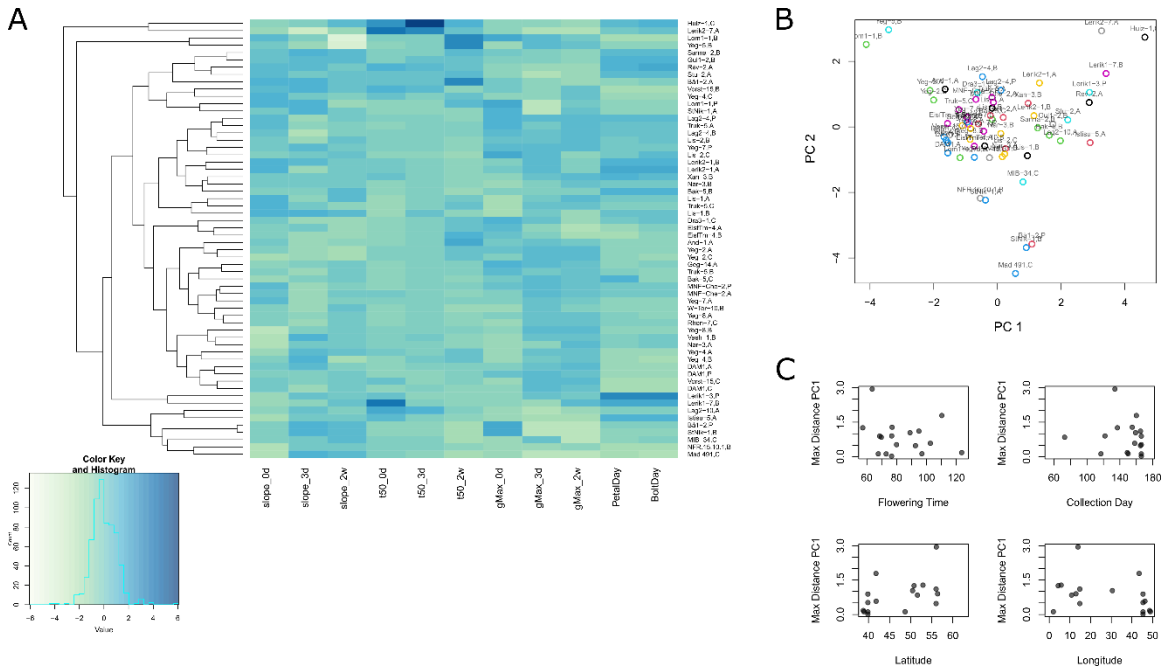


Figure S10: Variation in experimental phenology among maternal lines. A) Early flowering lines tended to have lower germination under 0 days stratification relative to 3 days or 2 weeks stratification, and later-flowering lines tended to have longer times to 50% germination. However, there was variation across traits. Replicate maternal lines of the same ecotype did not always cluster. B) Maternal lines of the same ecotype did not always cluster together in PCA of phenology traits. Lines belonging to the same ecotype are represented with the same color, although colors are not unique across ecotypes. PC1 was positively associated with time to 50% germination at 0 days and 3 days stratification and flowering time and negatively associated with time to 50% germination at 2 weeks stratification and max germination under all treatments. C. Flowering time, collection day, or geographic origin did not explain variance among maternal lines.



998 Table S1: Model statistics for other variables in models of within-population phenological
999 variation. P-values for the estimates are given in parentheses. Phenology breeding values did not
1000 significantly predict within-population variation in phenology.

Model	Non-Vernalized Flowering Time	Primary Dormancy	FT*Dormancy	Elevation	Adj R^2
Collection Date ~ μ + FT + Elevation	-3.25 (0.35)	NA	NA	**0.0148 (0.006)**	0.883
$PTU^{1/2}$ ~ μ + FT + Elevation	-1.21 (0.75)	NA	NA	**-0.0147 (0.006)**	0.876
Collection Date ~ μ + Dormancy + Elevation	NA	1.98 (0.56)	NA	**0.0126 (0.04)**	0.876
$PTU^{1/2}$ ~ μ + Dormancy + Elevation	NA	2.56 (0.48)	NA	**-0.0181 (0.009)**	0.858
Collection Date ~ μ + FT + Dormancy + Elevation	-8.19 (0.10)	2.19 (0.51)	NA	**0.0120 (0.05)**	0.881
$PTU^{1/2}$ ~ μ + FT*Dormancy + Elevation	-5.41 (0.56)	5.39 (0.43)	-6.86 (0.66)	**-0.0183 (0.009)**	0.864

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