

1 **Effects of two centuries of global environmental variation on phenology and physiology of**
2 ***Arabidopsis thaliana***

3 200 years of *A. thaliana* phenotypic variation

4

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45 **Abstract**

46 Intraspecific trait variation is caused by genetic and plastic responses to environment. This
47 intraspecific diversity is captured in immense natural history collections, giving us a window into
48 trait variation across continents and through centuries of environmental shifts. Here we tested if
49 hypotheses based on life history and the leaf economics spectrum explain intraspecific trait
50 changes across global spatiotemporal environmental gradients. We measured phenotypes on a
51 216-year time series of *Arabidopsis thaliana* accessions from across the native range and applied
52 spatially varying coefficient models to quantify region-specific trends in trait coordination and
53 trait responses to climate gradients. All traits exhibited significant change across space and/or
54 through time. For example, $\delta^{15}\text{N}$ decreased over time across much of the range and leaf C:N
55 increased, consistent with predictions based on anthropogenic changes in land use and
56 atmosphere. Plants were collected later in the growing season in more recent years in many
57 regions, possibly because populations shifted toward more spring germination and summer
58 flowering as opposed to fall germination and spring flowering. When climate variables were
59 considered, collection dates were earlier in warmer years, while summer rainfall had opposing
60 associations with collection date depending on regions. There was only a modest correlation
61 among traits, indicating a lack of a single life history/physiology axis. Nevertheless, leaf C:N
62 was low for summer- versus spring-collected plants, consistent with a life history-physiology
63 axis from slow-growing winter annuals to fast-growing spring/summer annuals. Regional
64 heterogeneity in phenotype trends indicates complex responses to spatiotemporal environmental
65 gradients potentially due to geographic genetic variation and climate interactions with other
66 aspects of environment. Our study demonstrates how natural history collections can be used to

67 broadly characterize trait responses to environment, revealing heterogeneity in response to
68 anthropogenic change.

69 **Keywords:** Generalized Additive Model, abiotic stress, climate change, $\Delta^{13}\text{C}$, phenology

70 **Introduction**

71 An organism's fitness is determined by the interaction between its traits and its environment.
72 Organisms respond to environmental gradients in diverse ways, including genetic and plastic
73 shifts in life history, phenology, and physiology (Burghardt, Metcalf, Wilczek, Schmitt, &
74 Donohue, 2015; Reich, 2014; Wright et al., 2004). Spatial and temporal environmental gradients
75 can promote phenotypic plasticity or generate varying selection across which populations adapt
76 to local conditions (Bradshaw, 1965; Henn et al., 2018; Joshi et al., 2001; Leimu & Fischer,
77 2008; Linhart & Grant, 2002; Matesanz, Gianoli, & Valladares, 2010; Turesson, 1922). By
78 studying how phenology and physiology change across environments through space and time we
79 can learn about mechanisms of adaptive environmental response and biological constraints.

80 Anthropogenic global change has led to dramatic phenotypic changes in many organisms. For
81 example, many species are shifting their ranges poleward and temperate spring phenology is
82 advancing (Parmesan & Yohe, 2003). However, anthropogenic global change is multi-faceted,
83 involving climate, nitrogen deposition, atmospheric CO₂, and land use. Our understanding of the
84 specific environmental drivers of phenotypic change has been hampered both by insufficient
85 long-term datasets and by the complexities of interacting and correlated environmental variables.
86 Furthermore, many populations and species do not exhibit the stereotypic advancing temperate
87 phenology and poleward range shifts (Both et al., 2004; CaraDonna, Iler, & Inouye, 2014; Park
88 et al., 2018). These diverse responses can be caused by geographic variation in the rate of
89 environmental change or by intraspecific genetic variation, clouding our understanding of
90 anthropogenic impacts. To address these challenges, we collected physiology and phenology
91 data for *Arabidopsis thaliana* (hereafter, *Arabidopsis*) specimens over the last 200 years and
92 across its native range, and we tested relationships between climate and traits using spatial

93 generalized additive models (GAMs) to account for geographic structure in environmental
94 response.

95 *Arabidopsis* is a powerful system for studying phenotypic change across space and climate
96 gradients. Past studies have found that *Arabidopsis* populations exhibit genetic differences
97 among populations likely due to isolation by distance (Alonso-Blanco et al., 2016; Horton et al.,
98 2012; Ostrowski et al., 2006; Platt et al., 2010) and local adaptation (Fournier-Level et al. 2011,
99 Hancock et al. 2011, Lasky et al. 2012) . Genetic differences in flowering time among
100 populations may be due to local adaptation (Atwell et al., 2010; Burghardt et al., 2015; Tabas-
101 Madrid et al., 2018), with northern genotypes having later flowering times (Atwell et al., 2010;
102 Stinchcombe et al., 2004). *Arabidopsis* physiology also shows evidence of a role in local
103 adaptation. Genotypes from regions of greater precipitation have faster growth and lower leaf
104 vein density, and genotypes from colder temperatures have increased leaf thickness and wider
105 leaf minor vein cross section (Adams, Stewart, Cohu, Muller, & Demmig-Adams, 2016; Sack et
106 al., 2012; Sartori et al., 2018). These findings provide a lens through which to interpret
107 phenotypic variation among plants in nature. In turn, museum collections offer broadly
108 distributed sampling in space and time to test the relationships between phenotypes and
109 environment in nature (Lang, Willems, Scheepens, Burbano, & Bossdorf, 2018; Willis et al.,
110 2017).

111 Multiple frameworks of plant life history and physiology variation correspond to a continuum of
112 fast to slow life histories (e.g. Grime, 1977; Westoby, 1998; I. J. Wright et al., 2004). Individuals
113 that use a fast strategy are characterized by fast relative growth, early reproduction, and intensive
114 use of nutrients or water, while individuals with a slow strategy are characterized by slow
115 growth, late reproduction, and more measured use of nutrients and water. The Leaf Economics

116 Spectrum (LES) hypothesizes that leaves vary along single life history-physiology axis from fast
117 to slow in association with large-scale climate gradients (Reich, 2014; Reich et al., 2003; Wright
118 et al., 2004). The LES predicts that lower nitrogen concentration leaves (high C:N, low
119 proportion N) are found in drier and in hotter areas, possibly in part because of investment in
120 non-photosynthetic leaf features, e.g. veins (Blonder, Violle, Bentley, & Enquist, 2011; Easlon et
121 al., 2014; Sack et al., 2012) (see Table 1 for phenotype/environment hypotheses and how they
122 relate to the fast/slow framework). Low N leaves are thicker (high mass to area) and provide
123 protection against stress (drought) at the expense of a N investment in photosynthesis, resulting
124 in a slower life cycle (Evans, 1989; Stocking & Ongun, 1962). Although community-wide
125 turnover in mean traits across natural environments often follows LES predictions, *within* species
126 trait variation often defies LES predictions (Anderegg et al., 2018; Hu et al., 2015; J. P. Wright
127 & Sutton-Grier, 2012). Nevertheless, *Arabidopsis* exhibits genetic variation in traits that
128 corresponds to LES predictions (Easlon et al., 2014; Sartori et al., 2018; Vasseur, Violle,
129 Enquist, Granier, & Vile, 2012); individuals with rapid life histories have physiological traits tied
130 to fast growth and resource acquisition (e.g. high stomatal conductance, high leaf area relative
131 mass (Specific Leaf Area, or SLA)) (Lovell et al., 2013; McKay, Richards, & Mitchell-Olds,
132 2003; Sartori et al., 2018; Wolfe & Tonsor, 2014).

133 Isotopic signatures provide clues to organismal life histories. In plants, $\Delta^{13}\text{C}$ measures
134 discrimination against ^{13}C in photosynthesis and is an indicator of pCO_2 within leaves (C_i)
135 relative to atmospheric pCO_2 (C_a) (Farquhar, O'Leary, & Berry, 1982). C_i declines when stomata
136 are closed, which may be a conservative life history response to soil drying, while C_a declines
137 with elevation. Thus, we expect $\Delta^{13}\text{C}$ to increase in moist growing environments and decrease
138 with elevation (Diefendorf, Mueller, Wing, Koch, & Freeman, 2010; Farquhar et al., 1982; Zhu,

139 Siegwolf, Durka, & Körner, 2010) (Table 1). $\delta^{15}\text{N}$ (the ratio of ^{15}N to ^{14}N) can be affected by
140 nitrogen allocation and so might reflect variation in C:N (Stock & Evans, 2006). However,
141 variation in leaf N and $\delta^{15}\text{N}$ may also directly reflect changing environments (N deposition,
142 biogeochemical cycling, e.g. Pardo *et al.* 2007) rather than plant traits, with both increasing with
143 temperature and leaf N increasing and $\delta^{15}\text{N}$ decreasing with rainfall (Table 1).

144 Fast/slow strategies also correspond to phenological variation, especially in annual plants. In
145 seasonal environments, phenology is constrained by seasonality and simultaneously determines
146 the environment encountered during vulnerable stages. *Arabidopsis* development can be highly
147 sensitive to moisture, temperature, and photoperiod (Burghardt *et al.*, 2015; Wilczek *et al.*,
148 2009). For example, although warmth can increase growth rates, many *Arabidopsis* genotypes
149 require winter cold cues (known as vernalization) to transition to spring flowering. Fast life
150 histories can allow spring or summer annual life cycles, where a plant germinates and flowers
151 within a single season, while slow life histories and vernalization requirements result in a winter
152 annual cycle, where a plant germinates in the fall and flowers the following spring. Rapid
153 development and reproduction can allow *Arabidopsis* plants to escape drought (McKay *et al.*,
154 2003), while slower flowering plants can exhibit drought avoidance strategies of minimizing
155 water loss (e.g. through stomatal closure) or maximizing water uptake (Kenney, Mckay,
156 Richards, & Juenger, 2014; Ludlow, 1989). Because herbarium specimens are typically
157 reproductive, it is challenging to infer germination times based on collection dates. However,
158 information on physiology and climate preceding collection may provide information on life
159 history variation.

160 Standardized metrics facilitate the comparison of ecologically relevant phenological variation
161 among sites that differ in climate and seasonal timing. Photothermal units (PTUs) integrate

162 developmental time under favorable temperatures and light and account for much of the
163 environmental influence on flowering dates in *Arabidopsis* (Brachi et al., 2010; Wilczek et al.,
164 2009). In essence, PTUs estimate how far along in a growing season an event occurs. Measures
165 of developmental time standardized to environmental conditions can better capture genetic
166 variation in development compared to raw flowering dates in *Arabidopsis*, the latter of which are
167 strongly driven by environment (Brachi et al., 2010). Without knowing an exact germination
168 date, it is impossible to perfectly approximate the climate experienced in the wild, yet even a
169 rough estimate using an arbitrary date allows us to compare changes in the climate experienced
170 at flowering. Variation in herbarium collection dates is a reliable proxy for variation in
171 phenology of flowering date (Davis, Willis, Connolly, Kelly, & Ellison, 2015; MacGillivray,
172 Hudson, & Lowe, 2010; Miller-Rushing, Primack, Primack, & Mukunda, 2006). In addition, low
173 PTUs at collection hints at a winter annual growing pattern (because growth is occurring before
174 PTU calculations begin), so we can use this measurement to search for regional variation in life
175 history. Combined with existing knowledge the phenology of ecotypes from different sites, PTUs
176 may help reveal phenological adaptation along spatial and temporal environmental gradients.

177 Here, we leverage the immense fieldwork underlying natural history collections to investigate
178 how intraspecific diversity is structured through time and along spatiotemporal climate gradients.
179 Specifically, we use thousands of *Arabidopsis* specimens that span over 200 years of sampling
180 across *Arabidopsis*' range in Eurasia and Northern Africa. We quantify the spatial patterns of
181 *Arabidopsis*' phenotypic variation along environmental gradients, which allows us to put
182 temporal trends in context. We hypothesized that for natural *Arabidopsis* populations,
183 phenotype-environment correlations would follow fast-slow predictions of LES and phenology

184 traits (Table 1). We combine these records with global gridded climate data to ask three
185 questions about *Arabidopsis* in nature:

- 186 1. To what degree does intraspecific trait variation among wild individuals fall along a
187 single coordinated life history-physiology axis?
- 188 2. Do life history and physiology vary across spatial environmental gradients in long-term
189 average conditions, suggesting adaptive responses consistent with the LES?
- 190 3. Have life history and physiology changed over the last two centuries? In particular, have
191 changes tracked climate fluctuations, suggesting adaptive responses consistent with the
192 LES?

193

194 **Materials & Methods**

195 *Samples*

196 Our set of samples (N= 3443) included *Arabidopsis thaliana* herbarium and germplasm
197 accessions with known collection dates between 1794 and 2010 from the native range of
198 *Arabidopsis* in Europe, the Middle East, Central Asia, and North Africa (Hoffmann, 2002).
199 Wild-collected germplasm accessions with known collection date and location (N = 447) were
200 included only in models of phenology. Information on germplasm accessions came from the
201 *Arabidopsis* Biological Resource Center (<https://abrc.osu.edu/>). For each herbarium specimen
202 (N=2663) we visually verified species identification and reproductive status as simultaneously
203 flowering and fruiting. Samples that were only fruited/senesced, only flowering, or had neither
204 open flowers nor fruits were excluded to focus on a relatively uniform developmental stage (see
205 Supplementary Table 1). This consistency is important for assessing C:N, since progression of
206 plant development involves reallocation of nutrients, and for a meaningful characterization of
207 phenology with collection date (Himmelblau & Amasino, 2001). Furthermore, too few samples
208 (178/2663) were in other phenological stages to allow for a rigorous comparison. We excluded
209 dozens of misidentified specimens, highlighting the importance of verification of information in
210 natural history collections (cf. unverified data in some online databases). Samples with too low
211 precision in collection date (less precise than a single month) were excluded from phenological
212 analysis.

213

214 *Leaf traits*

215 To test LES hypotheses for response to environment, we measured $\Delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N from
216 leaf tissue of herbarium samples. We removed and pulverized leaf samples (mean weight = 2.75
217 mg) of a subset of our quality-checked herbarium specimens and sent them to the UC-Davis
218 Stable Isotope Facility. In total, we obtained values for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C:N, and proportion N in 459
219 accessions, although 5 samples failed for $\delta^{13}\text{C}$ and 1 sample was missing failed for both C:N and
220 proportion N values.

221 We measured leaf $\delta^{13}\text{C}$ (isotope ratio), but atmospheric $\delta^{13}\text{C}$ has changed dramatically over the
222 time period of this study due to fossil fuel emissions. Thus we converted leaf isotope ratio ($\delta^{13}\text{C}$)
223 to discrimination ($\Delta^{13}\text{C}$) using an estimate of the atmospheric $\delta^{13}\text{C}$ time series (McCarroll &
224 Loader, 2004) from 1850 to 2000, continuing linear extrapolation beyond 2000, using the 1850
225 value for earlier specimens, and the equation of Farquhar et al. (1989), $\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}$, where δ_a is the
226 isotope ratio in the atmosphere and δ_p is the isotope ratio in plant tissue (ratios relative to a
227 standard).

228

229 *Phenology*

230 To estimate accumulated photothermal units (PTU) at date of collection, we used the equation of
231 Burghardt et al. (2015) to model the hourly temperature values for the accumulation of sunlight
232 degree hours between January 1 and dusk on the date of collection at each accession's
233 coordinate. Daylength was approximated with the R package geosphere (Hijmans, 2017).
234 Monthly temperature values for the period 1900-2010 came from the Climate Research Unit time
235 series dataset v4.01 (Harris, Jones, Osborn, & Lister, 2014). PTUs were only calculated for
236 specimens collected after 1901 (N = 2488) due to the historical limit of the monthly temperature

237 data. Daily temperatures were interpolated from monthly temperatures using the function
238 splinefun in R on the “periodic” setting. PTUs calculated from January 1st will not completely
239 account for the climate experienced by plants that germinate in the fall. However, for the same
240 developmental time (PTUs), determined by weather conditions, winter annuals are expected to
241 flower earlier in a growing season compared to spring annuals. Comparing changes in PTU at
242 collection to changes in date of collection might provide clues as to where climate is driving
243 flowering time shifts and where flowering time is responding to pressures other than
244 temperature.

245

246 *Statistical analysis*

247 *Arabidopsis* displays substantial genetic diversity in environmental response between genotypes
248 from different regions (e.g. Exposito-Alonso et al., 2018; Lasky, Forester, & Reimherr, 2018).
249 Thus, we employed a regression model with spatially varying coefficients (generalized additive
250 models, GAMs) to account for regional differences in responses to environment, much of which
251 may have a genetic component (Wheeler & Waller, 2009; Wood, 2006). GAMs allow fitting of
252 parameters that vary smoothly in space (i.e. parameter surfaces) and can thus capture spatially
253 varying relationships between predictors and the response of interest, such as we see in
254 ecological processes (Yee & Mackenzie, 2002; Yee & Mitchell, 2006). The spatially varying
255 coefficients fit by GAM allow us to infer from the data where relationships between variables
256 change, as opposed to binning data into a set of fixed (and possibly artificially defined) regions.
257 In a standard linear statistical model, the effect of a covariate x (i.e., the effect of x = January
258 Minimum Temperature) at site i is a linear function of the covariate: $x_i * \beta$. Note that differences
259 across sites in $x_i * \beta$ are completely controlled by differences in the covariate x at different sites.

260 In the spatially-varying GAMs we consider in this study, we allow the effect of covariates to
261 vary across space, with the effect of a covariate x at location i being $x_i * \beta_i$ where β_i is the linear
262 effect of x at the i^{th} spatial location. That is, we model allow the effect of the covariate x to vary
263 across space. Thus, the effect of (for example) January Minimum Temperature might be different
264 in Europe than it would be in Southeast Asia.

265 It would be impossible to uniquely identify a completely different effect β_i at every location, as
266 we only have one replicate at each site, and there would be no replication. Spatially-varying
267 GAMs address this identifiability by smoothing β_i across space and requiring that effects β_i and
268 β_j for sites i and j that are close in space be very similar to each other. The degree of spatial
269 smoothness in the GAM is chosen by cross-validation to ensure that the GAM does not overfit
270 the data. Thus, spatially-varying coefficient models provide an approach for modeling variability
271 in the effect of covariates across space, with the effect constrained to vary slowly across space to
272 ensure identifiability and combat overfitting.

273 Each cell in our 140x200 grid model rasters corresponded to 53.1 km East/West at the lowest
274 latitude (28.16°, versus 20.3 km at 68.18° N) and 31.8 km North/South (calculated using
275 Vincenty ellipsoid distances in the geosphere package). Smaller grid cells allow for more finely
276 smoothed slope values. Model predictions farther than 200km from a sampled accession were
277 discarded when visualizing results.

278 We selected climate variables based on knowledge of critical *Arabidopsis* developmental times
279 and likely environmental stressors: average temperature in April, when warmth is expected to
280 accelerate development (AprilMean in the models), minimum temperature in January, when
281 vernalization cues are likely accumulating or when Mediterranean plants are in early growth
282 (JanMinimum), and July aridity index (AI), when summer drought may be most likely (Fournier-

283 Level et al., 2013; Hoffmann, 2002; Lasky et al., 2012; Wilczek, Cooper, Korves, & Schmitt,
284 2014). Our analyses should not be highly sensitive to the exact calendar month chosen, given the
285 high correlation in conditions between consecutive months (e.g. warm Aprils tend to be followed
286 by warm Mays). Aridity index was calculated from July precipitation divided by July potential
287 evapotranspiration (PET) (United Nations Environment Program, 1997). These climate gradients
288 were generally not strongly correlated (July Aridity to April Mean Temperature $r = -0.33$; July
289 Aridity to January Minimum Temperature $r = -0.12$; January Minimum Temperature to April
290 Mean Temperature $r = 0.71$ by Pearson's product-moment correlation). We took temperature,
291 precipitation, and PET values from the Climate Research Unit time series dataset, using values
292 for the year of collection (New, Hulme, & Jones, 2000).

293

294 First, to study trait correlations that might indicate a fast-slow life history (Question 1), we
295 performed a Principal Components Analysis of flowering time, $\Delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio and
296 tested pairwise associations between traits. We considered how traits co-vary by calculating the
297 Pearson's correlation coefficients between traits and by Principal Components Analysis. We also
298 fit GAMs (described in detail below) with spatially varying intercepts allowing measured
299 phenotypes as both response and predictor variables to observe how the correspondence of traits
300 changes through space.

301

302 Second, to study phenotypic responses to spatial gradients in long-term average climates
303 (Question 2), we fit models with spatially varying coefficients for long-term, 50-year climate
304 averages at each location ("spatial climate models", Equation 1). We scaled these climate

305 covariates and year of collection to unit standard deviation (Hijmans, Cameron, Parra, Jones, &
306 Jarvis, 2005). In these models of responses to long-term average conditions, year of collection
307 can be considered a nuisance variable, accounting for temporal variation at a location that may
308 be important but is not the focus of this specific model. In spatial climate models, we used a
309 single global intercept. Spatial climate models included specimens from all years with phenotype
310 data.

311

$$312 \quad \text{Phenotype}_{ij} = \beta_1 \text{year}_j + \beta_2 \text{AI}_j + \beta_3 \text{JanMinimum}_j + \beta_4 \text{AprilMean}_j + \mu + \text{error}_{ij} \quad (\text{eqn 1})$$

313

314 In all models, the subscript j denotes location and i denotes year of collection. For the temporal
315 climate anomaly model, the spatially varying intercept is denoted by μ_j , where the “ j ” subscript
316 indicates that the intercept varies with location. The errors are assumed to be independent, be
317 normally distributed, and have constant variance.

318

319 Next, to assess how phenotypes have changed across the last two centuries (Question 3), we
320 tested a model with spatially-varying coefficients for the effect of year, allowing for geographic
321 variation in temporal trends (hereafter, “year models”). The model also included spatially
322 varying intercepts to account for regional differences in long-term mean phenotypes. Year
323 models included all specimens with data for a particular phenotype.

324 Finally, to assess how temporal fluctuations in climate drive phenotypic change (Question 3), we
325 fit models with the three climate covariates for the year of collection (Equation 2). We converted

326 climate covariates to local anomalies by standardizing them relative to the entire time-series for a
327 given grid cell to unit standard deviation and mean zero (“temporal climate anomaly models”).
328 In standardizing climate fluctuations to the climate record of a location, we assume that the effect
329 of an anomaly on the response variable is best captured by the relative strength (and direction) of
330 an anomaly (relative to an average anomaly) rather than how extreme an anomaly is in general.
331 The model also included spatially varying intercepts to account for regional differences in long-
332 term mean phenotypes. Temporal climate models only included specimens from after 1900,
333 when we had data on monthly climate from CRU. These models had the following structure

334

$$335 \quad \text{Phenotype}_{ij} = \beta_1 \text{year}_{\text{dev}, ij} + \beta_2 \text{AI}_{\text{dev}, ij} + \beta_3 \text{JanMinimum}_{\text{dev}, ij} + \beta_4 \text{AprilMean}_{\text{dev}, ij} + \mu_j + \text{error}_{ij}$$

336 (eqn 2)

337

338 Models were fit in R (version 3.5.0, R Core Team 2011) using the ‘gam’ function in package
339 mgcv (version 1.8-17, Wood 2011). We allowed the model fitting to penalize covariates to 0 so
340 that covariates weakly associated with phenotypes could be completely removed from the model;
341 thus, using the mgcv package we can achieve model selection through joint penalization of
342 multiple model terms. Coefficients in spatially varying coefficient models represent the
343 relationship between each term and phenotype at each geographic point (indexed by j in our
344 models).

345 We considered two other spatially-varying environmental variables of interest: elevation and N
346 deposition. We left elevation and nitrogen deposition covariates out of the final models because

347 inclusion resulted in instability in the numerical routines the GAM software (mgcv) used to
348 estimate parameters and approximate Hessian matrices needed for confidence intervals. See
349 supplemental material for more information on these covariates. Including only the variables of
350 the three climate covariates and year resulted in numerically stable estimates. In addition, scaling
351 of year and climate variables tended to reduce the concavity of variables and increase stability.

352

353 Code for all the models and plots will be included as a supplement and will be available on
354 github.

355

356 **Results**

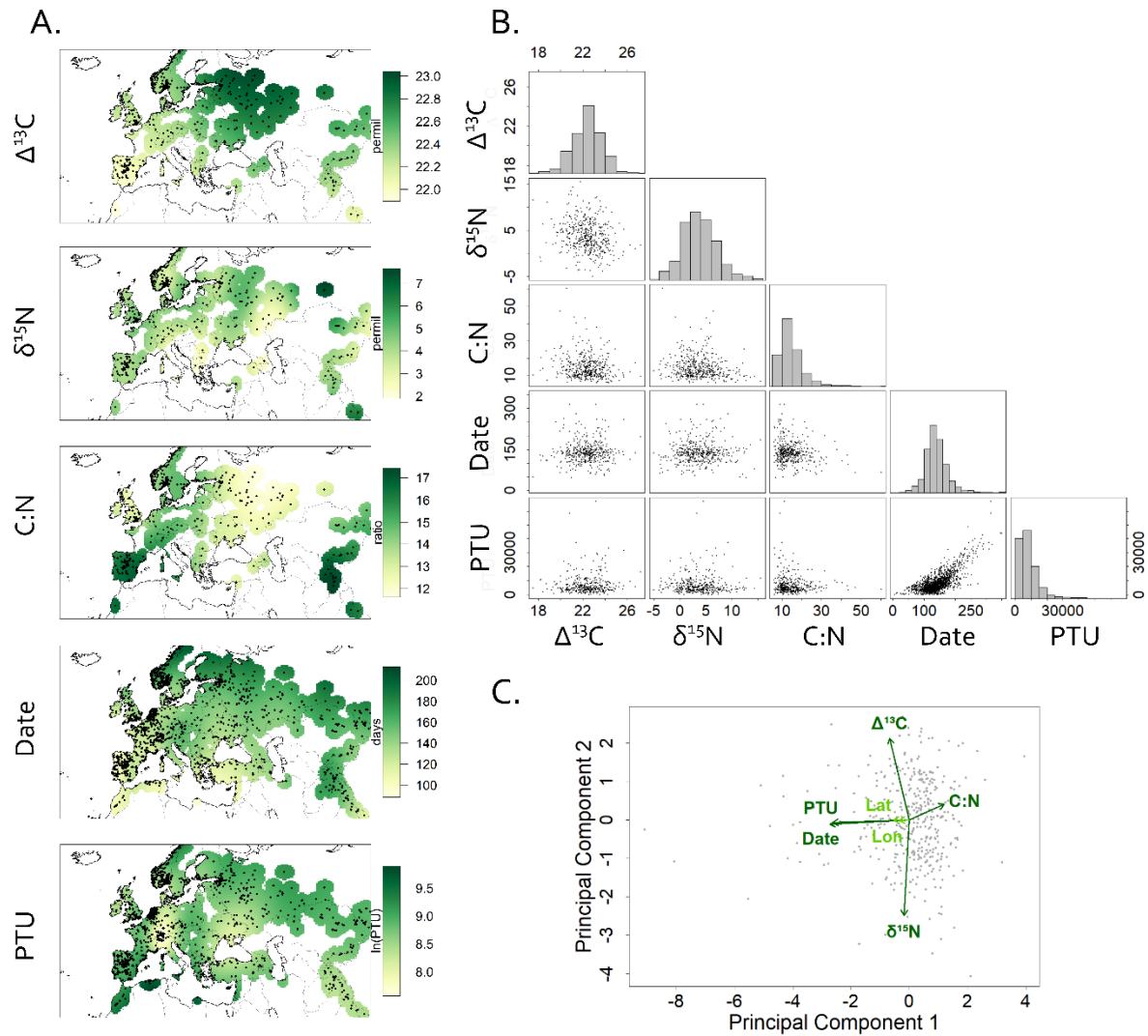
357 *Distribution of samples through time and space*

358 Samples were broadly distributed, with dense collections in Norway/Sweden, the Netherlands,
359 and Spain (reflecting major herbaria used in the study), and sparser collections to the east (Figure
360 1). The subset of samples with tissue analysis spanned the extent of the geographic distribution
361 of all samples. Tissue sampling was most dense in Norway, Spain, and the UK and sparser
362 elsewhere in the range. The earliest collection date we used was 1794, but a greater number of
363 samples were available from the 1900s onwards.

370 *Correlations among phenotypes (question 1)*

371 We found generally weak correlations among phenotypes of *Arabidopsis* individuals (Question
372 1). The first two principal components explained only 36.2% and 24.1%, respectively, of the
373 variance in the five phenotypes of $\Delta^{13}\text{C}$, $\delta^{15}\text{N}$, date of collection, C:N, and PTU (N = 397). The
374 first principal component corresponded to a negative correlation between C:N versus day of
375 collection (bivariate $r = -0.189$) and PTU (bivariate $r = -0.101$). Inspecting the relationship
376 between collection date and C:N further revealed a triangle shape (Figure 2B). That is, there
377 were no late-collected individuals with high C:N, potentially indicating that the late-collected
378 plants that we hypothesize are spring/summer annuals also exhibit fast growth strategies. Plants
379 with the lowest C:N have a less negative relationship to day of collection than plants with a
380 higher C:N, and ANOVA showed there to be a significant difference between the slopes of the
381 regression of the 25th and 75th percentiles of C:N ($p = 0.0002$, Figure S2) (Koenker & Koenker,
382 2011). When allowing the relationship between C:N and phenology to vary spatially (GAM with
383 spatially varying coefficients), we found both date of collection and PTU were negatively
384 correlated with C:N across the *Arabidopsis* native range, but this correlation was not
385 significantly different from 0 (the 95% confidence interval included 0) (Figure S3). The second
386 PC corresponded to a negative correlation between $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (bivariate $r = -0.218$). C:N and
387 leaf proportion N are highly correlated (bivariate $r = -0.816$, Figure S1), so we focus on C:N. See
388 supplementary material for leaf N results (Figure S16 and S17).

389



390

391 Figure 2: (A) Variation in phenotypes across the native range of *Arabidopsis* for $\Delta^{13}\text{C}$, $\delta^{15}\text{N}$,
392 C:N, collection date, and photothermal units (PTU) at collection. Color indicates the fitted mean
393 value (spatially-varying intercept) of the phenotype from the year-only model. For example,
394 collection date is earlier in the Mediterranean in comparison to other regions; however, PTUs are
395 lowest in the central part of the range. (B) Correlations between phenotypes in this study.
396 Histograms of the measured values of each phenotype are plotted along the diagonal. (C) PCA of
397 phenotypes. Correlations of phenotypes with principal components are plotted as arrows, with
398 length multiplied by 3 for ease of viewing. Latitude (correlation with first PC $r = -0.182$) and

399 longitude (correlation with first PC $r = -0.115$) are plotted for geographic context, though they
400 were not included in PCA. Arrows for latitude and longitude are scaled equally to the arrows for
401 phenotype correlations.

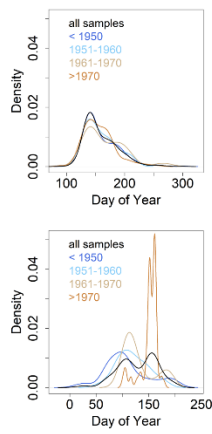
402

403 *Spatial variation in long-term average phenotypes (questions 2 and 3)*

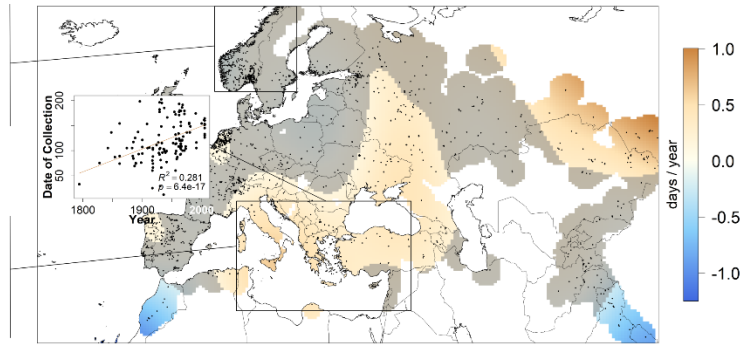
404 We visualized spatial diversity in phenotypes by plotting the spatial intercept surfaces in the year
405 only models (Figure 2A). All phenotypes showed significant spatial variation (all GAM smooth
406 terms significantly different from zero). $\Delta^{13}\text{C}$ was lower in the Iberian Peninsula and higher in
407 Russia (GAM smooth term, $p = 0.0002$). $\delta^{15}\text{N}$ varied across the range, but with less pronounced
408 spatial gradients (GAM smooth term, $p = 0.01$). C:N was higher in the Iberian Peninsula and
409 central Asia and lower in Russia (GAM smooth term, $p = 9\text{e-}05$). Collection day was earlier
410 along the Atlantic coast and Mediterranean (GAM smooth term, $p = <2\text{e-}16$). Despite this, PTU
411 at collection still was higher in the Mediterranean region as well as at far northern, continental
412 sites (GAM smooth term, $p = <2\text{e-}16$).

413 *Temporal*

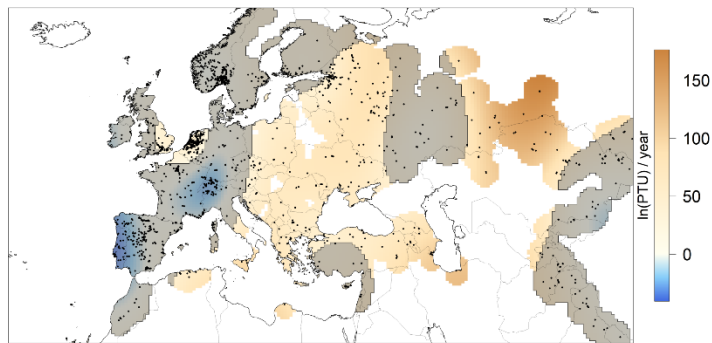
change in
phenotypes



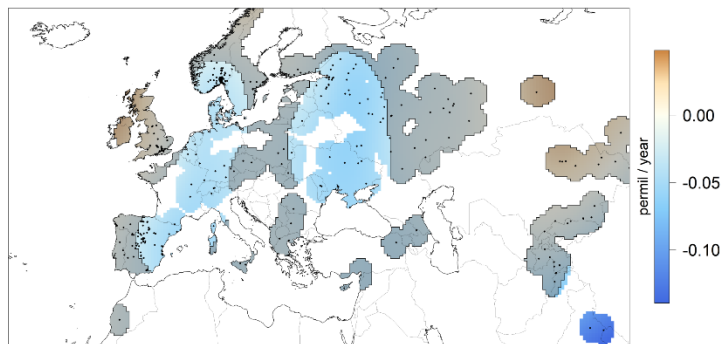
A. Day of Collection



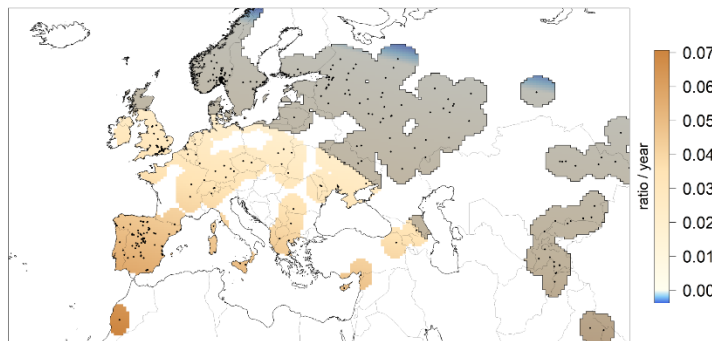
B. Photothermal Units



C. $\delta^{15}\text{N}$



D. C:N



431

432

433 Figure 3: Change in phenotypes across years for collection date (A), photothermal units (B), δ
434 nitrogen (C), and C:N (D). Color indicates the value of the coefficient for year in the model
435 excluding climate variables, gray shading indicates regions where estimated coefficient is not
436 significantly different from 0. For example, day of collection and photothermal units have
437 significantly increased over time in most of the range, but with some exceptions for day of
438 collection in the south. Inset scatterplot in A shows the significant increase in collection date
439 with year for samples in the boxed Mediterranean region. Plots to the left of A show the density
440 of collection dates through the year remains stable through time for Scandinavian collections
441 within the boxed region (top) but shift toward more collections late in the year in the boxed
442 Mediterranean collections (bottom).

443

444

445 Several phenotypes have changed significantly across large regions over the study period (1794-
446 2010, Figure 3 above). For example, C:N ratio increased in later years in much of southwestern
447 Europe. $\delta^{15}\text{N}$ decreased significantly throughout most of the range. Collection date and PTUs
448 became significantly later in many regions from the Mediterranean to Central Asia, although
449 collection date became significantly earlier in the extreme south (Morocco and Himalayas).
450 There was no significant temporal trend in $\Delta^{13}\text{C}$ (not shown).
451 The year trends in phenotypes across the study period were likely partly related to underlying
452 climate variation. However, collection date, C:N, and $\delta^{15}\text{N}$ were still significantly associated

453 with year of collection even when accounting for temporal anomalies in climate from 1901-2010
454 (Figures S6, S12, S14). PTUs were even more negatively related to year of collection after
455 controlling for temporal anomalies (Figure S8). A notable discrepancy is that Iberian collections
456 were collected significantly earlier in later years when yearly climate anomalies were accounted
457 for (Figure S6). There was still no significant temporal trend in $\Delta^{13}\text{C}$.

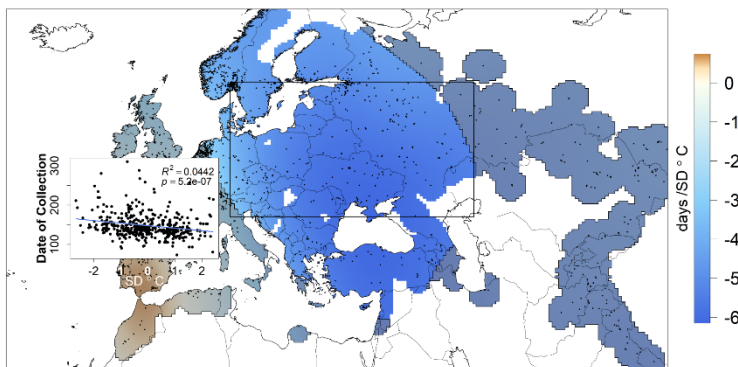
458

459 *Phenotype associations with spatiotemporal climate gradients (questions 2 and 3)*

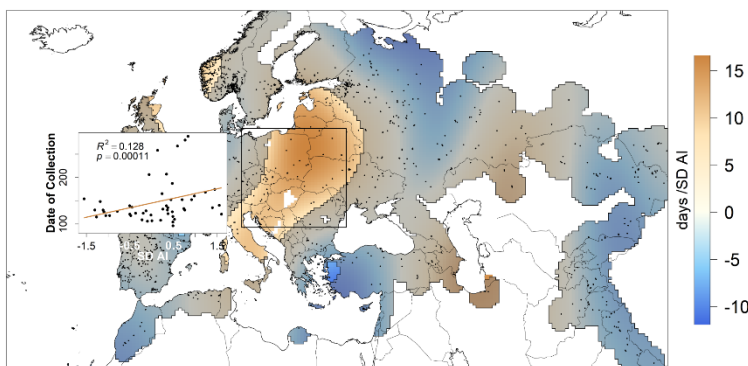
460 **Date of collection** – In years (temporal climate anomaly models) with a relatively warm April
461 plants were collected significantly earlier (Figure 4A). Similarly, in locations (spatial climate
462 models) with warmer temperatures plants were on average collected earlier in the year, though in
463 many regions these coefficients were non-significant (Figure S7A, B). We also tested
464 associations with July aridity index (precipitation/PET) and found that plants were collected
465 significantly earlier in years (temporal climate anomaly models) with dry summers in
466 Central/Eastern Europe (Figure 4B).

467

A. Mean April Temperature



B. July Aridity Index



468

469 Figure 4: Association between collection day of *Arabidopsis* temporal mean April temperature
470 anomalies (A) and July aridity index anomalies (B) (compared to 50-year average). Color
471 indicates the value of the coefficient of the April mean temperature anomaly or July aridity index
472 anomaly term. In years where April was warmer (positive anomalies), plants were collected
473 earlier (a negative relationship). In wetter years, plants were collected later in Eastern Europe.
474 Shading indicates regions where estimated coefficient is not significantly different from 0.
475 Scatterplots of phenotype measures for individuals within the boxed areas show a decreasing
476 collection date with mean April temperature anomaly and an increasing collection date with July
477 aridity index anomaly in Eastern Europe and a decreasing collection date in Central Asia.

478

479

480 **Photothermal units** – To standardize spatiotemporal variation in developmental periods, we
481 also modeled climate associations with PTUs. As expected, there were few areas where
482 temperature anomalies were significantly associated with PTUs, likely due to the ability of PTUs
483 to account for plastic responses (Figure S8). However, in some areas, accumulated PTUs at
484 collection changed significantly in association with spatial temperature gradients, perhaps
485 indicating spatial genetic differences in phenology (Figure S9). Locations with warmer Aprils
486 had plants collected at more PTUs in East-Central Europe and fewer PTUs around the Aegean
487 and Northern Asia. In the spatial climate model, plants from wetter areas in the Mediterranean
488 were collected at lower PTUs (Figure S8, S9).

489

490 **$\Delta^{13}\text{C}$ Carbon** – Wet summers were not significantly related to $\Delta^{13}\text{C}$ in any region in either the
491 temporal climate anomaly or spatial climate models (Figure S10, S11). Spatial variation in mean
492 April temperatures was not significantly related to $\Delta^{13}\text{C}$, but plants from locations of colder
493 Januaries did have lower $\Delta^{13}\text{C}$ in Northern Asia and the Iberian Peninsula. (Figure S11B).
494 Although elevation was not included in final models for reasons discussed above, replacing year
495 with elevation in the temporal climate anomaly model showed a significant negative association
496 between elevation and $\Delta^{13}\text{C}$ (Figure S18D). Including elevation reduced the significance of the
497 relationship between spatial variation in January temperature and $\Delta^{13}\text{C}$.

498

499 **$\delta^{15}\text{Nitrogen}$** – $\delta^{15}\text{N}$ was significantly higher in wetter years in Iberia, Asia, and Central Europe,
500 (Figure S12C), but lower in the North of France. Spatial variation in minimum January
501 temperatures was significantly positively related to $\delta^{15}\text{N}$ around the North Sea (Figure S13B).

502

503 **Leaf C:N** – For the temporal climate anomaly model, plants collected in years with warmer
504 winters in Iberia had significantly lower C:N ratios (Figure S14B). Leaf C:N differed in response
505 to April mean temperature and January minimum temperature among locations (spatial climate
506 models, Figure S15), although the patterns were mostly insignificant.

507 **Discussion**

508 Widely distributed species often exhibit considerable phenotypic diversity, a large portion of
509 which may be driven by adaptive plastic and evolutionary responses to environmental gradients.
510 Previous studies of intraspecific trait variation in response to environment have tended to focus
511 on genetic variation of environmental responses in common gardens (*e.g.* Wilczek *et al.* 2009;
512 Kenney *et al.* 2014), temporal trends in phenology from well-monitored sites (*e.g.* CaraDonna *et*
513 *al.*, 2014), or field sampling of individuals from a small number of sites (*e.g.* Jung, Violle,
514 Mondy, Hoffmann, & Muller, 2010). Here, we complement this literature by studying change in
515 traits across an entire species range over two centuries, giving us a window into drivers of
516 intraspecific diversity and regional differences in global change biology. From the accumulated
517 effort contained in natural history collections, we tested hypotheses about variation in life history
518 and physiology in response to environment. We observed modest evidence of coordinated
519 phenological-physiological axes of variation for *Arabidopsis* in nature. We found later flowering
520 times and higher accumulated photothermal units over the study period across most of the range
521 and lower $\delta^{15}\text{N}$ and higher C:N in more recent collections. Additionally, we observed distinct
522 regional differences in phenology, $\Delta^{13}\text{C}$, and C:N in response to rainfall and temperature,
523 potentially due to genetic differences among populations.

524

525 *Intraspecific variation in life history and physiology shows little coordination along a single*
526 *major axis (question 1)*

527 We found little evidence for tight coordination among studied phenotypes, fitting with some past
528 studies that found weak to no support for a single major axis in intraspecific trait variation in

529 response to environment across diverse plant growth forms (e.g. Albert *et al.* 2010; Wright &
530 Sutton & Grier 2012). Common garden experiments often find substantial genetic covariation
531 between the traits we studied possibly due to pleiotropy or selection maintaining correlated
532 variation (Des Marais *et al.*, 2012; Kenney *et al.*, 2014; McKay *et al.*, 2003). By contrast, the
533 massively complex environmental variation organisms experience in the wild may combine with
534 genotype-by-environment interactions to generate high dimensional trait variation among
535 individuals in nature.

536 Nevertheless, we found modest evidence of a life history-physiology axis: plants collected later
537 in the year had low leaf C:N, indicative of a fast-growing resource acquisitive strategy with low
538 investment in C for structure and high investment in N for photosynthesis. The C:N/collection
539 date axis is probably not due to later collections being at later developmental stages, since we
540 would expect plants collected later in development to have allocated nitrogen away from leaves,
541 lowering C:N. Instead, a strategy of higher leaf C:N may be adaptive for rapid-cycling plants
542 germinating and flowering within a season (spring/summer annuals), which we expect to be
543 collected later in the year due to later germination, contrasted with slower-growing genotypes
544 known to require vernalization for early spring flowering over a winter annual habit. Indeed, Des
545 Marais *et al.* (2012) found that vernalization-requiring (winter annual) *Arabidopsis* genotypes
546 had lower leaf N than genotypes not requiring vernalization for flowering, the latter of which
547 could also behave as spring or summer annuals.

548 The negative correlation we observed between $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ has been reported by other authors
549 and suggested to be a result of independent responses to multiple correlated environmental
550 variables rather than a biological constraint. Environmental variables with opposing effects on
551 $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ include soil, temperature, and rainfall patterns (Hartman & Danin, 2010; Liu *et*

552 al., 2007; Peri et al., 2012), due to depletion of soil N and changes in stomatal opening, and
553 atmospheric carbon (Bloom, Burger, Asensio, & Cousins, 2010), which increases carbon uptake
554 while suppressing nitrate assimilation.

555

556 *Arabidopsis* life history and physiology vary across spatial environmental gradients, suggesting
557 adaptive responses to long-term environmental conditions (question 2)

558 Geographic clines in traits in nature may be due to adaptive responses to environment. However,
559 the spatial differences in traits or trait changes through time we observed are difficult to ascribe
560 to genetic or plastic causes because of unknown genotype-environment interactions in the field
561 and the confounding of environmental gradients and population genetic structure. The 1001
562 Genomes Project identified genetic clusters of *Arabidopsis* that were somewhat geographically
563 structured but noted that these clusters overlapped and were distributed across a wide range of
564 environments (Alonso-Blanco et al., 2016) (see Figure S5 for a map of the clusters). The patterns
565 of significant phenotype-environment relationships we observed spanned multiple genetic
566 clusters, making it unclear how much of a role these broad genetic groupings play in determining
567 environmental response. The phenotype-environment relationships we observed followed our
568 expectations for how phenology could affect fitness through earlier flowering times in response
569 to warmth and both earlier and later flowering in response to drier environments. Physiological
570 traits were less well aligned with our predictions for adaptive response; we did not find low $\Delta^{13}\text{C}$
571 and high C:N associated with environments or years of water stress.

572

573 *Physiology, lack of correspondence to the Leaf Economic Spectrum*

574 The Leaf Economic Spectrum and fast/slow life history predictions were not well supported by
575 our results for how C:N, $\Delta^{13}\text{C}$, and $\delta^{15}\text{N}$ respond to climate, since we saw both positive and
576 negative trends with temperature and aridity depending on geographical region. This may be due
577 to the intraspecific nature of our study, as opposed to the interspecific data often used to support
578 the LES (Albert et al., 2010; Elmore, Craine, Nelson, & Guinn, 2017). In addition, our study may
579 have overlooked the effects of edaphic conditions on C:N and $\delta^{15}\text{N}$. C:N over most of the native
580 range was insignificantly related to spatial and temporal gradients of temperature and aridity
581 index (July precipitation/PET) but increased with year as seen in grassland communities due to
582 rising C_a (Gill et al., 2002). Likewise, $\delta^{15}\text{N}$ over most of the range neither decreased with aridity
583 index nor responded to temperature as expected, but did decrease with year as previously
584 reported (McLauchlan, Ferguson, Wilson, Ocheltree, & Craine, 2010), possibly due to CO_2
585 enrichment.

586 Similarly, we did not see strong relationships between aridity index and $\Delta^{13}\text{C}$. $\Delta^{13}\text{C}$ was
587 expected to be related to rainfall and temperature due to $\Delta^{13}\text{C}$ being a proxy for stomatal gas
588 exchange (Diefendorf et al., 2010; Farquhar et al., 1989). There are at least three potential
589 explanations for weak $\Delta^{13}\text{C}$ relationships with climate. First, we observed both positive and
590 negative trends for aridity and date of collection, consistent with the hypothesis that *Arabidopsis*
591 exhibits both drought escaping and drought avoiding genotypes. The phenological response to
592 moisture of rapid flowering (drought escape strategy) could confine growth to periods of high
593 moisture, obviating any stomatal closure in response to soil drying (and hence no effect on
594 $\Delta^{13}\text{C}$). Stated simply, phenology and physiology cannot be treated as completely independent
595 traits. Second, variation in plant traits we did not directly consider may affect $\Delta^{13}\text{C}$. Gas
596 exchange and carbon assimilation depend in part on leaf architecture and physiology traits like

597 venation, root allocation, and mesophyll conductance (Brodribb, Feild, & Jordan, 2007; Easlon
598 et al., 2014; Schulze, Turner, Nicolle, & Schumacher, 2006), which could limit responses in
599 $\Delta^{13}\text{C}$. For example, given the role of roots in sensing drought and triggering stomatal response
600 (Christmann, Hoffmann, Teplova, Grill, & Muller, 2004), greater investment in roots could allow
601 plants in relatively drier conditions to maintain open stomata, preventing decreases in C_i and
602 leading to no observed climate effect on $\Delta^{13}\text{C}$. Third, elevated atmospheric partial CO_2 could
603 mitigate climate effects on $\Delta^{13}\text{C}$ by increasing the efficiency of stomatal gas exchange (Drake,
604 Hanson, Lowrey, & Sharp, 2017). Local investigations of the patterns we found could
605 complement our results by characterizing the underlying ecophysiological and life history
606 mechanisms driving intraspecific variation.

607

608 *Phenology, high variation across space*

609 We found strong spatial gradients in two measures of phenology, suggesting that adaptive
610 responses to climate drive long-term trait differences among regions. Locations that were
611 warmer than average in either April or January corresponded to significantly earlier collection
612 dates, consistent with temperature's positive effect on growth rate (Wilczek et al., 2009). In
613 addition, our models provided support that some phenological variation did reflect seasonality of
614 moisture availability. We found that *Arabidopsis* was collected significantly earlier in years with
615 dry summers in central Europe and at significantly lower PTU in regions of wet summers around
616 the Mediterranean, suggesting drought escape or avoidance strategies, respectively, could be
617 important in those regions. Alternatively, later collections in wetter years could be the result of
618 multiple successful generations due to the extra rainfall. This ambiguity illustrates an important
619 caveat in using collection date and PTU to study *Arabidopsis* in the wild. Because we cannot

620 differentiate changes in total life span from changes in germination start date, shifts in phenology
621 (the timing of an organism's life to seasonal conditions) does not necessarily indicate shifts in
622 life history. Nevertheless, the observed regional variation in the response of collection date and
623 PTU to climate variables provides valuable insight into how different populations experience
624 their environment and suggest areas for more direct study.

625

626 *Changes in Arabidopsis life history and physiology over the last two centuries track climate,*
627 *suggesting adaptive responses (question 3)*

628 Increasing global temperatures were expected to increase relative growth rate and hasten
629 germination, decreasing flowering time as measured by collection date. In addition, atmospheric
630 CO₂ enrichment was expected to increase $\Delta^{13}\text{C}$ (Drake et al., 2017) and C:N and decrease $\delta^{15}\text{N}$
631 (Bloom et al., 2010). With increasing environmental nitrogen due to human activity (Galloway et
632 al., 2004), we expected $\delta^{15}\text{N}$ to decrease.

633 Our findings were largely consistent with these hypotheses in the year models for leaf
634 physiology. $\Delta^{13}\text{C}$ did not significantly change through time across the native range, which could
635 be due to life history shifts as mentioned above or differential response to aridity gradients across
636 the locations sampled masking the effect of elevated CO₂ (Drake et al., 2017). Geographic
637 variation in the strength of the relationships for other traits could be due to underlying genetic
638 variation or interaction with environmental factors we did not account for. Nevertheless, C:N
639 increased and $\delta^{15}\text{N}$ decreased as expected across large portions of the native range.

640 For collection date and PTU, however, our models returned the surprising result of later
641 collection rather than earlier, despite earlier collections in warmer years. The fact that the

642 relationship between warmth and collection date was spatially variable, and insignificant in some
643 regions, may indicate areas of contrasting phenological response, perhaps due to lost
644 vernalization signal or variable effects on germination (Burghardt, Edwards, & Donohue, 2016).
645 Variation across space in phenological response to climate change has been shown before and
646 may be due to genetic differences among populations or due to interactions with other
647 environmental variables (Park et al., 2018). *Arabidopsis* is known to complete a generation
648 within a single season, climate permitting, and warmer climates may even allow for fall
649 flowering (Fournier-Level et al., 2013; Wilczek et al., 2009). If warmer temperatures enable a
650 greater number of spring or summer germinants to flower before winter in regions such as
651 Central Europe, we would expect to see later collection dates in more recent years (Burghardt et
652 al., 2015). Regions that did not show later collection dates through time might be limited in
653 generational cycles due to summer drought or very short growing seasons. For instance, early
654 flowering in the spring has been implicated as an important strategy for *Arabidopsis* in the
655 Iberian Peninsula to escape seasonal heat and water limitation that curtail growth in later months
656 (Wolfe & Tonsor, 2014).

657 Our findings of later collection dates through the study period (1798-2010) may surprise some
658 readers due to previously observed acceleration of temperate spring phenology (Parmesan &
659 Yohe, 2003). However, we modeled changes in mean phenological response to environment,
660 which can be weakly related to either tail of phenology trait distributions (CaraDonna et al.,
661 2014). Individuals on the extremes, such as first-flowering individuals, are often the primary
662 focus of studies showing accelerated spring phenology in recent years. Why might *Arabidopsis*
663 flower later even as global temperatures rise? First, anthropogenic land use change may drive
664 phenology by favoring spring germinants, *e.g.* if disturbances favor faster life cycles. Second,

665 warming climate or increasing atmospheric pCO₂ may favor alternate life histories by increasing
666 relative growth rate, thus allowing spring or fall germinants to complete their life cycle before
667 conditions degrade at the end of a growing season. Later collections in more recent years might
668 represent an increasing proportion of fast-growing spring or summer annuals as opposed to
669 winter annuals. Whatever the cause of *Arabidopsis* flowering later, these phenological changes
670 may have important ecological effects, such as altered biotic interactions.

671 We found evidence that trait correlations may be changing through time. For example, in some
672 regions both C:N and date of collection have significantly increased over the past 200 years
673 (around the Eastern Mediterranean), while in other regions date of collection decreased while
674 C:N increased over time (Morocco and the Iberian Peninsula) (Figure 1). If the negative
675 relationship between leaf C:N and flowering time that we observed is truly an axis of adaptive
676 tradeoff between fast and slow life histories, this tradeoff may be changing at different rates
677 among regions with time. Changing environments might reshape biological constraints on
678 adaptive plant responses (Sgrò & Hoffmann, 2004).

679

680 *Our approach, technical limitations in herbaria data to surmount in future studies*

681 Understanding how environmental variation drives the intraspecific diversity in broadly
682 distributed species has been challenging due to logistics of large spatiotemporal scales. However,
683 advances in digitization of museum specimens and the generation of global gridded
684 spatiotemporal environmental data are opening a new window into large scale patterns of
685 biodiversity. One challenge of herbarium specimens is that they typically present a single
686 observation of a mature, reproductive individuals. Thus, these specimens contain limited

687 information on phenology and physiology at earlier life stages (*e.g.* seedling plants), which can
688 have subsequently strong impacts on later observed stages. Use of developmental models
689 (Burghardt et al., 2015) might allow one to backcast potential developmental trajectories using
690 herbarium specimens and climate data, to make predictions about phenology of germination and
691 transition to flowering. In addition, herbaria collections are often biased by factors such as
692 geography, species, and climate (Daru et al., 2018; Loiselle et al., 2008). Hierarchical sampling
693 through repeated collections in the same region could improve the confidence of our model in
694 representing phenotypic change through time.

695 Generalized additive models are a flexible approach to model phenotype responses to
696 environment that might differ spatially among populations (MacGillivray et al., 2010). These
697 models allow the data to inform on spatial variation in the trends studied, unlike approaches that
698 bin individuals into discrete and arbitrarily bounded regions. Herbarium records represent
699 imperfect and biased samples of natural populations (Daru et al., 2018), and future efforts may
700 benefit from additional information that might allow us to account for these biases. Here, we
701 sampled a very large number of specimens across continents and centuries, perhaps reducing the
702 effect of biases associated with specific collectors. Nevertheless, as museum informatics advance
703 it may become possible to explicitly model potential sources of bias, for example those arising
704 from collecting behavior of specific researchers.

705

706 *Conclusion*

707 Widely distributed species often harbor extensive intraspecific trait diversity. Natural history
708 collections offer a window into this diversity and in particular allow investigation of long-term

709 responses to anthropogenic change across species ranges. Here we show that spatiotemporal
710 climate gradients explain much of this diversity but nevertheless much of the phenotypic
711 diversity in nature for the model plant remains to be explained.

712

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726 would represent a conflict of interest.

727

728 **Tables**

729 Table 1: Hypothesized responses of phenotypes to increases in temperature, rainfall, or year, or
 730 how traits would change along a faster life history strategy.

731 Year trends are predicted due to elevated CO₂, nitrogen deposition, or elevated temperatures.

732 Citations for hypotheses: (Amundson et al., 2003¹; BassiriRad et al., 2003²; Burghardt et al.,

733 2015³; Craine et al., 2009⁴; Diefendorf et al., 2010⁵; Drake, Hanson, Lowrey, & Sharp, 2017⁶;

734 Gill et al., 2002⁷; McLauchlan, Ferguson, Wilson, Ocheltree, & Craine, 2010⁸; Menzel et al.,

735 2006⁹; Ordoñez et al., 2009¹⁰; Peñuelas et al., 2004¹¹; Reich, Hungate, & Luo, 2006¹²; Seibt,

736 Rajabi, Griffiths, & Berry, 2008¹³; Sparks & Carey, 2006¹⁴; Stock & Evans, 2006¹⁵; I. J. Wright

737 et al., 2004¹⁶)

	Temperature	Rainfall	Year	Fast Life History
$\Delta^{13}\text{C}$	+ ¹³	+ ⁵	+ ⁶	+
$\delta^{15}\text{N}$	+ ^{1,4}	- ^{1,4}	- ^{2,8,15}	No change
C:N	- ¹⁰ in cold regions + ¹⁶ in warm regions	- ¹⁶	+ ^{7,8,12}	-
Photothermal Units	+ or no change	+	No change	-
Collection Date	- ^{3,9}	+ ¹¹	- ^{9,14}	-

738

739 *Comparison of results to hypotheses*

740 Table 2: Expected phenotype responses to increases in temperature, rainfall, or year and

741 observed model output.

742 For some phenotypes a single trend over time was observed; however, most phenotypes showed

743 variation in responses to temperature and rainfall across the range.

	Temperature		Rainfall		Year	
	Expected	Observed	Expected	Observed	Expected	Observed
$\Delta^{13}\text{C}$	-	-, mainly insignificant	+	-, mainly insignificant	+	insignificant
$\delta^{15}\text{N}$	+	+, -, mainly insignificant	-	+	-	-
C:N	- +	+, -, mainly insignificant	-	insignificant	+	+
Photothermal Units	+	+, -	+	+, -	No change	+
Collection Date	-	-, +	+	+, -	-	+, -

744

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