Modeling the Influence of Genetic and Environmental Variation on the Expression of Plant Life Cycles across Landscapes

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ABSTRACT: Organisms develop through multiple life stages that differ in environmental tolerances. The seasonal timing, or phenology, of life-stage transitions determines the environmental conditions to which each life stage is exposed and the length of time required to complete a generation. Both environmental and genetic factors contribute to phenological variation, yet predicting their combined effect on life cycles across a geographic range remains a challenge. We linked submodels of the plasticity of individual life stages to create an integrated model that predicts life-cycle phenology in complex environments. We parameterized the model for *Arabidopsis thaliana* and simulated life cycles in four locations. We compared multiple "genotypes" by varying two parameters associated with natural genetic variation in phenology: seed dormancy and floral repression. The model predicted variation in life cycles across locations that qualitatively matches observed natural phenology. Seed dormancy had larger effects on life-cycle length than floral repression, and results suggest that a genetic cline in dormancy maintains a life-cycle length of 1 year across the geographic range of this species. By integrating across life stages, this approach demonstrates how genetic variation in one transition can influence subsequent transitions and the geographic distribution of life cycles more generally.

Keywords: Arabidopsis thaliana, flowering time, germination, life history, phenotypic plasticity, population ecology.

Plant life cycles are composed of multiple life stages (e.g., seed, vegetative, reproductive) that differ in environmental sensitivities and tolerances. In seasonal environments, the timing, or phenology, of life-stage transitions (e.g., germination, flowering, seed dispersal) may have important implications for fitness, and the optimal phenology may change temporally or spatially. Life-cycle phenology can influence fitness (survival and fecundity) directly by determining the environment to which each differentially stress-tolerant life stage is exposed (Donohue et al. 2010; Munguía-Rosas et al. 2011). Moreover, by changing the rate at which organisms transition through life-cycle stages, phenology can influence crucial demographic measures such as generation length (Caswell 1983; Forrest and Miller-Rushing 2010; Kimball et al. 2010). Both phenology and generation time vary across species' ranges and influence organismal responses to climate change (Chuine and Beaubien 2001; Morin et al. 2007; Aitken et al. 2008; Willis et al. 2008).

Geographic variation in life cycle can be caused by both environmental and genetic factors, but how environmental cues combine with genetic variation across the range to determine those responses is largely unknown. In this era of rapidly changing climate, disentangling the contributions of these multiple factors to phenological variation is especially crucial. Here we present a modeling approach that predicts the life cycles expressed by differentially sensitive genotypes in response to environmental variation and apply it to understand life-cycle variation in the plant *Arabidopsis thaliana*.

Phenological transitions are often environmentally sensitive, or phenotypically plastic, to multiple seasonal factors such as moisture, temperature, and photoperiod (Bradshaw 1965; Sultan 2000). The environmental sensitivity of life-cycle variation is most likely a result of past selection that allows each life stage to be expressed in favorable conditions in the face of spatial or temporal environmental variation in climate. For instance, cuing allows bud burst to happen later in the spring (Ducousso et al. 1996) and fewer insect generations to occur each

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year (Roff 1980, 2002) at higher latitudes where favorable conditions arrive later and last for a shorter period of time. Similarly, organisms often use abiotic cues as a way to synchronize phenology with each other or with pollinators (e.g., flowering in outcrossing species; Wolkovich 2013).

Often these phenology patterns are not caused by plastic response to a single cue; instead, organisms integrate information from multiple environmental factors. These factors, moreover, can affect responses at different timescales (e.g., hourly, daily, seasonally). For instance, the flowering transition in *A. thaliana* responds not only to instantaneous temperature conditions but also to the cumulative effects of long-term cold exposure and daily photoperiod (Wilczek et al. 2009; Andrés and Coupland 2012). As such, plastic responses of life-stage transitions to complex environmental variation can cause life-cycle variation across time and geography.

Genetic variation in the environmental sensitivity of life-stage transitions can either magnify or reduce life-cycle variation across heterogeneous environments. This variation is commonly observed (Long et al. 2007; Li et al. 2010; Anderson et al. 2011; Olson et al. 2012) and is often spatially structured (Paaby et al. 2010; Blackman et al. 2011). This variation is commonly observed (Long et al. 2007; Li et al. 2010; Anderson et al. 2011; Olson et al. 2012) and is often spatially structured (Paaby et al. 2010; Blackman et al. 2011). This allelic variation can contribute to differences in life cycle across a species' range. For example, allelic variation in the amount of accumulated cold required for flowering is thought to cause variation in the number of generations possible each year in *A. thaliana* (Simpson and Dean 2002; Michaels et al. 2003). However, allelic variation can also reduce phenotypic variation across environmental gradients (termed countergradient variation by Levins 1969; reviewed in Conover and Schultz 1995). For instance, genetic clines in growth rate across latitude in *Rana temporaria* compensate for differences in average temperature and lead to the same observed growth rates across the range (Laugen et al. 2003).

Moreover, the effects of both allelic and environmental variation on one phenological transition can have ramifying effects on the timing of the entire life cycle by changing the seasonal environment experienced by later life stages, which influences the phenotypic expression of subsequent plastic traits (Donohue et al. 2005; Galloway and Burgess 2009; Saarinen et al. 2011; Chiang et al. 2012). It is therefore important to consider these cascading effects when predicting how variation in any given stage influences overall life-cycle dynamics (Post et al 2008).

In sum, to understand geographic patterns of any one life-stage transition and of entire life cycles, we must consider (1) the multiple environmental factors that affect the timing of different transitions, (2) the genetic variation that could either augment or mask the sensitivity of a transition to those environmental factors, and (3) the cascading effects of one transition on the timing of other life-cycle transitions.

Here we take a modeling approach to predict the joint contributions of environmental and allelic variation on life-cycle phenology and generation length. Using an individual-based model (IBM), we link together phenology models (essentially, models of plasticity) that predict the timing of each of the multiple life-stage transitions that compose the life cycle. Unlike models that focus on a single life-stage transition, this integrated framework incorporates the important dynamic that the timing of one life stage determines the seasonal conditions experienced by subsequent life stages. Previous models for trees (Morin et al. 2008) and crops (Hoogenboom et al. 1994; White and Hoogenboom 1996) have linked multiple life-stage transitions within a generation. Here we extend such an approach to investigate dynamics across multiple generations (see Stoeckli et al. 2012 for an example in insects).

This modeling approach permits investigation of how fixed parameters that describe environmental sensitivities interact with environmental variation to produce complex phenotypes (i.e., phenology). Because genotypes differ measurably in environmental sensitivities, different model parameterizations can represent allelic variation in how organisms respond to diverse environmental factors (Morin et al. 2007; Wilczek et al. 2009; Zhao et al. 2013). As such, this modeling approach supplies an extremely flexible tool for predicting the reaction norms of particular genotypes in response to complex and variable environments (Buckley and Kingsolver 2012). It even provides a method for predicting environment-dependent differences among genotypes.

We present results of this integrated life-cycle model based on parameters estimated in the annual plant *A. thaliana*. This species displays wide variation in life cycle across its native European range, and a great deal is known about the environmental sensitivity of germination, flowering, and seed dispersal. Using this integrated model, we predicted the effects of known allelic variation in germination and flowering time on the life cycle in four locations across the native range.

In this article, we explore the causes of geographic variation in life-cycle phenology and length. Specifically, we ask: (1) How does environmental variation influence life-cycle variation within and among locations? (2) What is the effect of genetic variation in two traits that influence phenology: seed dormancy and floral repression? (3) Do environmental and genetic variation interact to magnify or reduce variation in life cycles? We found that a single genotype can produce very different life cycles depending on local conditions. Further, genetic variation interacted

with environmental variation to determine life-cycle phenology, reducing variation in life-cycle length across the geographic range.

Methods

Study System

Arabidopsis thaliana displays life-cycle variation between populations (Pigliucci 2002; Koornneef et al. 2004; Lundemo et al. 2009). Life-cycle designations in this species focus on the primary season experienced by the vegetative stage. Winter annuals germinate in the fall, overwinter as a rosette, and flower in spring. In contrast, spring, summer, and fall annuals all flower in the same season in which they germinate. However, the annual designation can be misleading because it refers only to the fact that the plant is aboveground for less than a year. Because we know very little about the seed dynamics of *A. thaliana* in natural populations, an individual could hypothetically spend years as a dormant seed before it germinates or complete multiple generations in a single year. Within some populations, mixtures of life cycles occur whereby, for example, some individuals germinate in autumn and others in spring (Lawrence 1976; Pico 2012). Whether genetic variation underlies this phenological variation and whether this variation is caused by discrete or overlapping generations are unknown.

Variation in the phenology of natural populations of *A. thaliana* has been documented in the four European locations for which we present model results. Wilczek et al. (2009) found that in a northern site near Oulu, Finland, germination primarily occurred in early fall and flowering occurred in early summer (C. Lopez-Gallego and R. Petipas, personal communication). In a southern coastal site near Valencia, Spain, germination occurred primarily in late fall (D. Eaton, personal communication) and flowering occurred in early spring. Therefore, in both locations, *A. thaliana* behaves as a winter annual; but the life cycle of the northern population is dominated by the rosette stage, while the southern population is dominated by the seed stage (see also Ratcliffe 1961; Montesinos-Navarro et al. 2010; Ågren and Schemske 2012). In contrast, in eastern Europe (at the center of the native range in Halle, Germany) and in the United Kingdom, winter-annual, spring-flowering life cycles are common, but flowering can also occur in the summer and late fall (Ratcliffe 1961 and citations therein; Thompson 1994; Wilczek et al. 2010). See figure B1 for illustrated summaries of these observed life cycles (figs. A1, B1–B19 available online).

Allelic variation occurs in genes that influence the timing of flowering and germination. Increasing the expression level of the floral repressor *FLOWERING LOCUS C* (*FLC*) delays flowering, but if the plant experiences prolonged exposure to cold, expression is reduced and flowering occurs (Sheldon et al. 2000; Bastow et al. 2004; Sung and Amasino 2004; Dennis and Peacock 2007), potentially imposing a winter-annual life cycle. Genotypes with high floral repression occur throughout the European range, while low–floral repression genotypes are primarily restricted to central Europe (A. M. Wilczek, unpublished data) and northern Spain (Mendez-Vigo et al. 2011).

In addition, *A. thaliana* accessions display a latitudinal cline in primary seed dormancy levels, driven in part by variation in the *DELAY OF GERMINATION 1* (*DOG1*) locus (Kronholm et al. 2012): primary dormancy levels are higher in accessions from lower latitudes (Atwell et al. 2010; Chiang et al. 2011). Primary seed dormancy is a strong determinant of germination timing and represses germination, despite exposure to environments that usually promote germination. As seeds age (afterripen), primary dormancy decreases (Finch-Savage and Leubner-Metzger 2006; Graeber et al. 2012).

Developmental Threshold (Phenology) Models

Phenological models of development, derived first to aid crop production (Wang 1960), predict the timing of life-stage transitions as a function of temporal variation in multiple environmental factors (Xinyou et al. 1997; Alvarado and Bradford 2002; Hammer et al. 2005). Parameterized mathematical functions describe the rate of development in response to current and cumulative environmental factors. Developmental transitions from one life stage to the next occur when organisms accrue enough developmental progress to cross a transition threshold. The models therefore predict the amount of time required to proceed from one developmental stage to the next, given environmental conditions. Such models have been used to accurately describe the timing of life-stage transitions such as flowering (Welch et al. 2005; Wilczek et al. 2009; Satake 2010; Satake et al. 2013), bud burst (Cannell and Smith 1983; Hunter and Lechowicz 1992; Chuine 2000), and seed germination (Gummerson 1986; Alvarado and Bradford 2002) under controlled and field conditions. Currently, these models investigate the effects of climatic factors but not the effects of biotic factors such as inter-/intraspecific competition, herbivores, pathogens, or pollinators. Incorporating biotic factors into such models remains an area for future development as the physiological responses to these factors become better characterized.

Integrated Life-Cycle (ILC) Model

We created an integrated model that predicts whole life cycles by connecting three independent, phenological sub-

models that describe how germination, flowering, and seed-dispersal timing depend on specific environmental factors (fig. 1). We linked these submodels such that the timing of germination determines the seasonal conditions experienced by rosettes, which in turn influences flowering time, the timing of seed dispersal, and the germination time of the subsequent generation. Our models use hourly environmental inputs to capture known effects of diurnal variation and environmental extremes on developmental rates.

ILC Model Details

We built our individual-based model in the R statistical environment (R Development Core Team 2008) and have deposited the code in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.nv0p1 (Burghardt et al. 2014). The simulation begins with a cohort of 1,000 seeds of one "genotype" (reflected by a fixed set of phenological parameters). These parameters remain constant for the entire simulation and therefore can be interpreted as fixed, genetic attributes of a lineage. These seeds are binned into dormancy categories according to a normal distribution, the mean and variance of which are defined by maternal parameter values (genotype). This distribution reflects commonly observed variation in initial primary dormancy within a maternal seed cohort found in *A. thaliana* and many other species (for details, see "Germination Submodel" in app. A; apps. A, B available online). As the simulation proceeds, the rate of progression through each life stage depends on environmental inputs each hour (fig. 2*a*).

Seeds accumulate developmental progress at a rate influenced by water potential, soil temperature, and dormancy level, according to a hydrothermal model of germination (Gummerson 1986; Alvarado and Bradford 2002; app. A, "Germination Submodel"). Seeds with different dormancy levels progress at different developmental rates toward germination (i.e., seeds with lower dormancy develop more quickly in a given environment), so seeds from the same genotype (parameter set) dispersed on the same day may germinate on different days due to normally distributed variation in initial dormancy level (fig. 2*b*; gray lines surrounding mean). Once seeds attain the germination threshold, they germinate and the vegetative stage begins.

Vegetative plants accumulate progress toward reproduction according to a photothermal model of flowering (Wilczek et al. 2009). Long photoperiods and high daytime temperatures promote development, whereas high floral repression levels reduce developmental rate. Over time, floral repression decreases as a function of cumulative exposure to cold temperatures indicative of winter (fig. 2*c*; for details, see "Flowering Submodel" in app. A). Once flowering occurs, reproductive plants accumulate progress toward seed dispersal at a rate influenced by temperature (fig. 2*d*; for details, see "Seed Dispersal Submodel" in app. A). As soon as the seeds disperse, they are assigned a dormancy level based on the maternal parameters and

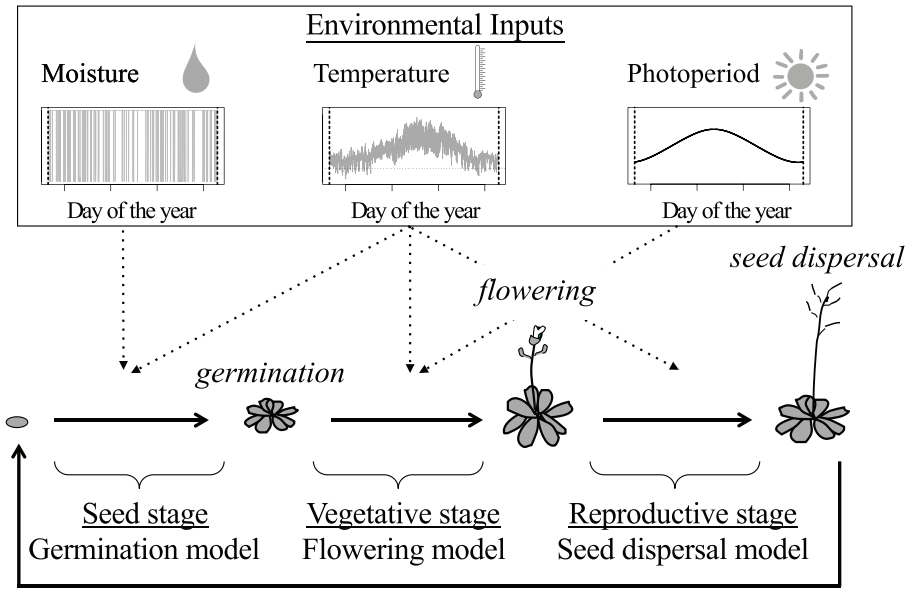

Figure 1: Basic structure of the integrated life-cycle model. Dotted arrows indicate which environmental inputs are used for each stage in the model. Solid arrows indicate the direction of progression through life stages.

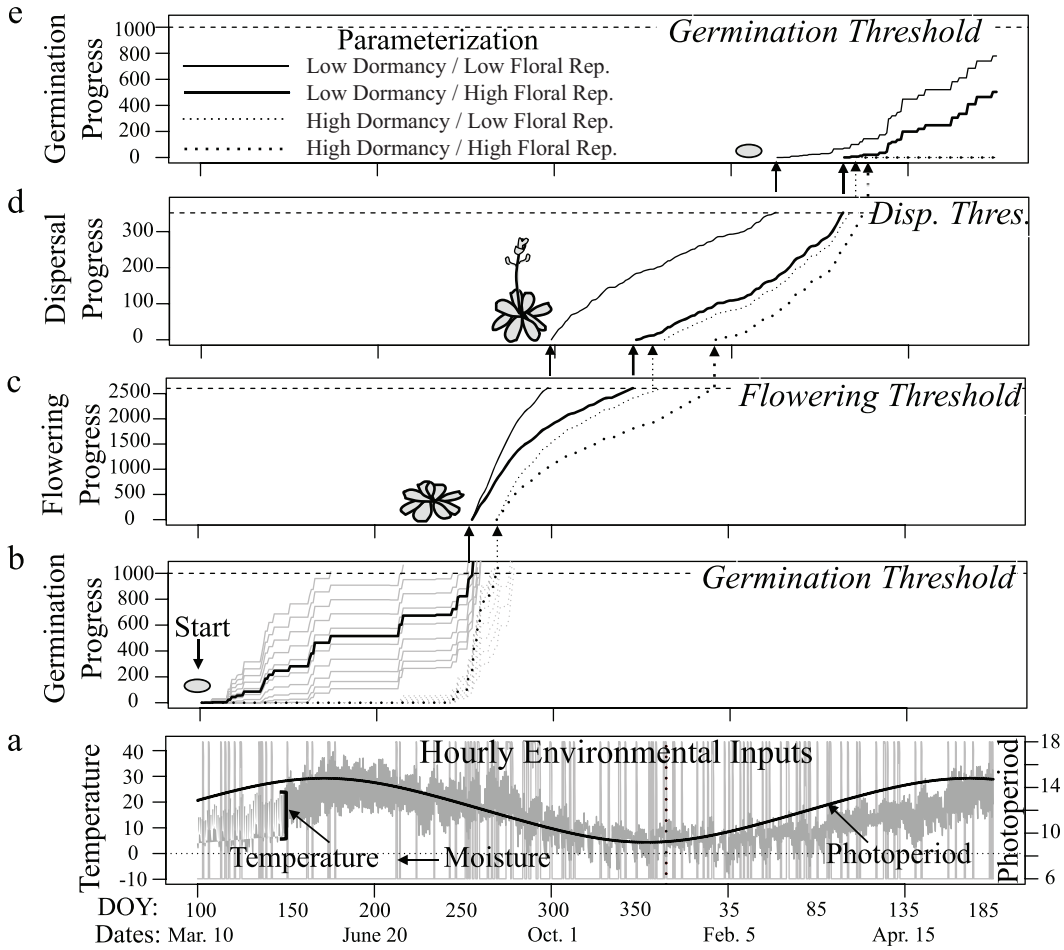

Figure 2: Schematic of four genotypes (all combinations of low/high dormancy and low/high floral repression) accumulating developmental progress in Valencia, Spain, through each of the phenology submodels. *a*, Environmental inputs are used to determine hourly progress. The black line is photoperiod, the light gray parallel lines are precipitation events, and the dark gray lines are hourly temperature inputs. *b*, All seeds are dispersed March 10 and immediately begin developing at a rate determined by the germination model. The darker lines indicate the mean dormancy class whose phenology will be followed throughout the graphic. The gray lines surrounding the mean class depict the behavior of different initial dormancy levels within a cohort. Both floral repression genotypes show the same germination behavior and are therefore superimposed on top of one another in the first panel that shows germination progress (*b*). Seeds germinate when they accumulate enough development to cross the germination threshold. *c*, *d*, Individuals progressing through the vegetative and reproductive phases, respectively. *e*, Germination of the next generation.

begin progressing toward germination (fig. 2*e*). The process repeats for many generations.

At a daily scale, we tracked the number of germination, flowering, and seed-dispersal events. Seeds produced on the same day by different individuals were pooled together for assignment of initial dormancy level, and there was no spatial heterogeneity within a simulation; that is, all individuals present at a given time experienced the same environment inputs. The model was simulated over 60 years using environmental factors from a given location (fig. 2*a*); the first 15 years were discarded, and the last 45 years of data were summarized. The 15-year burn-in is conservative; sensitivity analyses indicate that life-cycle expression stabilized in 5–10 years (for most genotypes) and that results are robust to the date of initial seed dispersal.

This study concerns how physiological parameters and environmental factors combine to produce life-cycle variation independently of natural selection on those life cycles. Environment-dependent survival and fecundity will be investigated in the future as the data required for parameterization become available. In our simulations, every plant survived and produced one seed to maintain a constant population size. This analysis, therefore, reveals the baseline phenological expression of different genotypes

across the native range. If natural selection has shaped genetic variation in parameter levels, we would expect the life stages of local genotypes to reflect adaptive outcomes, making our results relatively unbiased by the assumption of a lack of selection.

Environmental Inputs

We ran five randomly assembled environmental replicates based on climate data from four European environments spanning a latitudinal gradient: Valencia, Spain; Halle, Germany; Norwich, England; and Oulu, Finland. We used randomly assembled environments to (*a*) allow replication to ensure that results were independent of the exact series of environmental inputs and (*b*) avoid including the effects of climate change in the 60-year simulations. Halle and Norwich have similar photoperiod amplitudes, but temperature is milder in Norwich due to proximity to the ocean (see fig. B2 for examples of environmental inputs for each location and app. A for details on climate data sources and methods). We chose these locations not only because of the breadth of environmental conditions there but also because the flowering model of Wilczek et al. (2009) was validated in those locations and phenology has been observed in field experiments at those sites (Wilczek et al. 2009; Fournier-Level et al. 2013) and in nearby natural populations (Wilczek et al. 2009). The environmental series used to create the results summarized in this article have been deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.nv0p1 (Burghardt et al. 2014).

Parameterization

All submodels were fit to the common laboratory genotype (Columbia) via chamber experiments or using estimates from the literature (see table 1 for details about our confidence in each parameter value). To determine parameters for the germination model, a common maternal growth environment of 20°C was used. To ensure that seed dispersal and germination submodels were reasonable approximations, we tested each model on an independent data set generated in chambers (L. T. Burghardt, unpublished data; see app. A for details). Because the germination model and afterripening model were the least empirically validated, we ran simulations while varying three crucial parameters to confirm that our main results were not specific to the exact parameterization of those models (see figs. B3, B4 for results).

Parameter Levels That Resemble Observed Genotypic Variation

Although we parameterized the initial model based on the Columbia ecotype, as described above, we compared outcomes using different parameter values that span known natural genetic variation for two key parameters: floral repression and initial dormancy level. This analysis does not investigate other genetic interactions or genetic-background effects because of lack of information on realistic parameter values. For the floral repression parameter (F_i), we used initial values derived from Wilczek et al. (2009). These values corresponded to parameter estimates for strong ($F_i = 0.737$) and null ($F_i = 0.598$) alleles of the *FLC* activator, *FRI*, expressed in the Columbia background in field conditions. We also explored an extremely high F_i level (.88) to mimic some ecotypes that appear to have an almost obligate winter requirement for flowering.

We explored the phenotypic impact of an observed latitudinal dormancy cline. While there is considerable regional variation in dormancy, we chose values that corresponded to the average dormancy level found in each portion of Europe (Atwell et al. 2010; Chiang et al. 2011; Kronholm et al. 2012). We modeled populations at northern, central, and southern latitudes as needing 0, 50, and 100 days, respectively, of dormancy loss at 22°C before 50% germination of the seed cohort. This corresponds to a primary dormancy parameter (Ψ_{mean}) of 0 (low), 1.25 (mid), and 2.5 (high). All populations were assumed to have the same within-cohort variation in dormancy level. The summarized data that underlie figures 3–5 have been deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.nv0p1 (Burghardt et al. 2014).

Results

Model Behavior

Examination of individual model trajectories revealed how the seasonal environment shapes the expression of genetic variation in threshold traits. To illustrate the mechanics of the model, we first present in figure 2 results from a single model run for four different parameterizations/genotypes in Valencia as they respond to the environment after being dispersed on March 10. Immediately, the two low-dormancy genotypes started accumulating progress toward germination, while the high-dormancy ones did not (solid lines vs. black dotted lines, respectively). However, because very little rain occurred in the summer, germination progress of nondormant seeds was minimal while dormant seeds continued to lose dormancy during these dry months. The seasonal environmental context reduced the expression of genetic variation such that seeds with highly disparate dormancy levels germinated only a few weeks

Table 1: Model parameter descriptions and values for the Columbia accession

Submodel, parameter	Units	Level	Description	Confidence[a]
Germination:				
Θ_{germ}	°C MPa	1,000	Threshold for germination	b
Temperature:				
$T_{b.g}$	°C	3	Base temperature for germination	c
T_o	°C	22	Optimal temperature for germination	b
k_T	MPa/°C	.12	Dormancy increase for each °C above T_o	d
Initial dormancy:				
Ψ_{mean}	MPa	0	Mean dormancy (Ψ_b) at dispersal	b
$\Psi_{breadth}$	MPa	1	Difference between lowest and highest dormancy classes	b
cl_{seed}	None	10	No. seed dormancy classes	b
Ψ_{min}	MPa	−1	Minimum dormancy possible	NA
Afterripening				
$T_{b.ar}$	°C	3	Base temperature for afterripening	c
Ψ_{max}	MPa	−5	Maximum moisture for afterripening	d
Ψ_l	MPa	−350	Lower moisture limit for afterripening	d
Ψ_u	MPa	−50	Upper moisture breakpoint for afterripening	d
d_{sat}	Days	40	Days from 0 Ψ_b to −1 Ψ_b	b
Ψ_{scale}	MPa	1	Scalar for Ψ_b loss	d
Flowering:				
$\Theta_{flowering}$	°C h	2,604	Threshold for flowering	a
Temperature:				
$T_{b.f}$	°C	3	Temperature base for flowering	a
Photoperiod:				
d_s	h	10	Critical short photoperiod	a
p_s	None	.626	Rate of development at d_s	a
d_l	h	14	Critical long photoperiod	a
p_l	None	1	Rate of development at d_l	a
Floral repression:				
WC_{sat}	°C	960	Winter chilling saturation point	a
F_i	None	.598	Initial floral repression	a
F_u	None	0	Floral repression at WC_{sat}	a
$T_{v.min}$	°C	−3.5	Temperature minimum for winter chilling	a
$T_{v.max}$	°C	6	Temperature maximum for winter chilling	a
κ	None	−5.1748	Parameters for shape of winter chilling effectiveness function	a
ω	None	2.2256	...	a
ξ	None	.99590	...	a
Seed dispersal:				
$\Theta_{dispersal}$	°C	8,448	Threshold for dispersal	b
Temperature:				
$T_{b.d}$	°C	3	Temperature base for dispersal	b

Note: Parameter levels resembling the Columbia ecotype were used for the submodels of life-stage transitions. Mean dormancy level (Ψ_{mean}) and initial floral repression (F_i) were the variable parameters in this study. Those parameter choices are explained in "Methods." All others were held constant at the values below.

[a] Our confidence in the parameter estimates: a = previously published parameterization on field data and validation with chamber data; b = estimates derived from chamber experiments (L. T. Burghardt, unpublished data) or *A. thaliana* literature search; c = parameter copied from another *A. thaliana* life-stage transition; d = estimates taken from models parameterized for other species; NA = not applicable.

apart in fall (fig. 2*b*). Also note that maternally induced variation in dormancy within a single parameterization (light gray traces surrounding the mean) resulted in larger variation in germination timing in the low-dormancy genotype than in the high-dormancy genotype.

After germination, genotypes that differed in initial floral repression started to diverge, and flowering times spread out across 3 months (fig. 2*c*). However, by the time seed dispersal occurred, genotypic differences again diminished. Because of cooler temperatures during reproduction, early genotypes did not progress much early in the season, so their head start due to earlier flowering was limited. Later in the season, development was faster due to warmer temperatures, allowing later-germinating or later-flowering in-

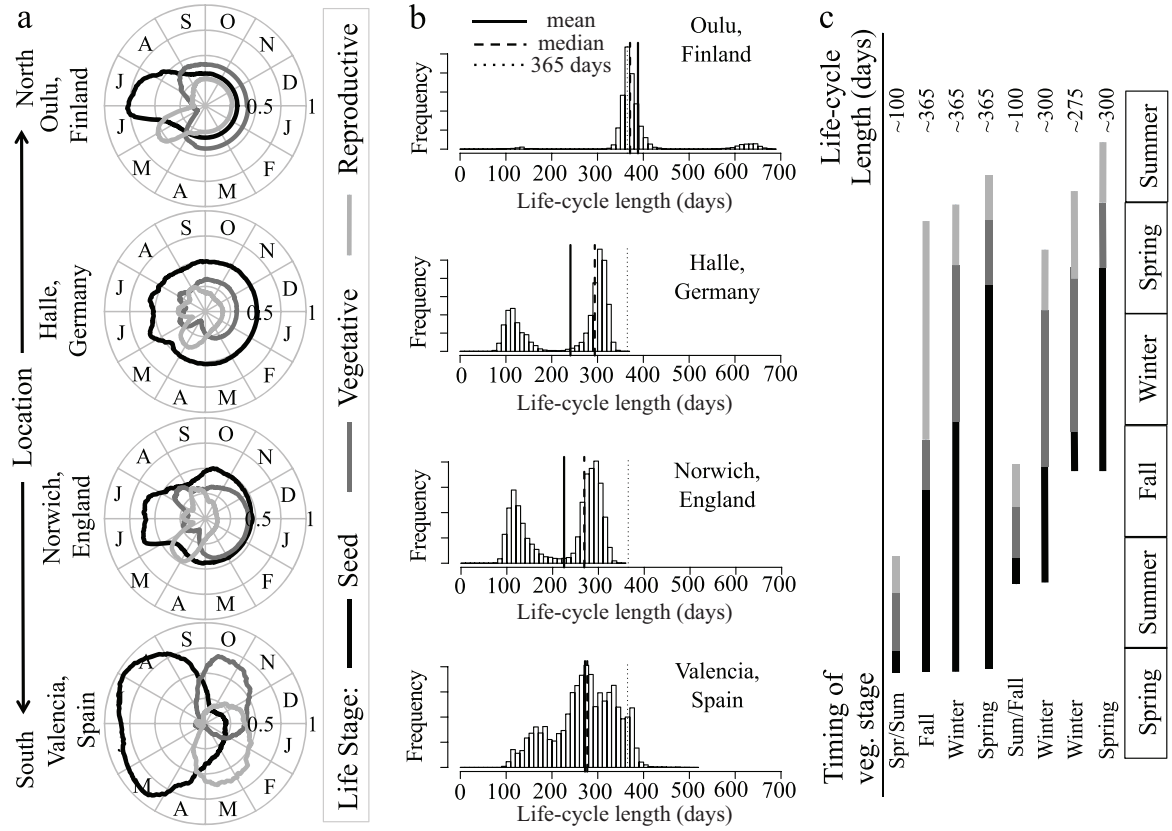

Figure 3: Model results for a genotype with low floral repression and low dormancy in each of four locations across a latitudinal gradient in Europe. *a*, Circle graphs represent the proportion of individuals in each life stage over the course of the year: seed (black), vegetative (dark gray), and reproductive (light gray) life stages. Distance from the center of the circle indicates the proportion of individuals in the life stage at a particular time of year. All graphs are scaled so that the outermost diameter represents 100% of individuals. January 1 starts at 3 o'clock, and the year proceeds clockwise. *b*, Frequency distribution of observed life-cycle lengths in the population. The solid line indicates the average life-cycle length, the dashed line indicates the median, and the dotted line indicates a 365-day life cycle. *c*, Examples of phenological scenarios that occurred as model output that can create the life-cycle lengths graphed in *b*. The length of the bar indicates the proportion of time spent in each life stage. The color code is the same as in *a*. See figure B5, available online, for a color version.

dividuals to catch up in calendar time (fig. 2*d*). Ultimately, seed-dispersal time was fairly synchronous; only the low-dormancy/low–floral repression genotype had a substantially shorter life cycle. Therefore, the seasonal environment can cause compensation among transitions that reduces genotypic differences in generation length.

In addition to the environment reducing differences between genotypes, we also found that it could magnify differences under some conditions. For instance, developmental progress toward flowering is extremely slow in the winter, so small differences in developmental rates between genotypes in the fall can determine whether they flower in the fall or wait up to 9 months for spring to arrive. These differences were also expressed within genotypes, as seeds with different initial dormancy levels may germinate at different times and therefore be at slightly different developmental stages when winter arrives, creating bimodel life-stage lengths (Wilczek et al. 2010). Overall, we found the seasonal environment to be a potent force shaping variation between individuals and genotypes.

Life-Cycle Differences within and between Locations

Next, we present results for a single parameterization (genotype) across all four locations. The parameterization resembling the Columbia accession (see table 1 for parameters describing this genotype) produced remarkable life-cycle variation across European environments (fig. 3; see fig. B6 for life-cycle timing of all genotypes). In Oulu, seeds germinated in late summer, initiated flowering in either the early fall or late spring (see fig. B7 for distribution of flowering times at all sites), and matured seeds in the summer, creating a life cycle with aboveground stages present most of the year. In contrast, in Valencia,

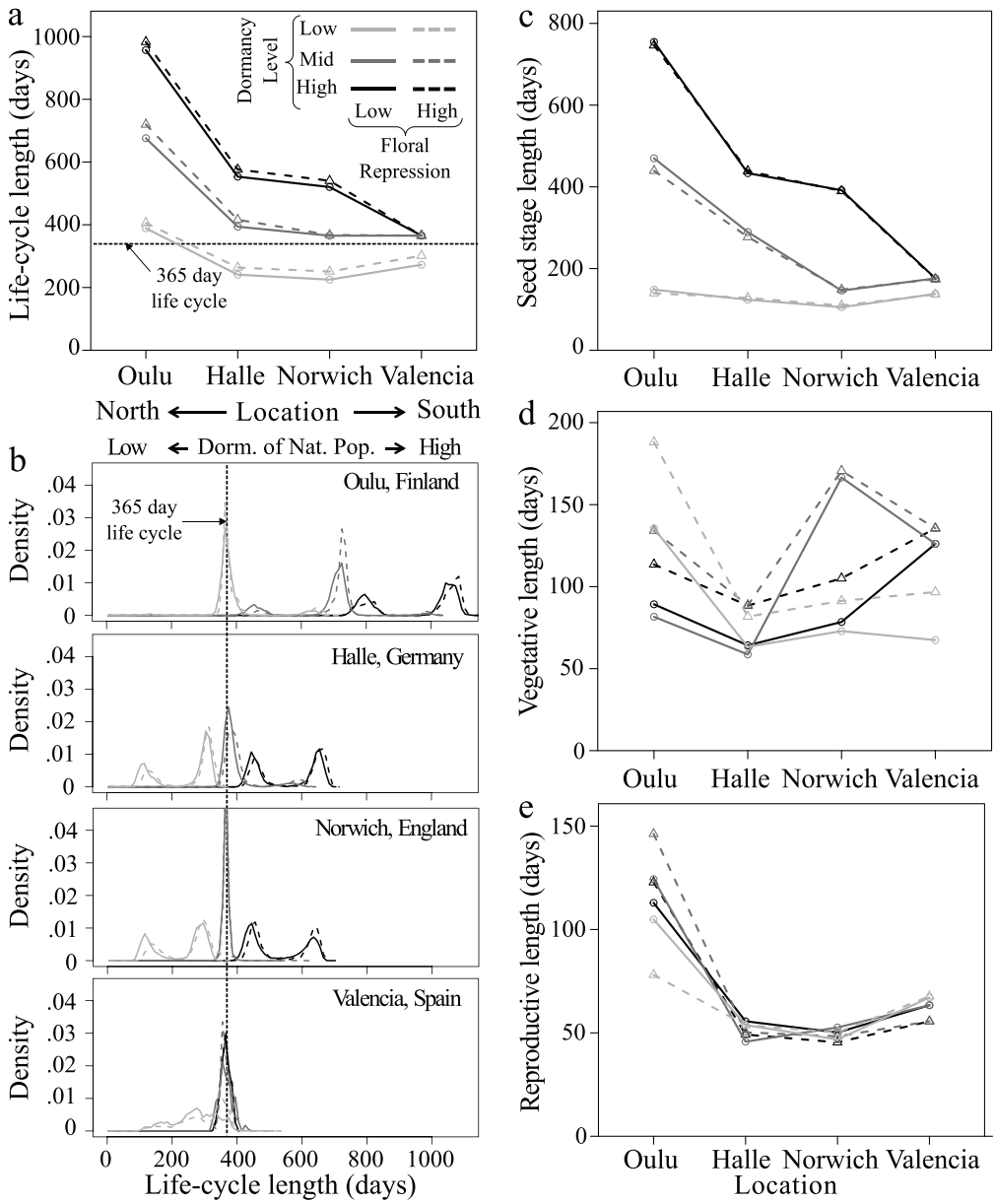

Figure 4: Summary of how primary dormancy level and floral repression level alter life-cycle length. Dormancy level measured in natural populations has been observed to be low in northern Europe and high in southern Europe. *a*, Reaction norms of life-cycle length in response to the four locations. Lines denote different "genotypes" that vary in dormancy level from low to high (black = high, dark gray = mid, light gray = low) and vary in floral repression level from low to high (solid = low, dashed = high). Averages are derived from the last 45 years of a 60-year model run. *b*, Graphs of the density distribution of life-cycle lengths that define the mean values graphed in the reaction norms depicted in *a*. Similar reaction norms for seed stage length (*c*), vegetative length (*d*), and reproductive length (*e*). See figures B14–B17, available online, for color versions of graphs and frequency distributions of *c*–*e*.

overwintering plants spent a large portion of their lives as seeds; they germinated in late fall and flowered in early spring (fig. 3*a*). In Valencia, there were also summer- and fall-flowering cohorts (fig. B6), leading to wide variation in life-cycle length (fig. 3*b*). In central Europe (Halle and Norwich), our model predicted this genotype would have flowering bouts in spring, summer, and fall (fig. 3*a*).

Three flowering bouts occurred in Norwich and Halle, but this is not necessarily because three generations occurred each year. The average life-cycle length was more

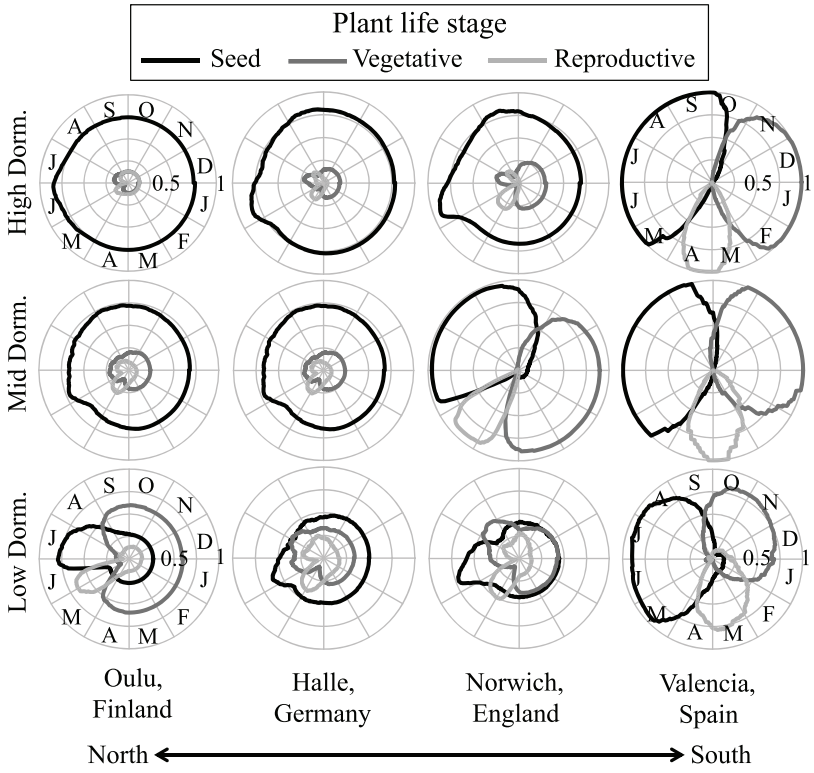

Figure 5: Effect on life-stage phenology of altering dormancy level in a high–floral repression background. Moving from right to left changes location, and moving from top to bottom varies dormancy level. For each graph, distance from the center of the circle indicates the proportion of individuals in a given life stage, January 1 occurs at 3 o'clock, and the year moves clockwise. All graphs are identically scaled so that the outermost diameter represents 100% of individuals. Low–floral repression results differ in small ways and are in figure B5, available online. See figure B19, also online, for a color version.

than 200 days at both of these sites (fig. 3*b*). This suggests that the multiple flowering bouts were created via overlapping generations and not via rapid cycling through three discrete generations. For example, less-dormant seeds dispersed in spring may germinate immediately, producing a summer-flowering cohort with a short life cycle; the more dormant ones may wait until fall to germinate, creating a longer life cycle. In fact, life cycles often fell into these short and long categories, creating a bimodal distribution of life-cycle lengths for a single genotype (fig. 3*b*). A diversity of phenological patterns can lead to similar life-cycle lengths, although all do not occur in all locations (see fig. 3*c* for examples of phenological patterns).

Three factors contributed to the variability in life cycles observed within a location. First, in locations where flowering occurred in multiple seasons, newly dispersed seeds experienced different environmental conditions and therefore germinated at different times of year. Second, differences in initial dormancy level between seeds within a cohort distributed germination events temporally and therefore caused differences in the timing of flowering and seed dispersal. Third, life-cycle variation can be caused by environmental differences between years. For instance, warmer years may yield more flowering in the fall, and wetter years may create larger and differently timed summer cohorts (for examples of between-year variation of this genotype, see figs. B8, B9; for genotypes expected to occur in each location, see figs. B10–B13).

Effects of Varying Phenological Parameters

Next, we examined the effects on life cycle of varying two key parameters that influence flowering time (initial floral repression level) and germination time (initial dormancy level). Both of these parameters are known to exhibit genetic variation among natural populations of *Arabidopsis thaliana*, and the parameter values chosen for this analysis reflect the range of that variation.

Low floral repression had a small effect on total life-cycle length, decreasing it by ~15 days at most locations and dormancy levels (fig. 4*a*) compared to high floral repression. Changes in mean life-cycle length were often

caused both by plants remaining in the vegetative stage longer and by shifts in the proportion of individuals that expressed a short versus long life cycle (figs. B16 and 4*b*, respectively).

Although floral repression had a small effect on total life-cycle length, it did influence the lengths of some individual life stages: it had a large effect on the vegetative interval, which varied by location (fig. 4*d*), and a smaller effect on the reproductive interval (fig. 4*e*; see figs. B15–B17 for frequency distributions for the durations of all component life stages). These results were robust to changes in three important germination model parameters (see figs. B3, B4).

Genotypes with higher initial floral repression produced slightly more individuals expressing a winter-annual life cycle as compared to a spring/summer-annual life cycle, but this effect was highly dependent on dormancy level (fig. B18). We also tested whether a very high ($F_i = 0.88$) floral repression level might create an obligate winter chilling requirement, as found in a few northern (Shindo 2005) and southern (Mendez-Vigo et al. 2011) accessions. While extremely high floral repression levels often led to high proportions of winter rosettes, this was not always the case (see fig. B18, Halle).

Primary dormancy level had a large effect on life-cycle length, and this effect was environment dependent, as shown by the reaction norms of different dormancy genotypes across sites (fig. 4*a*). Overall, dormancy level strongly influenced the length of the life cycle, but differences between dormancy genotypes (parameters) were much greater in Oulu (~600 days) than in Valencia (~100 days). Life-cycle lengths of less than 1 year occurred only at the lowest dormancy level, despite the observation that multiple flowering bouts in a year can occur at many dormancy levels (fig. 5). Dormancy level not only altered the duration spent as a seed but also had ramifying effects on the vegetative and, to a lesser extent, reproductive interval (fig. 4*c*, 4*d*).

The three dormancy levels tested here represent a genetically based, latitudinal cline in primary dormancy documented in this species. When we evaluated genotypes in the locations in which they occur, the model predicted this cline to result in a 365-day life-cycle length at all sites: low dormancy in Oulu (light gray), mid-dormancy in Halle and Norwich (dark gray), and high dormancy in Valencia (black; fig. 4*a*). Therefore, the observed geographic distribution of allelic variation in dormancy level may counteract environmental effects and reduce life-cycle length variation across the species range.

Dormancy also played a crucial role in determining the phenology of life-stage transitions. Different levels of dormancy resulted in a winter-annual life cycle in different locations (defined by the presence of an overwintering rosette; fig. 5). In Oulu, a winter-annual life cycle occurred only at extremely low dormancy levels and was more pronounced with high floral repression levels (figs. B6, B18). In Valencia, winter annuals occurred across all dormancy and floral repression levels (figs. 5, B18) but were only limited to that life cycle at higher dormancy levels (fig. B18).

In Norwich, the mid-dormancy level canalized the life cycle to that of a winter annual; all seeds lost dormancy and germinated during the fall, leaving no individuals to germinate in the spring (figs. 5, B18). However, in Halle, that same parameterization did not lead to a canalized winter-annual life cycle because the high dormancy caused progressively later germination each year, leading to a gradual change in the life cycle from a winter annual to a summer annual over the course of the simulation (fig. B13; this phenological instability also occurred at a few other location/parameter combinations). Lowering the dormancy level from Ψ_{mean} of 1.25 to 0.875 (50 vs. 35 days till 50% germination), however, did canalize a winter-annual life cycle. Thus, small differences in environment (e.g., between Norwich and Halle) can lead to dramatic differences in life-cycle phenology even if life-cycle length is predicted to remain unchanged.

Comparing Model Results to Observed Life-Cycle Variation

Because we know the parameter combinations that often occur in natural populations at each location, we next compared our predictions to known life cycles of natural populations. In the far north, many populations have high initial floral repression and low dormancy (Atwell et al. 2010; Brachi et al. 2010). The model predicted that this parameterization in Oulu would result in a rosette-dominated winter-annual life cycle (fig. 5, *bottom left*), matching observations of populations near Oulu. If floral repression levels were low, many individuals flowered in the fall due to the strong promotion of flowering by long photoperiods. Thus, in northern Europe, high floral repression may prevent fall flowering.

In lowland Spain, natural accessions are most often strongly dormant (Atwell et al. 2010; Chiang et al. 2011) and have high initial floral repression (Mendez-Vigo et al. 2011). In our simulations, this parameterization resulted in a seed-dominated, winter-annual life cycle (fig. 5, *top right*) similar to that observed in Spanish populations. Low dormancy resulted in earlier germination and flowering, with a small proportion of the population completing a generation in the summer, while decreases in floral repression led to progressively earlier flowering in the winter (fig. B6).

At middle latitudes, variation in floral repression and dormancy parameters is large (Le Corre 2005; Atwell et

al. 2010; Brachi et al. 2010). In some locations, canalized winter-annual life cycles are observed in natural populations, and in others, multiple flowering bouts occur per year. The mid-dormancy level we tested predicted a 365-day life cycle in both Halle and Norwich, but the phenology was that of a winter annual only in Norwich. At low dormancy levels, multiple flowering bouts (and multiple generations) were predicted, but mixtures of life-cycle phenologies can occur in a calendar year even at high dormancy levels (fig. 5). Thus, we predict that both winter-annual and multiple-flowering-bout life cycles are possible at middle latitudes and that dormancy parameters will be key determinants of life-cycle expression.

Discussion

Despite extensive knowledge of genetic variation in *Arabidopsis thaliana*, very little is known about how this variation is manifest as phenotypic variation across the species' range. We used a model to predict reaction norms for specific *A. thaliana* genotypes in response to complex environments. The model predicted wide variation in life-cycle phenology across locations and parameterizations, and the predictions for genotypes known to exist in each location broadly matched known patterns of phenology in situ. This result suggests that systems of environmentally regulated phenology are highly effective at restricting life stages to occur only at particular times of year even without seasonal entrainment by mortality and fecundity processes. Life-cycle length was shaped by environmental conditions, initial primary dormancy level, and, to a lesser extent, initial floral repression. The model also predicted that a known genetic cline in dormancy is expected to interact with local environmental conditions in a manner that reduces variation in generation time across the latitudinal range of *A. thaliana*.

Life-Cycle Plasticity of A. thaliana

Diverse environmental conditions (temperature, moisture, and photoperiod) across *A. thaliana*'s range strongly influenced phenology and generation time. The environmental sensitivity (plasticity) of this species not only generated life-cycle variation between locations, it also generated for a single genotype mixtures of life cycles within a location. These phenological mixtures occurred across a diversity of life-cycle lengths. In all locations, the model predicted that some individuals would germinate in the fall and flower in the spring (an overwintering life cycle); however, their offspring did not necessarily express that same life cycle. For instance, because of within-cohort dormancy variation, some might germinate late in the spring, in which they were dispersed, and others may wait until the following fall. The idea that a single genotype can produce mixtures of life cycles is supported empirically in *A. thaliana* populations (Thompson 1994; Donohue 2009; Montesinos-Navarro et al. 2012; Pico 2012) and has been suggested as a bet-hedging strategy in other systems (Bradford and Roff 1997; He et al. 2010). Understanding the causes and adaptive significance of this variation presents a compelling challenge for future research.

Predicted Effects of Allelic Variation

Overall, initial floral repression level (resembling allelic variation in *FRIGIDA/FLC*) had a smaller effect on life-cycle length than dormancy, partly because longer vegetative periods were compensated for by shorter reproductive periods. Floral repression did, however, alter the amount of time spent in the vegetative stage. Increasing floral repression shifted a portion of the population to a winter-annual life cycle, but unless floral repression levels were extremely high, it rarely canalized that life cycle. Therefore, our results do not support the hypothesis that floral repression levels similar to those typically observed in natural populations are capable of canalizing a winter-annual life cycle across the range of *A. thaliana* (Simpson and Dean 2002; Michaels et al. 2003) or that reduced floral repression has repeatedly evolved to create rapid-cycling populations (Toomajian et al. 2006). On a cautionary note, we tested only one parameter related to winter chilling: initial floral repression. It is possible that other parameters not included in the model, such as time to winter chilling saturation (Shindo 2005) or temporal dynamics of vernalization (Chew et al. 2012), could contribute to observed clinal variation in flowering time in natural populations (Caicedo 2004; Stinchcombe et al. 2005) and influence life cycles to a larger extent.

Our results add further support to mounting evidence in this species that dormancy levels influence life-cycle phenology and length (Chiang et al. 2012; Montesinos-Navarro et al. 2012; Pico 2012; Footitt et al. 2013). Initial primary dormancy level strongly influenced the life cycle expressed within a population and the number of generations completed in a year. Further, our models agree with the proposition that some genotypes of *A. thaliana* may have generation times of up to 3–4 years in northern Europe (Lundemo et al. 2009). Because of the short growing season in Oulu, relatively low dormancy levels can still result in generation times longer than 1 year, despite the fact that we are likely underestimating life-cycle length by focusing on seed dynamics on the soil surface (i.e., we model only physiologically dormant seeds, not seeds that are dormant because of burial).

Finally, our results suggest that the observed north-to-south dormancy cline in *A. thaliana* may buffer the effect

of environmental variability on life-cycle length (Conover and Schultz 1995; for examples, see Arendt and Wilson 1999; Colautti et al. 2009). The dormancy cline is predicted to produce an annual life cycle at all four sites, reducing differences in life-cycle length across environments. Recently, latitudinal (Wagmann et al. 2012) and altitudinal (Fernández-Pascual et al. 2013) dormancy clines in the same direction have been found in other species, suggesting that this may be a common mechanism of life-cycle control across latitudes. In sum, careful work on dormancy in this species may untangle the causes of life-cycle variation.

Model Applications

The modeling framework we demonstrate here can create predictions of which environments reveal and which environments mask genetic differences, providing an unusual tool for predicting environment-dependent genotypic effects. This is important because the environment has been found to strongly influence the phenotypic consequence of allelic variation. For instance, in *A. thaliana*, many flowering and germination quantitative trait loci are environment specific, both in controlled environments (Atwell et al. 2010; Huang et al. 2010; Li et al. 2010) and field experiments (Wilczek et al. 2009; Ågren and Schemske 2012; Fournier-Level et al. 2013). In this study, we predict allelic variation in primary dormancy level to have a larger phenotypic effect in northern sites than in southern sites. By predicting multiple phenotypes, this approach could also aid in understanding geographic patterns of covariation among traits and their relationship with environmental variation. For instance, modeling may help understand the recently described latitudinally dependent relationships among flowering time, seed dormancy, and growth rate in this species (Debieu et al. 2013).

This approach can also predict pleiotropic effects of allelic/parameter changes on subsequent life-stage transitions. Pleiotropy occurs when a single allele influences more than one trait. While this can occur through a direct effect of the gene on both traits, it can also occur because one trait changes the environment that determines a subsequent plastic trait. This environmentally induced pleiotropy (Donohue 2014) is accommodated naturally by the structure of the integrated model. For instance, the model of *A. thaliana* predicts that changes in seed dormancy will alter the amount of time spent in all three life stages and further suggests that reductions in reproductive period will compensate for increases in vegetative period due to increasing floral repression levels. Such pleiotropy and compensatory responses between life-stage lengths were reported from a recent field experiment with *A. thaliana* (Chiang et al. 2012).

Finally, these models are well suited to encapsulate individual-level trait variation, as we do here by including variance in initial dormancy level. Such variation can play an important role in organismal success, particularly in response to stochastic environmental variation (Brown and Venable 1986; Simons and Johnston 2006). In sum, integrating phenology models provides novel perspectives on the relationship between genes and environment.

Future Directions

The phenological models implemented here are intended to be part of an iterative modeling process; as knowledge of underlying developmental processes and background-specific effects grows, they will be refined and improved. In fact, the flowering model used here has already been augmented to include additional genetic effects (Chew et al. 2012), although these additions do not qualitatively change our results. While several processes that may play a role in life cycles were omitted here, starting from simpler models facilitates interpretation and creates a null baseline for comparison with more complex versions.

As we move forward, the next step is to augment the germination formulations, test model predictions empirically under seasonally variable conditions using genotypes that differ in physiological sensitivities, and explore genotypic fitness given environment-dependent survival and fecundity. Hypotheses suggested by the model can be tested with near-isogenic lines in the Columbia background with alleles that alter flowering or germination timing introgressed. In particular, explorations of how secondary dormancy cycling (i.e., mechanisms that change dormancy levels based on the environment after dispersal) affects phenology may be particularly informative. Finally, because our current knowledge of seed dynamics in this species is extremely limited, the fit of the model is mostly assessed via behavior of aboveground life stages. Careful demographic work that links dispersal and germination phenotypes will be critical to future refinements.

This model does not address the potential influence of biotic interactions on the expression of phenology (Elzinga et al. 2007; Revilla et al. 2014), and this offers a rich area for future development. However, as suggested by Wolkovich et al. (2013), plants often use abiotic cues to synchronize or avoid interactions with others, so modeling effects of abiotic factors could provide information on their corresponding consequences for biotic interactions (Brachi et al. 2012). Ultimately, incorporating explicit intraspecific density-dependent processes would be necessary to fully explore these dynamics, and species interactions could be addressed by linking ILC models of different species.

The general integrated approach of linking phenology

models across life stages could be applied to any organism whose life cycle is regulated by environmental factors. We chose to model an organism that can be easily experimentally manipulated and for which much genetic information exists, but agronomists have built successful models of phenology for numerous plant and insect species without detailed genetic knowledge. Because of this flexibility, the integrated modeling approach could be used to predict population or species responses to future climatic conditions or to aid predictions of which seasonal environmental factors are most likely to influence particular life stages. Finally, because experiments studying whole life cycles are extremely time-consuming and challenging, using a modeling approach first could suggest hypotheses that can then be targeted for testing in the field.

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Literature Cited

Ågren, J., and D. W. Schemske. 2012. Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. New Phytologist 194:1112–1122.

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1:95–111.

Alvarado, V., and K. J. Bradford. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. Plant, Cell and Environment 25:1061–1069.

Anderson, J. T., C. Lee, and T. Mitchell-Olds. 2011. Life-history QTLs and natural selection on flowering time in *Boechera stricta*, a perennial relative of *Arabidopsis*. Evolution 65:771–787.

Andrés, F., and G. Coupland. 2012. The genetic basis of flowering responses to seasonal cues. Nature Reviews Genetics 13:627–639.

Arendt, J. D., and D. S. Wilson. 1999. Countergradient selection for rapid growth in pumpkinseed sunfish. Ecology 80:2793–2798.

Atwell, S., Y. S. Huang, B. J. Vilhjálmsson, G. Willems, M. Horton, Y. A. N. Li, D. Meng, et al. 2010. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. Nature 465:627–631.

Bastow, R., J. S. Mylne, C. Lister, and Z. Lippman. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. Nature 427:164–167.

Blackman, B. K., S. D. Michaels, and L. H. Rieseberg. 2011. Connecting the sun to flowering in sunflower adaptation. Molecular Ecology 20:3503–3512.

Brachi, B., C. Aimé, C. Glorieux, J. Cuguen, and F. Roux. 2012. Adaptive value of phenological traits in stressful environments: predictions based on seed production and laboratory natural selection. PLoS ONE 7:e32069.

Brachi, B., N. Faure, M. Horton, E. Flahauw, A. Vazquez, M. Nordborg, J. Bergelson, J. Cuguen, and F. Roux. 2010. Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genetics 6:e1000940.

Bradford, M., and D. A. Roff. 1997. An empirical model of diapause strategies of the cricket *Allonemobius socius*. Ecology 78:442–451.

Bradshaw, A. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13:115–155.

Brown, J. S., and D. L. Venable. 1986. Evolutionary ecology of seed-bank annuals in temporally varying environments. American Naturalist 127:31–47.

Buckley, L. B., and J. G. Kingsolver. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate change. Annual Review of Ecology, Evolution, and Systematics 43:205–226.

Burghardt, L. T., C. J. E. Metcalf, A. M. Wilczek, J. Schmitt, and K. Donohue. 2014. Data from: Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.nv0p1.

Caicedo, A. L. 2004. Epistatic interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal cline in a life-history trait. Proceedings of the National Academy of Sciences of the USA 101:15670–15675.

Cannell, M. G. R., and R. I. Smith. 1983. Thermal time, chill days and prediction of budburst in *Picea sitchensis*. Journal of Applied Ecology 20:951–963.

Caswell, H. 1983. Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences. American Zoologist 23:35–46.

Chew, Y. H., A. M. Wilczek, M. Williams, S. M. Welch, J. Schmitt, and K. J. Halliday. 2012. An augmented *Arabidopsis* phenology model reveals seasonal temperature control of flowering time. New Phytologist 194:654–665.

Chiang, G. C. K., M. Bartsch, D. Barua, K. Nakabayashi, M. Debieu, I. Kronholm, M. Koornneef, W. J. J. Soppe, K. Donohue, and J. de Meaux. 2011. *DOG1* expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. Molecular Ecology 20:3336–3349.

Chiang, G. C. K., D. Barua, E. Dittmar, E. M. Kramer, R. R. de Casas, and K. Donohue. 2012. Pleiotropy in the wild: the dormancy gene *DOG1* exerts cascading control on life cycles. Evolution 67:883–893.

Chuine, I. 2000. A unified model for budburst of trees. Journal of Theoretical Biology 207:337–347.

Chuine, I., and E. G. Beaubien. 2001. Phenology is a major determinant of tree species range. Ecology Letters 4:500–510.

Colautti, R. I., J. L. Maron, and S. C. H. Barrett. 2009. Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. Evolutionary Applications 2:187–199.

Conover, D. O., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecology and Evolution 10:248–252.

Debieu, M., C. Tang, B. Stich, T. Sikosek, S. Effgen, E. Josephs, J. Schmitt, M. Nordborg, M. Koornneef, and J. de Meaux. 2013. Co-variation between seed dormancy, growth rate and flowering time changes with latitude in *Arabidopsis thaliana*. PLoS ONE 8:e61075.

Dennis, E. S., and W. J. Peacock. 2007. Epigenetic regulation of flowering. Current Opinion in Plant Biology 10:520–527.

Donohue, K. 2009. Completing the cycle: maternal effects as the missing link in plant life cycles. Philosophical Transactions of the Royal Society B: Biological Sciences 364:1059–1074.

———. 2014. Why ontogeny matters during adaptation: developmental niche construction and pleiotropy across the life cycle in *Arabidopsis thaliana*. Evolution 68:32–47.

Donohue, K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. R. Polisetty, and J. Schmitt. 2005. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evolution 59:758–770.

Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, postgermination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics 41:293–319.

Ducousso, A., J. P. Guyon, and A. Kremer. 1996. Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (*Quercus petraea* (Matt) Liebl). Annals of Forest Science 53: 775–782.

Elzinga, J. A., A. Atlan, A. Biere, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. Trends in Ecology and Evolution 22:432–439.

Fernández-Pascual, E., B. Jiménez-Alfaro, J. Caujapé-Castells, R. Jaén-Molina, and T. E. Díaz. 2013. A local dormancy cline is related to the seed maturation environment, population genetic composition and climate. Annals of Botany 112:937–945.

Finch-Savage, W. E., and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. New Phytologist 171:501–523.

Footitt, S., Z. Huang, H. A. Clay, A. Mead, and W. E. Finch-Savage. 2013. Temperature, light and nitrate sensing coordinate *Arabidopsis* seed dormancy cycling, resulting in winter and summer annual phenotypes. Plant Journal 74:1003–1015.

Forrest, J., and A. J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3101–3112.

Fournier-Level, A., A. M. Wilczek, M. D. Cooper, J. L. Roe, J. Anderson, D. Eaton, B. T. Moyers, et al. 2013. Paths to selection on life-history loci in different natural environments across the native range of *Arabidopsis thaliana*. Molecular Ecology 22:3552–3566.

Galloway, L. F., and K. S. Burgess. 2009. Manipulation of flowering time: phenological integration and maternal effects. Ecology 90: 2139–2148.

Graeber, K., K. Nakabayashi, E. Miatton, G. Leubner-Metzger, and W. J. J. Soppe. 2012. Molecular mechanisms of seed dormancy. Plant, Cell and Environment 35:1769–1786.

Gummerson, R. J. 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. Journal of Experimental Botany 37:729–741.

Hammer, G. L., S. Chapman, E. van Oosterom, and D. W. Podlich. 2005. Trait physiology and crop modelling as a framework to link phenotypic complexity to underlying genetic systems. Australian Journal of Agricultural Research 56:947.

He, X. Z., Q. Wang, J. T. S. Walker, D. J. Rogers, and P. L. Lo. 2010. A sophisticated life-history strategy in a parasitoid wasp: producing univoltine and multivoltine phenotypes in a local population. Biological Control 54:276–284.

Hoogenboom, G., J. W. White, J. W. Jones, and K. J. Boote. 1994. BEANGRO: a process-oriented dry bean model with a versatile user interface. Agronomy Journal 86:182–190.

Huang, X., J. Schmitt, L. Dorn, C. Griffith, S. Effgen, S. Takao, M. Koornneef, and K. Donohue. 2010. The earliest stages of adaptation in an experimental plant population: strong selection on QTLs for seed dormancy. Molecular Ecology 19:1335–1351.

Hunter, A. F., and M. J. Lechowicz. 1992. Predicting the timing of budburst in temperate trees. Journal of Applied Ecology 29:597–604.

Kimball, S., A. L. Angert, T. E. Huxman, and D. L. Venable. 2010. Contemporary climate change in the Sonoran Desert favors cold-adapted species. Global Change Biology 16:1555–1565.

Koornneef, M., C. Alonso-Blanco, and D. Vreugdenhil. 2004. Naturally occurring genetic variation in *Arabidopsis thaliana*. Annual Review of Plant Biology 55:141–172.

Kronholm, I., F. X. Pico, C. Alonso-Blanco, J. Goudet, and J. de Meaux. 2012. Genetic basis of adaptation in *Arabidopsis thaliana*: local adaptation at the seed dormancy QTL *DOG1*. Evolution 66: 2287–2302.

Laugen, A. T., A. Laurila, K. Rasanen, and J. Merila. 2003. Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates: evidence for local adaptation. Journal of Evolutionary Biology 16:996–1005.

Lawrence, M. J. 1976. Variation in natural populations of *Arabidopsis thaliana* (L.) Heynh. Pages 167–190 *in* J. G. Vaughan, A. J. MacLeod, and B. M. G. Jones, eds. Conference on the biology of the Cruciferae. Academic Press, London.

Le Corre, V. 2005. Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. Molecular Ecology 14:4181–4192.

Levins, R. 1969. Thermal acclimation and heat resistance in *Drosophila* species. American Naturalist 103:483–499.

Li, Y., Y. Huang, J. Bergelson, M. Nordborg, and J. O. Borevitz. 2010. Association mapping of local climate-sensitive quantitative trait loci in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the USA 107:21199–21204.

Long, Y., J. Shi, D. Qiu, R. Li, C. Zhang, J. Wang, J. Hou, et al. 2007. Flowering time QTL analysis of oilseed *Brassica* in multiple environments and genome-wide alignment with *Arabidopsis*. Genetics 177:2433–2444.

Lundemo, S., M. Falahati-Anbaran, and H. K. Stenoien. 2009. Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in northern Europe. Molecular Ecology 18:2798–2811.

Mendez-Vigo, B., F. X. Pico, M. Ramiro, J. M. Martinez-Zapater, and C. Alonso-Blanco. 2011. Altitudinal and climatic adaptation is mediated by flowering traits and *FRI*, *FLC*, and *PHYC* genes in *Arabidopsis*. Plant Physiology 157:1942–1955.

Michaels, S. D., Y. He, K. C. Scortecci, and R. M. Amasino. 2003. Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. Proceedings of the National Academy of Sciences of the USA 100:10102–10107.

Montesinos-Navarro, A., F. X. Pico, and S. J. Tonsor. 2012. Clinal variation in seed traits influencing life-cycle timing in *Arabidopsis thaliana*. Evolution 66:3417–3431.

Montesinos-Navarro, A., J. Wig, F. X. Pico, and S. J. Tonsor. 2010. *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. New Phytologist 189:282–294.

Morin, X., C. Augspurger, and I. Chuine. 2007. Process-based mod-

eling of species' distributions: what limits temperate tree species range boundaries? Ecology 88:2280–2291.
Morin, X., D. Viner, and I. Chuine. 2008. Tree species range shifts at a continental scale: new predictive insights from a process-based model. Journal of Ecology 96:784–794.
Munguía-Rosas, M. A., J. Ollerton, V. Parra-Tabla, and J. A. De-Nova. 2011. Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. Ecology Letters 14:511–521.
Olson, M. S., N. Levsen, R. Y. Soolanayakanahally, R. D. Guy, W. R. Schroeder, S. R. Keller, and P. Tiffin. 2012. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. Molecular Ecology 22:1214–1230.
Paaby, A. B., M. J. Blacket, A. Hoffmann, and P. S. Schmidt. 2010. Identification of a candidate adaptive polymorphism for *Drosophila* life history by parallel independent clines on two continents. Molecular Ecology 19:760–774.
Pico, F. X. 2012. Demographic fate of *Arabidopsis thaliana* cohorts of autumn- and spring-germinated plants along an altitudinal gradient. Journal of Ecology 100:1009–1018.
Pigliucci, M. 2002. Ecology and evolutionary biology of *Arabidopsis*. *Arabidopsis* Book 22:1.
Post, E. S., C. Pedersen, C. C. Wilmers, and M. C. Forchhammer. 2008. Phenological sequences reveal aggregate life-history response to climatic warming. Ecology 89:363–370.
R Development Core Team. 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
Ratcliffe, D. 1961. Adaptation to habitat in a group of annual plants. Journal of Ecology 49:187–203.
Revilla, T. A., F. Encinas-Viso, and M. Loreau. 2014. (A bit) earlier or later is always better: phenological shifts in consumer-resource interactions. Theoretical Ecology 7:149–162.
Roff, D. 1980. Optimizing development time in a seasonal environment: the "ups and downs" of clinal variation. Oecologia (Berlin) 45:202–208.
Roff, D. A. 2002. Life history evolution. Sinauer, Sunderland, MA.
Saarinen, T., R. Lundell, H. Åström, and H. Hänninen. 2011. Parental overwintering history affects the responses of *Thlaspi arvense* to warming winters in the North. Environmental and Experimental Botany 72:409–414.
Satake, A. 2010. Diversity of plant life cycles is generated by dynamic epigenetic regulation in response to vernalization. Journal of Theoretical Biology 266:595–605.
Satake, A., T. Kawagoe, Y. Saburi, Y. Chiba, G. Sakurai, and H. Kudoh. 2013. Forecasting flowering phenology under climate warming by modelling the regulatory dynamics of flowering-time genes. Nature Communications 4:1–8.
Sheldon, C. C., D. T. Rouse, E. J. Finnegan, W. J. Peacock, and E. S. Dennis. 2000. The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). Proceedings of the National Academy of Sciences of the USA 97:3753–3758.
Shindo, C. 2005. Role of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of *Arabidopsis*. Plant Physiology 138:1163–1173.
Simons, A. M., and M. O. Johnston. 2006. Environmental and genetic sources of diversification in the timing of seed germination: implications for the evolution of bet hedging. Evolution 60:2280–2292.
Simpson, G. G., and C. Dean. 2002. *Arabidopsis*, the Rosetta stone of flowering time? Science 296:285–289.
Stinchcombe, J. R., A. L. Caicedo, R. Hopkins, C. Mays, E. W. Boyd, M. D. Purugganan, and J. Schmitt. 2005. Vernalization sensitivity in *Arabidopsis thaliana* (Brassicaceae): the effects of latitude and *FLC* variation. American Journal of Botany 92:1701–1707.
Stoeckli, S., M. Hirschi, C. Spirig, P. Calanca, M. W. Rotach, and J. Samietz. 2012. Impact of climate change on voltinism and prospective diapause induction of a global pest insect—*Cydia pomonella* (L.). PLoS ONE 7:e35723.
Sultan, S. E. 2000. Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5:537–542.
Sung, S., and R. M. Amasino. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427:159–164.
Thompson, L. 1994. The spatiotemporal effects of nitrogen and litter on the population dynamics of *Arabidopsis thaliana*. Journal of Ecology 82:63–68.
Toomajian, C., T. T. Hu, M. J. Aranzana, C. Lister, C. Tang, H. Zheng, K. Zhao, P. Calabrese, C. Dean, and M. Nordborg. 2006. A nonparametric test reveals selection for rapid flowering in the *Arabidopsis* genome. PLoS Biology 4:e137.
Wagmann, K., N.-C. Hautekèete, Y. Piquot, C. Meunier, S. E. Schmitt, and H. Van Dijk. 2012. Seed dormancy distribution: explanatory ecological factors. Annals of Botany 110:1205–1219.
Wang, J. Y. 1960. A critique of the heat unit approach to plant response studies. Ecology 41:785–790.
Welch, S. M., Z. Dong, J. L. Roe, and S. Das. 2005. Flowering time control: gene network modelling and the link to quantitative genetics. Australian Journal of Agricultural Research 56:919–936.
White, J. W., and G. Hoogenboom. 1996. Simulating effects of genes for physiological traits in a process-oriented crop model. Agronomy Journal 88:416–422.
Wilczek, A. M., L. T. Burghardt, A. R. Cobb, M. D. Cooper, S. M. Welch, and J. Schmitt. 2010. Genetic and physiological bases for phenological responses to current and predicted climates. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3129–3147.
Wilczek, A. M., J. L. Roe, M. C. Knapp, M. D. Cooper, C. Lopez-Gallego, L. J. Martin, C. D. Muir, et al. 2009. Effects of genetic perturbation on seasonal life-history plasticity. Science 323:930–934.
Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. Proceedings of the National Academy of Sciences of the USA 105:17029–17033.
Wolkovich, E. M., B. I. Cook, and T. J. Davies. 2013. Progress towards an interdisciplinary science of plant phenology: building predictions across space, time and species diversity. New Phytologist 201:1156–1162.
Xinyou, Y., M. J. Kropff, H. Nakagawa, and T. Horie. 1997. A model for photothermal responses of flowering in rice. II. Model evaluation. Field Crops Research 51:201–211.
Zhao, M., C. Peng, W. Xiang, X. Deng, D. Tian, X. Zhou, G. Yu, H. He, and Z. Zhao. 2013. Plant phenological modeling and its application in global climate change research: overview and future challenges. Environmental Reviews 21:1–14.

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