The Influence of Genetic and Environmental Factors on the Phenology and Life-Cycle Expression of *Arabidopsis thaliana*

by

Liana Twardosz Burghardt

Department of Biology
Duke University

Date:_______________________

Approved:

___________________________
Kathleen Donohue, Supervisor

___________________________
Mark D. Rausher

___________________________
Sonke Johnsen

___________________________
Justin P. Wright

___________________________
William F. Morris

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School of Duke University

2015
ABSTRACT

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Abstract
This dissertation examines the processes that generate phenotypic variation in life cycles in seasonal environments. Collectively, a life cycle describes the stages an organism passes through during a generation. The timing, or phenology, of these transitions is often influenced by both environmental and allelic variation. Using the model organism Arabidopsis thaliana and both empirical and modeling approaches, I examine how correlations between life-cycle transitions, environment-dependent allelic effects, and epistasis generate patterns of life-cycle variation both within and between generations. In my first chapter, I use experiments to determine that many combinations of genetic, environmental, and developmental factors can create similar germination phenotypes, that maternal effects can influence phenotypes more than genetic differences, and that cross-generational effects can reduce variation in germination timing despite variation in flowering and dispersal time. In my second chapter, I use a modeling approach to consider the entire life cycle. I find that environmental variation is a major driver of phenotypic variation, and that considering the known geographic distribution of allelic variation across the range improves the match of model predictions to phenotypes expressed in natural populations. Specifically, variation in dormancy generated in the previous generation is predicted to cause life-cycle differences within a location, and the geographic distribution of allelic variation in
dormancy interacts with local climatic environments to canalize an annual life history across the range. Finally, I test if allelic and environmental variation that affects early life stages can influence the environment experienced during reproduction. This environment determines both the time available for reproduction and the environment experienced during senescence. By implementing simple survival rules for flowering plants in the model, I show that time available for a plant to reproduce depends on earlier phenological traits and varies widely from year to year, location to location, and genotype to genotype. If reproductive trade-offs that underlie the evolution of senescence are environmentally sensitive, these results suggest that genetic variation in earlier life-stage transitions might shape senescence rates and whether they are environmentally responsive. In sum, my dissertation demonstrates the importance of pleiotropy, environment-dependent allelic expression, and epistasis in defining life-cycle variation, and proposes a novel way of predicting these relationships and complex life cycles under seasonal conditions.
Dedication

To my father for always having the highest of expectations, to my mother for instilling a sense of the wonder in the world, and to my sister for her understated honesty and insight.
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Introduction

BROAD OVERVIEW

Collectively a life cycle describes the series of stages an organism progresses through during a generation. Historically, much work has been devoted to describing the dizzying diversity of life cycles across the tree of life: from those species that pass through only one or two life-stages that inhabit similar environments to species with complex life cycles where multiple, distinct life stages inhabit disparate abiotic or biotic contexts. Examples of complex life cycles include jellyfish that progress through both free floating larval stages and sessile polyp stages before becoming adults and amphibians and many insects that have both aquatic and terrestrial life stages.

While life-cycle stages are often well described, there has been a dearth of work examining the timing, or phenology, of life stages within the whole life cycle (Bell 2011). Specifically, as organisms grow and develop, what factors shape when organisms transition from life stage to life stage? This gap leaves many questions open for investigation. Can the evolution of one transition influence the timing, or expression, of later transitions? How does the timing of the life cycle vary across the range of a species? What is the relative importance of genes and the environment to determining life-cycle expression? How will climate change and long-distance/human-mediated dispersal influence life cycles? And ultimately, how does changing life-cycle timing change fitness? Further, despite their cyclical nature, life cycles are often studied linearly in
ways that de-emphasize connections between generations (Donohue 2009). How important are these trans-generational connections for life-cycle timing?

In addition to the compelling evolutionary and ecological motivations for studying life-cycle phenology, there are also practical and social reasons for pursuing these avenues of research. Understanding how life-stage transitions are influenced by the environment, influence each other, and evolve will be critical for predicting how species will respond to climate change. This is particularly salient given that humans are changing organismal environments both temporally through greenhouse gas emissions and spatially through massive species introductions. Further, research on life-cycle phenology has many applications such as timing the sowing of crops, defining breeding programs in novel environmental conditions (Hoogenboom and White 1994), and predicting the behavior of insect pests that threaten crop yields with increasing numbers of generations per year (Stoeckli 2012). Additionally, an increased ability to predict life-cycle phenology could augment niche modeling used for conservation planning by providing information on life-stage specific niches (Donohue et al. 2014) and allow for the incorporation of allelic variation and plasticity into predictions of potential responses to change (Valladares et al. 2014).

Fundamentally, my dissertation investigates the processes that generate and limit variation in life-cycle timing both within and between locations. I address the following specific questions about sources of variation in life cycles:
1) In seasonal environments, how does the timing of earlier life-stage transitions shape the timing of later life-stage transitions both within and between generations?

2) How does allelic variation for life-cycle transitions and environmental variation combine to influence the synchrony and geographic distribution of life cycles?

3) How do seasonal environments and genetic variation for life-cycle transitions influence the environment experienced during reproduction and how does that influence the time available for reproduction and other traits such as senescence?

I use the model plant *Arabidopsis thaliana* to answer these questions because the behavior of individual life-stage transitions in response to environmental factors is well characterized. However, as with most organisms, comparatively little is known about the temporal expression of the whole life cycle.

I argue throughout my dissertation that three processes are of critical importance to the expression of life-cycle variation in seasonal environments: pleiotropy (Figure 1a), environment-dependent allelic expression (GxE; Figure 1b), and epistasis (GxG; Figure 1c). These processes will also generate correlations between environment, phenotypes, and fitness (Figure 1d) that will shape the evolution of life cycles. The design of experiments and creation of methods that allow prediction of these processes will be vital to understanding the expression and evolution of life cycles moving forward and is thus a major emphasis of my dissertation work.
In this introduction, first I describe how the environment influences life-cycle timing, or expression. Next, I introduce the themes that pervade my dissertation. At the end of each section, I explain how each of my chapters addresses that theme. Finally, I provide brief summaries of each of my chapters.

Figure 1: Schematic of the factors that shape a life cycle. Each life-stage transition (P1-P3) determines the environment (E) experienced by the subsequent life stage. This environment interacts with the genotype (G) of the individual to determine the timing of the subsequent life-stage transition. The timing of each of these life-stage transitions (P) determines the environment experienced by the subsequent life stage thus the fitness at the end of each life stage (F1-F3). The environment is thus depicted as both generating phenotypes (ExG) and determining selection on those phenotypes (ExP). Processes explored in this dissertation are highlighted: correlations between life-stage transitions caused by sequential expression of traits in seasonal environments (a); environment-
dependent allelic expression (b); and epistasis (c). These processes are important because earlier life-stage transitions can have correlated effects on the timing of later life stages by determining the selective environment the subsequent life stage experiences (d). Arrows indicate direct pathways of causation, X’s indicate interactions, and dashed lines depict correlations caused by the direct pathways.

INFLUENCES OF TEMPORAL ENVIRONMENTAL VARIATION ON PHENOTYPES

The environment influences life-cycle expression in two ways: First, in many organisms the timing of life-stage transitions (or phenological events) is strongly influenced by the environment. In other words, these traits are phenotypically plastic (Bradshaw 1965; Forrest and Miller-Rushing 2010; Nicotra et al. 2010; Sultan 2000); and thus, a single genotype can produce a diversity of phenotypes depending on environmental conditions. For instance, the same genotype of the understory herb *Campanulastrum americana* is an annual if it germinates in the fall but is a biennial if it germinates in the spring (Galloway and Burgess 2009; Galloway and Etterson 2007). This diversity occurs because exposure to winter chilling is a requirement for flowering—fall seedlings receive chilling in their first winter, but spring seedlings do not until the following year (Galloway L., personal communication). Thus, the environment strongly influences the timing of life-stage transitions.

Second, the environment also determines selection on expressed life-stage transitions with successful variants determining which environmental responses persist. Adaptive responses to environmental variation have been demonstrated empirically for specific life-stage transitions in plants such as flowering time and germination (Aronson
et al. 1992; Clauss and Venable 2000; Donohue 2000; Sultan 2000) and more broadly for the timing of life-stage transitions in other organisms (Charmantier et al. 2008; Visser et al 1998). For example, the fitness of anuran species that lay eggs in temporary ponds is determined by the timing of metamorphosis. If the pond dries up before metamorphosis is complete, the tadpoles die (Richter-Boix et al. 2011). Given that temporal pools vary widely in drying rates, some species have evolved the ability to speed up metamorphosis in response to pool drying (Enriquez-Urzelai et al. 2013). This response is selected for because it allows individuals to maximize size in slow-drying ponds while ensuring survival in fast-drying ponds. In these species, the timing of metamorphosis (the expressed phenotype) \textit{and} the fitness of the organism are both determined by a single environmental factor—water availability. Thus, environmental factors can both define and determine selection on individual life-stage transitions.

However, organismal fitness is not solely defined based on the timing of a single life-stage transition. Within a generation, the combined effects of sequential life-stage transitions together determine the environment that each life-stage experiences and thus overall fitness. For instance, as my dissertation demonstrates, the timing of seed dispersal of \textit{A. thaliana} determines the environment the seed experiences and thus both its survival and when it germinates. The timing of germination determines the environment the vegetative stage experiences and thus its survival and the timing of flowering. And the timing of flowering determines the rate of seed development,
senescence time, and thus dispersal timing. When the fitness effects of multiple life-stage transitions and other associated traits have been examined in concert, strong correlations are often found (Fournier-Level et al. 2013; Saastamoinen et al. 2013; Valimaki et al. 2013; Willink et al. 2014).

Ultimately, the plasticity of life-stage transitions serves to determine both the speed of progression through the life cycle and the environmental conditions that each life stage is exposed to. Over a long selective history in a particular location, we might expect the plastic life-cycle system of an organism to have evolved such that each life stage is expressed at the time of year when it is best able to survive. However, life-cycle adaptation is very difficult to study empirically across generations due to technical limitations and thus strong empirical evidence is lacking in most systems. Further, the tight connections and feedbacks in this system between the environment, genotype, and phenotype generate numerous correlations that shape the expression of phenotypic variation (Figure 1). Due to the complexity of these interactions, modeling approaches that simplify these systems to their component parts can play an important role in deciphering how each of these factors contribute to the expression of life cycles.

SEASONAL CAUSES OF PLEIOTROPY

Because seasonal environments are cyclical, it is possible that the correlations mentioned above could be predictable once the mechanisms that generate them are understood. One important type of correlation that emerges when these plastic life-cycle
transitions are combined is called environmentally-induced pleiotropy (Donohue 2014), and it occurs when the phenotypic expression of two traits are both influenced by the environment (Figure 1a). For instance, because the warmth of summer always follows spring, and the cool of winter always follows fall, if a plant germinates in the spring it will flower much faster than if it germinates in the fall. Therefore, due to the structure of the seasonal environment, spring germination will always be correlated with rapid flowering and fall germination will always be correlated with late flowering. Thus, allelic changes that influence germination may have pleiotropic effects on the expression of other life stages through changing the environment experienced by the subsequent life stage.

The possibility for pleiotropy among life-stage transitions is not restricted to a single generation. For many species, cross-generational effects have been documented where parents can influence offspring phenotypes. This phenomena has been documented in invertebrates and birds (Basso et al 2014; Tolrian 1995) and is particularly prevalent in plants where environments experienced by the mother during reproduction (Donohue 2009; Fenner 2005;) or even earlier in the life cycle (Chen et al. 2014) can often have lasting effects on germination behavior. Further, these maternal effects are sometimes adaptive (reviewed in Mousseau and Fox 1998). For instance, in the Campanulastrum example above, the maternal light environment determines how many progeny are annual versus biennial. In some environments it is advantageous to
have annual offspring and in others it is advantageous to have biennial offspring (Galloway and Ettersen 2009). Thus, the predictable components of seasonal environments create the potential for adaptive cross-generational effects.

In *A. thaliana*, the timing of life-stage transitions in the maternal generation can influence offspring transitions in multiple ways. First, the timing of the maternal life cycle, specifically flowering time, determines seed-dispersal timing, and thus the environment seeds will experience once they are dispersed (Figure 1c). Maternal effects through dispersal timing have been shown to strongly effect future life-stage transitions and fitness (Donohue et al. 2005, Chiang et al. 2012). Secondly, the maternal plant can alter the life-stage timing of her progeny by altering them physiologically or physically (Figure 1d). For instance, dormancy levels of seeds are set by maternal environmental conditions via changes in gene expression in the seed (Kendall et al. 2011), seed provisioning, (Finch-Savage and Leubner-Metzger 2006), and seed coat properties (MacGregor et al. 2015). These changes shift how a seed responds to the environment and thus can change the timing of germination. Therefore, to truly understand the phenotypic expression of any given generation, life-stage transitions of previous generations must be considered.

These correlations can also have important implication for fitness (Figure 1d). Because the timing of one transition determines the selective environment of the subsequent life stage, selection on a trait may actually occur through its correlated
effects on later traits. Thus, the processes that generate these correlations can also have
direct fitness consequences and therefore may play an important role in shaping the
evolution of life cycles.

Unfortunately, little research to date has investigated the processes that generate
these observed correlations between traits, and we still do not have a firm grasp on how
important these effects are likely to be. Further, such within and between generation
pleiotropic effects have rarely been considered when projecting organismal responses to
climate change (Walck et al. 2011). In my first chapter, I address these gaps by using
empirical data to determine the importance of flowering time on the germination
behavior of offspring. In my second chapter, I consider this question using a multi-
generational modeling approach that explicitly includes between life stage and inter-
generational dynamics. I use this approach to ask what combinations of life-stage
phenotypes are possible for the same genotype in the different seasonal contexts that
exist across a species range. Lastly, in chapter three and Appendix D, I extend the
discussion to consider how pleiotropy between transitions may influence time available
for reproduction—a rough measure of fitness.

**GENETIC EFFECTS ON LIFE-CYCLE EXPRESSION ACROSS RANGES**

Allelic variation in genes that influence life-stage transitions occur across species
ranges (Anderson et al. 2011; Blackman et al. 2011; Li et al. 2010; Ma et al. 2010) and can
sometimes be obviously correlated with environmental clines (Olson et al. 2013;
Prendeville et al. 2013). For instance, clinal variation in ten SNP’s was shown to underlie *Populus* phenology. Trees in the south required cumulative exposure to warmer temperatures before bud burst and shorter critical photoperiods to set bud than those adapted to northern environments (Olson et al. 2013). This allelic variation, combined with the environment that occurs at each location across the range, determines the geographic distribution of life cycles. Two factors hinder our ability to understand how patterns of allelic variation relate to patterns of phenotypic variation in life cycles (Weinig et al. 2014): environment-dependent allelic expression (Figure 1b) and epistatic interactions across life stages (Figure 1c).

First, the pervasiveness of environment-specific allelic effects is a theme that has emerged from the plethora of QTL and GWAS studies conducted on phenological traits in the last twenty years (see Des Marais et al. 2013; El-Soda et al. 2014 for reviews) and can complicate interpretation of patterns of allelic variation. Often an allelic variant only influences a trait in a limited set of environments. Further, even if allelic effects are maintained across environments, the magnitude of the effect often changes. The *Populus* study highlighted above also illustrates this finding: different allelic variants were associated with bud flush/bud set in a northern common garden compared to a southern one (Olson et al. 2013). These effects can lead to complex patterns of phenotypic expression across ranges. For instance, allelic variation that has no phenotypic effect in one part of the range might strongly influence phenotypes in another part of the range.
This patterns could potentially also lead to conditional neutrality—wherein alleles are favored in one environment but not in others.

Another particularly salient example of the importance of environment-dependent allelic expression for the interpretation of allelic variation is illustrated by the phenomenon of counter-gradient variation (Conover and Schultz 1995). This occurs when allelic variation in combination with environmental variation leads to phenotypic stasis across ranges. In this case, allelic variation in how traits respond to the environment is necessary to compensate for geographic variation in environmental conditions. This allelic variation can be deciphered via common garden experiments. For instance, consider two genotypes that both flower one month after germination in the locations where they have evolved. One of those locations is warmer than the other. When measured in a common environment, the genotype that evolved in the cooler location will flower faster than the one from the warmer location. This is because the plant actually has to develop faster in the cooler environment to have the same flowering time as the genotype in the warmer location. Thus allelic variation may or may not be expressed phenotypically across ranges as environments change.

Secondly, epistatic interactions (GxG) across life-stage transitions could also complicate the interpretation of allelic variation (Friedman 2014; Figure 1c). For instance, in A. thaliana, allelic variation that influences germination time can determine whether allelic variation in the FRIGIDA locus (Chiang et al. 2013). In this scenario, a new allele
might sweep to fixation if it increased fitness, but only in populations that have previously evolved a certain allele at another life-stage transition. Such processes can generate a complicated pattern of allelic variation across the range. Ultimately, by masking or revealing allelic variation, the environment defines geographic distributions of alleles.

Interpreting the phenotypic consequences of observed geographic distributions of allelic variation remains a challenge because of the environmental sensitivity of life-stage transitions and the phenotypic correlations that occurs between them. In my dissertation, I address this challenge by using two approaches. First, I empirically measure allelic effects in many different environments and use that information to infer in what contexts allelic variation will be expressed. I use this approach to understand sources of germination-timing variation across generations.

Next, I develop a modeling framework that allows the prediction of life-cycle expression in complex environments. By incorporating knowledge of how genetic variation alters environmental sensitivity, I can account for environment-dependent allelic expression. By linking models across multiple life-stage transitions, moreover, I account for correlations and epistatic interactions across life stages. With knowledge of the geographic distribution of genotypes, this approach can potentially predict the geographic distribution of complex life histories across a species range. In my second
chapter, I use this method to predict how allelic variation influences the geographic
distributions of life-cycle expression patterns in the model organism *A. thaliana*.

Lastly, we might expect allelic distributions to reflect the selective history in each
location. Thus, by implementing simple survival rules, I begin to consider how life-cycle
expression may influence fitness. In my third chapter and Appendix D, I project how the
timing of germination and flowering shapes how long a plant has to reproduce.

**LIFE-CYCLE PHENOLOGY DETERMINES THE ENVIRONMENT EXPERIENCED BY EACH LIFE STAGE**

Up to this point, I have been primarily exploring how life-stage transitions
combine to form life cycles, but of course these life-stage transitions set the stage for the
environmental context of many other traits such as senescence and growth. A promising
use of the modelling framework defined in my second chapter is to use the model to
determine the environment experienced by each life stage and thus the selective
environment for traits expressed during that life stage. This aspect of selection is ignored
by many methods (*e.g.* niche modelling), despite evidence in many systems that it plays
a fundamental role in adaptation.

One such trait, whose expression has been shown to be environment-dependent
and whose expression is continually puzzling is senescence. Senescence, at the
demographic level, is measured as a decline in survival (or fertility) with age in a given
environment and is observed in many species across the tree of life (Jones et al. 2014;
Nussey et al. 2013), although is certainly not ubiquitous particularly in plants (Baudisch et al. 2013). There are several classic explanations for the evolution of senescence such as mutation accumulation (reviewed in Flatt and Schmidt 2009; Roach 2003), but recent models by Baudisch highlight the additional role of trade-offs between survival and fecundity in determining the diversity of senescence patterns observed (Baudisch 2008). The stronger the trade-off between survival and reproduction the earlier organisms are predicted to senesce, but there is evidence that the strength of this trade-off may be environment-dependent. Thus, through shaping these trade-offs, the environment that is experienced during reproduction may define the evolution of senescence.

In my third chapter and Appendix D, I outline how seasonal environments and patterns of phenology define stage-specific selective environments. I ask how variable the environments are that plants are exposed to while maturing seeds to infer if trade-offs between survival and fecundity may vary from year to year. By calculating how variable the time available for reproduction is both within and between locations and for different genotypes, I can speculate about whether environment-dependent senescence might be advantageous in a given location or not.

CHAPTER SUMMARIES

In my first chapter, I test whether maternal environmental effects influence the expression of phenotypic variation in their offspring and determine how correlations between life-stage transitions shape the expression of offspring transitions. Using
manipulative experiments, I show that many combinations of genetic, environmental, and developmental factors can create similar germination phenotypes, and that the seed-maturation environment plays a larger role in determining germination behavior than genetic variation. By explicitly measuring environment-dependent allelic effects and defining a simple model, I show that maternal effects may canalize germination timing, even when there is variation in flowering and dispersal time. This chapter thereby demonstrates that phenotypic variation is the outcome of interactions among genotypes and environments experienced in more than one generation. I designed, implemented, and analyzed the experiments in this chapter with the advisement of K. Donohue. B. Edwards is an additional collaborator that contributed to the implementation, data analysis and write-up. This chapter has been submitted for publication.

In my second chapter, I test how environmental and genetic variation combine to influence the synchrony and geographic distribution of life cycles. Using a modeling approach to predict life-cycle phenology in complex environments, I contextualize geographic patterns of allelic variation in this species after taking into account environment-dependent allelic effects, epistasis among life-stage transitions, and correlations between life stages generated by the environment. First, I ask how important environmental variation alone is for determining life-cycle length and phenology. I find that a single genotype can resemble the breadth of life-cycle variation across the range. Additionally, I show that both winter-annual and summer-annual life
cycles can be expressed by the same genotype in a location. This diversity of life cycles is created by dormancy variation within seed cohorts induced in the previous generation. Next, I consider how observed patterns of allelic variation across the range influence the geographic distribution of life cycles, and I find that incorporating genetic variation improves that match to observed life cycles. Lastly, I test whether these individually plastic traits combine to create phenotypic variation or stasis in higher order traits such as life-cycle length. I find that genetic variation at the level of one life-stage transition is necessary to maintain phenotypic stasis of life-cycle length given environmental variation across the range. I constructed the model and the resultant chapter under the supervision of K. Donohue and with the help of three additional collaborators (C. J. E. Metcalf, A. Wilczek, and J. Schmitt). It has been published in the American Naturalist (Burghardt et al. 2015).

Lastly, I test if allelic and environmental variation affecting early life stages can influence time available for reproduction and consider how that may shape the evolution of senescence. By implementing simple survival rules for flowering plants in the model, I show that the time available for a plant to reproduce depends on early developmental traits and varies widely from location to location and genotype to genotype. For A. thaliana, a semelparous species in which reproduction is fatal, these highly variable reproductive windows may select for differences in the timing of senescence between populations. Further, in locations with strong year-to-year variation
in the time available for reproduction or in which multiple life cycles are expressed in a single year in a single location, my work suggests that senescence rates should be environment-dependent. This suggestion is supported by recent research on the molecular and genetic basis of semelparity and senescence in this species. Thus, genetic variation in earlier life-stage transitions might shape whether senescence rates are environmentally responsive or not. This chapter was written by in collaboration with C. J. E. Metcalf with additional support from K. Donohue. It is accepted for publication in the edited book “Evolution of Senescence across the Tree of Life”.

Overall, I demonstrate the importance of environment-dependent allelic effects and pleiotropy via both empirical and modeling approaches. I find that environment-caused pleiotropic interactions between life stages can canalize life-cycle variation both within and between generations. The model can also predict the geographic distribution of life cycles in this species given known patterns of allelic variation in life-stage transitions across the range. Finally, I show that life cycles determine both the environment in which traits are generated and selected.
1. Multiple paths to similar germination behavior in
Arabidopsis thaliana

INTRODUCTION

Proper germination timing is vital for species persistence because it determines the environmental conditions experienced by later life stages (Donohue et al. 2010; Kimball et al. 2010). For many species, the timing of germination depends strongly on environmental factors such as temperature (Baskin and Baskin 2014; Fenner and Thompson. 2005). While progress is being made towards understanding how a changing climate may influence the timing of flowering and budburst (Chuine 2000; Morin et al. 2008), less work has been focused on the potential for climate change to alter germination timing (Walck et al. 2011).

Temperatures experienced by the maternal plant during seed development can influence germination timing by changing dormancy level (Donohue 2009; Galloway and Etterson 2007) and temperatures experienced by a seed after dispersal also strongly influence germination (Baskin and Baskin 2014; Donohue et al. 2005a). How seed-maturation temperature interacts with post-dispersal temperature to influence the probability of germination has not been well studied, but it is this interaction that will determine the timing of germination under seasonally-varying conditions (See Figure 2 for a schematic). Additional factors such as photoperiod influence germination of A. thaliana, but have a much smaller effect than temperature (Donohue et al. 2005b; Munir
et al 2001). Therefore in this study, we examine the interaction between seed maturation and post-dispersal temperature using the model organism *Arabidopsis thaliana*.

Environmental factors such as temperature can also strongly influence the expression of genetic variation. Some environmental contexts magnify phenotypic differences between allelic variants at loci, while others can reduce, or canalize, that variation (Byers 2005; Falconer and Mackay 1996; Roff 1997). Defining the environmental contingency of the expression of genetic variation is important because selection among genotypes occurs only under conditions in which phenotypic differences among genotypes are expressed. In this study, we also examine how seed-maturation and post-dispersal temperature influence the expression of genetic variation in germination.

In seasonal environments, seed dormancy is a major mechanism used by plants to delay germination, often thought to prevent germination until times of year during which seedling survival is high (Donohue et al. 2010). Dormancy is defined as the failure of seeds to germinate under environmental conditions that would otherwise permit germination. It has been frequently shown that less dormant seeds can germinate under a wider range of temperatures. One can therefore characterize seed dormancy by assessing temperature-dependent germination behavior (Finch-Savage and Footitt 2012). Here we refer to this description of germination behavior as the germination envelope (Figure 2a).
For many species, including the model organism *Arabidopsis thaliana*, seeds lose dormancy over time in a process called after-ripening. Dormancy loss via after-ripening is often characterized by a widening of the germination envelope over time (Finch-Savage and Leubner-Metzger 2006). For example, in fall-germinating populations, seeds are dispersed in the spring and are often dormant at high temperatures soon after dispersal, but gradually gain the ability to germinate at progressively higher temperatures over the summer (Baskin and Baskin 1972; Baskin and Baskin 1983; Footitt et al. 2011). We refer to these dynamic changes in germination envelopes with after-ripening as the germination trajectory (Figure 2a).

Interestingly, populations collected across Europe differ in how they respond to post-dispersal temperatures (Atwell et al. 2010; Chiang et al. 2009). For instance even though the two common lab ecotypes Columbia (Col) and Landsberg erecta (Ler) are both fairly non-dormant, they can respond differently to temperature cues (Chiang et al 2011). Further, some genes that influence germination behavior and vary among natural populations have been identified. The *DELAY OF GERMINATION 1 (DOG1)* locus from the Cape Verde Island (Cvi) accession strongly increases dormancy compared to the Landsberg erecta (Ler) allele (Alonso-Blanco et al. 2003; Bentsink et al. 2006). Graeber et. al (2014) recently showed that this gene is involved in defining the range of temperatures over which germination can occur. Additionally, *FLOWERING LOCUS C (FLC)*, a naturally variable locus that was originally implicated in flowering time, has
now been shown to influence dormancy as well (Chiang et al. 2009). Both genes have been shown to be under selection via their effects on germination timing in the field in the United States (Chiang et al. 2011; Chiang et al. 2009). However, the diverse conditions under which these allelic differences are expressed or masked are not well characterized.
Figure 2: Processes that influence germination timing in *A. thaliana*. 

*a*, Schematic of the multiple factors that are known to influence germination timing. The relationships between these factors are explored in this paper. 

*b-c*, Illustrations of how seed-maturation and post-dispersal environments combine to determine the expression of genetic variation. In the first example (b), two genotypes are matured and dispersed in cool conditions which results in differences in germination trajectory between the two genotypes. When those trajectories interact with the environment for three months after dispersal, differences in realized germination timing occur (bar graphs at bottom). In contrast, the second example (c) shows the same two genotypes matured and dispersed in warmer conditions. While germination trajectories still differ between genotypes, lower dormancy levels result in no differences in germination timing between the genotypes. Scales for Prop and Temp in b and c are the same as for a. In the bar graphs “No seeds” indicates that 100% of seeds have already germinated so even if environmental conditions are favorable no germination occurs.

Maternal temperature effects on seed dormancy operate through the seasonal timing of flowering. *Arabidopsis thaliana* has a broad geographic range and displays a range of life cycles that mature their seeds in a diversity of temperatures. Some populations have both fall- and spring-germinating cohorts that flower in the spring, while others behave as strict winter annuals, with all plants germinating in the fall and flowering in the spring. Still other populations have both fall- and summer-germinating cohorts that flower at different times and therefore mature seeds at different temperatures (Burghardt et al. 2015; Pico 2012; Pigliucci 1998).

Temperature experienced during seed maturation has been shown to have a strong influence on dormancy and germination (see Figure 2). Decreasing the temperature during seed development results in increased levels of dormancy (*i.e.* decreased probability of germinating; Donohue et al. 2005c; Kendall et al. 2011; Penfield and Springthorpe 2012). The changes in dormancy are mediated by altered gene
expression (Kendall et al. 2011), seed provisioning, (Finch-Savage and Leubner-Metzger 2006), and seed coat properties (MacGregor et al. 2015).

Differences between genotypes in germination envelopes and trajectories measured in the lab may (Figure 2b) or may not (Figure 2c) lead to differences in germination behavior in natural seasonal environments. This is because seeds only experience a small subset of the possible post-dispersal temperatures in any location and these conditions may or may not be ones in which allelic variation is expressed. The conditions that seeds experience are further limited by correlations between the seed-maturation and post-dispersal environments that occur in seasonal environments. For instance, seeds that are matured in warmer conditions of late spring are more likely to be dispersed in warm environments. Thus, it is important to view genetic variation measured in the lab in the context of relevant seasonal variation. A model based on lab measurements coupled with field data can translate lab germination behavior into reasonable expectations for behavior in the field.

The adaptive rationale for the existence of maternal effects on seed dormancy has not been firmly established for A. thaliana. One possibility is that the environment experienced during seed maturation provides information about the post-dispersal environment that enables germination to occur at an optimal time (Galloway and Etterson 2007; Wagmann et al. 2012). This process could operate both for seeds matured on the same plant across the dispersal season and for different plants that flower at
different times. For instance, in order to germinate at the same time, seeds matured and dispersed early in a season may need to be more dormant, whereas seeds dispersed later may need to be less dormant.

In this study, we explore whether seed-maturation effects can compensate for variation in flowering time and ask if different combinations of genotypes and temperatures can produce similar germination behaviors. To do this, we tested how the germination behavior of four genotypes known to vary in dormancy level respond to three seed-maturation temperatures and a range of post-dispersal temperatures as seeds after-ripened. To test how germination responses to seed-maturation and post-dispersal temperature might be manifest as germination timing under seasonally variable temperatures, we applied the data to construct a simple model that predicts the germination probabilities over the course of a year. Using climates from two geographic locations, we applied this model to project the seasonal conditions under which genotypic differences in germination timing would be expressed and tested how seed-maturation temperature altered germination timing across genotypes and dispersal cohorts. Specifically, we asked: 1) Which combinations of seed-maturation and post-dispersal temperature and after-ripening lead to similar germination behavior among genotypes? 2) When will genetic variation be expressed in seasonal environments? 3) Can effects of seed-maturation temperature compensate for differences in dispersal timing?
MATERIALS AND METHODS

Genetic material and seed production conditions

We used four genotypes known to differ in germination. First, we compared the behavior of the two standard lab accessions, Landsberg erecta (Ler) and Columbia (Col). Ler was originally collected in Germany and Col most likely was as well. Secondly, to isolate the effect of particular alleles on dormancy, we used two near isogenic lines (NILs) that contain the FLC or DOG1 allele from the highly dormant Cape Verde Island (Cvi) accession introgressed into the Ler background (LerFLC and LerDOG1 respectively; (Alonso-Blanco et al. 2003; Chiang et al. 2009). These NILs were obtained from Maarten Koornneef.

To test how the seasonal timing of flowering can influence germination behavior, we used three different seed-maturation temperatures for seed production. Growing conditions were selected to generate a range of dormancy levels and represent the breadth of conditions experienced by reproductive A. thaliana both within and between maturation seasons across the native European range (14°C and 20°C) and the invaded range of the southern US (25°C). Plants were grown under three constant temperature regimes (hot-25°C, warm-20°C, and cool-14°C) with a 12-hour photoperiod in EGC Model GC8-2 Plant Growth Chambers (Chagrin Falls, OH). Twelve plants per genotype were grown at each temperature. Replicate plants were randomly distributed over three replicate chambers.
Seed sowing was staggered in order to synchronize the seed harvest. After seven days of dark stratification at 4°C, seeds were sowed into pots filled with Metromix 360 (Scotts Sierra, Marysville, OH, USA). Seeds were then germinated under full spectrum light at 20°C/12-hr photoperiod. After 10 days, seedlings were transferred to vernalization (~5°C/10-hr photoperiod) for 28 days before being randomized and placed into their respective growing temperatures. Seedlings were fertilized on two occasions before bolting (Peter’s Professional 20-20-20 fertilizer, The Scotts Company, Marysville, OH, USA). Plants were watered as needed and pot positions were rotated on a weekly basis within each chamber. As siliques approached 70-90% maturity, water was withheld for two weeks and plants in all temperature treatments were harvested on a common date. Seeds from each plant were collected into eppendorf tubes and both dried and stored at low relative humidity in Secador® 4.0 Auto-Dessicator Cabinets (Bel-Art Products, Pequannock, NJ, USA) until used for germination assays.

Germination assays and measures

To determine the range of temperatures under which germination can occur, we assayed seeds in four constant temperatures that span those experienced throughout the life cycle (8, 16, 22, 31°C) and in a constant 12-hour photoperiod. Germination assays were conducted on seeds that had after-ripened for 3, 7, 19, and 48 weeks. Germination was assessed in controlled, Percival Model GR41LX incubation chambers (Percival Scientific Inc., Perry, IA) with new light bulbs installed at the beginning of the
experiment. For these assays, 12 seeds per genotype were sown onto 35 mm petri plates containing Whatman P5 filter paper saturated with sterile, double-distilled water. For each genotype x maturation treatment combination, we used twelve independent (biological) replicate plates in every germination-temperature treatment. Petri plates were randomized on trays and trays rotated within chambers every few days. Thus, petri plates represent our unit of analysis. This setup resulted in a total of 2304 plates (4 after-ripening x 4 genotypes x 3 maturation x 4 germination temperatures x 12 replicates). Plates were assessed for germination proportion on days 3, 5, 8, and 14. Germination had reached a clear plateau in all temperatures by day 14 with the exception of 8°C, where we saw high proportions but did not know for sure that germination had plateaued. Dead seeds, though rare, were excluded when they occurred. We calculated final germination proportion for each plate as the number of germinants on day 14 divided by the total number of viable seeds.

Analysis of data

We compared germination proportions among genotypes and treatments using generalized linear models (glm package in R). Genotype (Geno), maturation conditions (Mat), seed age or after-ripening time (AR), and germination temperature (Temp) were treated as predictors in the model, and interactions were evaluated as described below. The relationship between each predictor and germination probability was non-linear, so predictors were modeled as discrete factors. Separation (and quasi-separation) can occur
in generalized linear models with a binomial link when combinations of predictor
variables lead to an all or nothing response—cases in which all or no seeds germinate.
While separation should not influence AIC values or likelihood ratio tests, it can bias
estimates of model coefficients. Therefore we confirmed our inferences from glm using
Firth’s penalized likelihood (brglm package in R). Here, we report glm results except in
the cases we note where more accurate coefficient estimation was necessary, in which
case we report coefficients from brglm.

For all models, we used two different methods to assess the importance of
individual model terms and interactions: 1) via difference in AICc and 2) via a likelihood
ratio test. AICc is similar to AIC but has a larger penalty for increased number of
parameters and is recommended when the number of data points divided by the
number of parameters is <30 (Burnham and Anderson 2002). When comparing models
using AICc, terms that are considered important in the model will result in negative
AICc differences.

First, we used a full model (y= Mat x Geno x Temp x AR), which included all
possible interactions, to test for a significant four-way interaction amongst treatment
variables. The four-way interaction was significant, so we next tested the importance of
the four-way interaction separately for each of the other three genotypes compared to
the reference genotype Ler. All p-values were corrected for multiple comparisons using
sequential Bonferroni. When the four-way interaction was not highly important (P > .001
or AICc was positive), we proceeded to test the importance of each three-way interaction in a model that included all three-way interactions.

To test whether germination envelopes changed with after-ripening in a genotype- or maturation-specific manner, we analyzed each combination of genotype and maturation treatment separately and tested for a significant AR x Temp interaction. We used likelihood ratio tests with corrected p-values via sequential Bonferroni to determine whether model fit was significantly improved by the inclusion of this interaction.

Lastly, in order to determine which genotypes were the most divergent across all after-ripening periods and maturation temperatures assessed, we tested for differences in behavior across germination temperatures between genotype pairs (Ler vs Lerdogi, Ler vs Col, Ler vs Lerfic) that were the same age and matured at the same temperature. For all twelve combinations at each temperature, coefficients indicating the strength of genotypic effect were obtained and likelihood ratio tests were performed to determine significance. We corrected for false discovery rate using the p.adjust function in R (Benjamini & Hochberg 1995). Since the accurate estimation of coefficients was important, we used brglm (outlined above) instead of glm.

Cluster Dendrograms

A cluster analysis was used to examine similarities in germination behavior by clustering based on two phenotypes—germination envelopes and germination
trajectories. For the first analysis, we compared the germinations envelopes of 48 combinations of three factors (4 Genos x 3 Mat x 4 AR). Combinations of Geno x Mat x AR with similar germination responses to temperature (germination envelopes) were clustered together. In the second cluster analysis, we considered the similarity of germination trajectories of seed cohorts that differed in terms of genotype and maternal conditions (12 combinations: 4 Geno x 3 Mat). Clusters were, therefore, based on the similarity of germination trajectories—how germination envelopes changed with after-ripening. This analysis clusters combinations of genetic and maturation factors together that are more likely to respond similarly to seasonal environmental variation. Cluster dendrograms were drawn using the heatplot.2 function in the R package “gplot” with a pre-specified number of clusters (5) using the dist method.

*Germination Projections*

We used our measurements of germination trajectories for every genotype x maturation combination to project the proportion of seeds expected to germinate each subsequent month after dispersal. Using our germination envelope data, we linearly interpolated between temperatures to estimate the germination probability for every degree in the tested temperature range. Estimates below the lowest tested temperature (8°C) were extrapolated down to 4°C. We presumed germination did not occur below 4°C based on knowledge of base temperatures for other phenological transitions and previous germination experiments in the lab (Burghardt, unpublished data). As average
monthly temperatures for our locations did not exceed 31°C, it was not necessary to extrapolate estimates above this temperature. We then calculated monthly probabilities at each temperature by interpolating between time points.

We acquired seasonal climate data from two locations where *A. thaliana* grows and for which we know when seed dispersal occurs in natural populations. In Halle, Germany (longitude: 11.993, latitude: 51.495), in the middle of its native range, seeds disperse in May and June (Amity Wilczek personal communication) and in a naturalized population in Durham, NC USA (longitude: 35.9886, latitude: 281.0928) seeds disperse in April and May (L. Burghardt, personal observation). We extracted historical monthly temperature norms from [http://climexp.knmi.nl/](http://climexp.knmi.nl/). This tool uses time series data for 1901-2012 from the Climatic Research Unit (CRU) in East Anglia (version CRU TS3.21). We used all land-based near surface measurements in the 5 degree x 5 degree grid cell surrounding each location and only when at least 50% of data was available. For Halle, mean daily temperatures in °C beginning in January were: -1, 1, 4, 9, 13, 16, 18, 18, 14, 9, 4, 1 and for Durham they were: 5, 6, 10, 15, 20, 24, 26, 25, 22, 16, 10, 6.

Finally, we used the interpolated estimates of germination proportion, described above, and these monthly temperature averages to project germination behavior of seeds cohorts dispersed early and late within their observed dispersal season. We conducted separate predictions for seeds matured and dispersed at different times. Specifically, we used the observed temperature the month of dispersal and the
germination envelopes of the seeds to determine how many individuals in a 100 seed cohort would germinate that month. Germinated individuals were removed from the seed pool and the same calculation was conducted for each subsequent month’s observed temperature until the seed bank was exhausted. Germination envelopes shifted each month due to after-ripening.

To test for genetic influences, we tested naturally occurring combinations of seed-maturation and post-dispersal environments. For instance, in Durham, seeds matured in cool temperature conditions are dispersed in April and seeds matured in warm temperatures are dispersed in May, so the season of seed maturation is ‘matched’ with the natural season of dispersal. To test the importance of the plastic response to seed-maturation temperature on germination timing, we also included combinations of dispersal and maturation temperature that are mismatched \textit{i.e.} do not occur in nature. These combinations reflect genotypes that are insensitive to environmental cues.

\textbf{RESULTS}

\textit{Main effects and overall shape of germination envelopes}

As previously documented, cool seed-maturation temperature imposed higher dormancy leading to less germination, and longer durations of after-ripening released dormancy leading to more germination (Figure 17; Table 1). Regarding temperature-dependent germination, or “germination envelopes”, germination probability was the highest at 16°C with slightly lower probability of germination at 8°C and 22°C, and a
much lower probability at 31°C (Figure 17d). Germination envelopes changed
dynamically with after-ripening. Fresh seeds germinated primarily at 8°C and 16°C
(Table 1), but the germination envelope widened with after-ripening, and seeds became
increasingly more likely to germinate at high temperatures (Figure 3: a4-d4).

For every combination of genotype and seed-maturation temperature, we found
a significant temperature x after-ripening interaction, but the pattern of response
differed among them (discussed below; Table 3). Further, as determined via a likelihood
ratio test, all four factors (Geno, Mat, AR, and Temp) interacted to determine
germination probability (df=54, \( \chi^2 = 142 \), p<<.001). However, when we compared each
genotype directly to Ler, we found that this significant four-way interaction was
dominated by the difference in behavior between Ler and LerDOGI (Table 4).

Table 1: Summary describing general patterns of how the factors investigated
here individually influence germination proportions. Results are summarized from
boxplots in Figure 17.

<table>
<thead>
<tr>
<th>Factor</th>
<th>General trend in germination proportion (Fig S1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype—Geno</td>
<td>Landsberg erecta (Ler) accession = high germination</td>
</tr>
<tr>
<td></td>
<td>Columbia accession = mid-range germination</td>
</tr>
<tr>
<td></td>
<td>Ler(_{FLC-Cvi}) = high germination</td>
</tr>
<tr>
<td></td>
<td>Ler(_{DOGI-Cvi}) = low germination</td>
</tr>
<tr>
<td>Seed Maturation Temp.—Mat</td>
<td>Higher temperature = higher germination</td>
</tr>
<tr>
<td>Seed age or After-Ripening—AR</td>
<td>Older/more after-ripened seeds = higher germination</td>
</tr>
<tr>
<td>Germination temperature—Temp</td>
<td>8°C = mid-range germination</td>
</tr>
<tr>
<td></td>
<td>16°C = maximal germination</td>
</tr>
<tr>
<td></td>
<td>22°C = mid-range germination</td>
</tr>
<tr>
<td></td>
<td>31°C = minimum germination</td>
</tr>
</tbody>
</table>
Figure 3: The influence of maternal and genetic factors on germination temperature envelopes. Reaction norms of germination proportion in response to temperature ("germination temperature envelopes") across all after-ripening durations (columns) for each genotype (rows). Lines within each panel indicate the seed-maturation temperature (14°C-black squares, 20°C-dark grey circles, 25°C-light grey triangles). Data points depict mean germination proportion at each temperature for twelve replicates. Error bars indicate the standard error. Letters indicate a significant difference between warm-matured seeds and hot-matured seeds (h) or warm-matured seeds and cool-matured seeds (c). Significance at the P < 0.05 level was determined using likelihood ratio tests of glms comparing the germination probability of either hot- or cool-matured seeds directly to the warm-matured seeds. Significance was corrected for multiple tests using sequential Bonferroni correction.
Seed-maturation temperature modified germination trajectories

Changes in temperature-dependent germination over time, or germination trajectories, were modified by seed-maturation temperature, as indicated by significant Mat x AR x Temp interactions for each genotype (Table 5). Cool seed-maturation temperature (14°C) altered germination responses to post-dispersal temperature (Figure 17c and Table 1) by imposing stronger dormancy, thereby reducing germination proportions and narrowing the germination envelope. The germination envelope expanded more slowly for cool-matured seeds than for the envelopes of seeds matured in hot (25°C) and warm (20°C) temperatures (Figure 3).

Interestingly, the influence of seed-maturation temperature on germination probability persisted throughout the duration of after-ripening studied here. For most genotypes, seeds matured at cool temperatures had lower germination than those matured at the highest temperature after 48 weeks of after-ripening even though seeds were still viable (Figure 3).

Genotypes differed in germination trajectories

Genotypes differed in overall germination propensity: Ler and Ler bic consistently had the highest germination proportions, whereas Col had intermediate germination proportions and Ler dogi had the lowest (Figure S2). All genotypes germinated the most at 16°C and the least at 31°C, but genotypes differed in how germination envelopes changed with after-ripening (significant Geno x Temp x AR
interaction; Table 6). Ler and Lerflc acquired the ability to germinate at higher temperatures as they after-ripened much more than did Lerdog1 and Col (Figure 3). This pattern was observed for all seed-maturation temperatures. However, both Ler and Lerflc genotypes matured in the cold regained some dormancy at 48 weeks.

In general, genotypic differences in germination proportion were most apparent at temperature extremes. Differences between seeds of Lerdog1 and Ler were observed across seed maturation conditions, and the magnitude of divergence between genotypes increased with temperature (Figure 3, Figure 18). In contrast, Lerflc was surprisingly similar to Ler at all germination temperatures, with some notable exceptions at cool temperatures. Columbia behaved like to Ler at 16°C, but had less germination at 8°C and 22°C and considerably less germination at 31°C. Thus, allelic variation was frequently revealed at temperature extremes, but masked in intermediate conditions.

*Highly disparate combinations of factors can produce similar germination envelopes*

To determine which combinations of factors—genotype, seed-maturation temperature, and after-ripening duration—produced the most similar germination responses to temperature, we clustered our data based on similarity of germination envelopes (48 different combinations of factors). We identified five major germination responses to temperature; clusters 1-3 had high germination proportions, whereas little germination occurred in clusters 4-5 (Figure 4).
Specifically, in cluster 1 (the largest cluster), seeds germinated to high proportions at all temperatures except 31°C. Some of the recurrent treatment factors found in this cluster included seeds that were after-ripened for mid (7 or 19 weeks) or long (48 weeks) durations, warm and hot seed-maturation temperatures, or low dormancy genotypes matured in cool temperatures. Cluster 2 germinated to high proportions at all temperatures and included only the least dormant genotypes, Ler and Ler FLC, and after-ripened seeds that were matured in warm or hot temperatures. Cluster 3 was characterized by high germination at all temperatures except 8°C, and only one treatment combination produced this phenotype (cool-matured Ler seeds at 19 weeks). Germination was restricted to 8°C and 16°C in cluster 4, and included factors associated with high levels of dormancy: the Ler DOG1 genotype, cool seed-maturation temperature, and fresh seeds. Little to no germination occurred at any temperature in cluster 5. It primarily included the Ler DOG1 genotype, but also three-week old seeds of the Col genotype matured in cool temperatures.

This analysis shows that highly divergent combinations of factors can produce similar germination phenotypes. In particular, environmental factors that increase dormancy, cool seed-maturation temperatures or short after-ripening durations, can cause less dormant genotypes to resemble more dormant genotypes. Likewise, more dormant genotypes that have experienced germination-promoting conditions (warm maturation and long after-ripening) can resemble less dormant genotypes that have
Figure 4: Cluster dendrogram and heat map of similarity of germination envelopes for all combinations of seed age (AR), genotype (Geno), and maturation (Mat). The grey-scale boxed numbers on branches denote major clusters. The color scale indicates germination proportion, with darker color shades indicating higher germination proportions. On the far right are heuristics describing the representative shape of the germination envelopes for each major cluster. Clustering is based on how each genotype responded to temperature across all seed-maturation temperatures and seed ages. *LerDOG1* and *LerFLC* are abbreviated *DOG1* and *FLC* respectively.
been induced into stronger dormancy. For example, fresh seeds of LerFLC and Ler genotypes when matured in cool temperatures were phenotypically similar to fresh seeds of the LerDOG1 genotype matured in hot temperatures or LerDOG1 seeds that were matured in cool temperatures and after-ripened for 48 weeks. Combinations of factors can also cancel each other out. For example, the phenotype of Col seeds matured in hot temperatures after 3 weeks of after ripening resembles that of Col seeds matured in cool temperatures after 48 weeks of after-ripening. Thus, there are multiple routes by which to achieve to similar germination envelopes in *A. thaliana*.

*Clustering of germination trajectories reveals strong response to seed-maturation temperature and genetic variation in that response*

We next evaluated which combinations of genotypes and seed-maturation temperatures produced the most similar germination trajectories—changes in temperature-dependent germination over the course of after-ripening (Figure 5). Five clusters were identified. Cluster 4, the least dormant cluster, germinated to high proportions in every germination temperature except when seeds were 3 weeks after-ripened. This cluster included hot- and warm-matured Ler and LerFLC. Cluster 3 was similar, but had reduced germination at 31°C at all after-ripening durations. Factor values in this cluster included hot- and warm-matured Col seeds and hot-matured LerDOG1 seeds. In cluster 1, germination proportions were low until 7 weeks of after-ripening, and factor values in this group included cool-matured LerFLC and Col
genotypes as well as warm-matured *LerDOG1*. Cluster 5 was highly divergent from the other clusters; germination proportions in this cluster remained low throughout after-ripening and included only *LerDOG1* seeds matured in cool temperatures.

Figure 5: Cluster dendrograms describing the similarities in germination trajectories between genotypes with seeds matured at different temperatures. Clustering is based on the mean response of each Genotype x Maturation combination to each germination temperature over the course of after-ripening. The major clusters are indicated by grey-scale numbers (1 through 5) at the tips of the dendrograms. Germination proportion is indicated by the color key, with darker color shades indicating higher germination proportions. Cartoons on the right depict representative germination trajectories (temperature-dependent germination at four time points of after-ripening) of each of the 5 major clusters. *LerDOG1* and *LerFLC* are abbreviated *DOG1* and *FLC* respectively.

Neither genotype nor seed-maturation temperature solely determined how germination envelopes changed with after-ripening; instead, seed-maturation temperature influenced how similar or dissimilar genotypes were in response to after-ripening. For example, while Ler and Ler*FLC* clustered together when seeds were matured in warm and hot temperatures, they diverged when matured in cool temperatures. In fact, cool-matured Ler*FLC* seeds were more similar to cool-matured Col
and warm-matured LerDOG1 seeds. This is because cool-matured Ler seeds exhibited a distinctive decline in germination at low temperature with prolonged after-ripening. Moreover, genotypes differed in their responsiveness to seed-maturation temperature. Specifically, LerDOG1 was highly responsive to seed-maturation temperature, and it was distributed over three divergent clusters; in contrast, Col was least responsive to seed-maturation temperature and was restricted to two adjacent clusters (Figure 5).

Location and genotype-specific projections of germination timing

To predict the timing of germination in a seasonal temperature environment, we combined our germination trajectories with seasonal temperature data from Durham, NC (USA) and Halle, Germany (see schematic in Figure 6a). Projections of germination time were made for cohorts of seeds matured at 14°C (cool) and 20°C (warm) for each genotype. Based on observations of natural seed dispersal at these locations, seeds matured under cool temperature conditions were dispersed in April in Durham, NC (average temp at dispersal of 15°C, Figure 6b) and in May in Halle, Germany (average temp of 13°C, Figure 6d). Seeds matured under warm temperatures were dispersed in May in Durham (average temp of 20°C, Figure 6c) and June in Halle (average temp of 16°C, Figure 6e). While the average temperature in June in Halle is lower than our warm seed-maturation temperature (20°C), A. thaliana shows much lower dormancy above 15°C than below it (V. Springthorpe, in review). Therefore we believe that warm seed
maturation is a better reflection of the dormancy level of these seeds than cool seed-maturation temperatures.

For most genotypes, the projected time of germination is early summer in Durham and mid-summer in Halle. When seeds were dispersed early (and exposed to cooler maturation conditions), half of the seed cohort germinated one month prior to seeds that experienced warm maturation conditions and were dispersed on month later. The other half of the early seed cohort germinated synchronously with the warm-matured cohort (Figure 6: b-e). Thus half the warm-matured seeds actually germinated more quickly after dispersal than the cold matured seeds. While this was the overall trend, genotypes did differ in projected germination times, as discussed next.

May (later) dispersal in Durham revealed germination differences between Ler and Col that were not visible in any of the other scenarios. In this scenario, warm seed-maturation temperature caused Col to more closely resemble Lerdogi than Ler. In contrast, a subtle difference in germination time between Ler and Lerflc was expressed only when seeds were matured and dispersed (early) in cool conditions. Thus, we predict that Col will be the most differentiated from Ler and Lerflc in seasonal contexts that are warmer than that of central Europe.

As was indicated in the cluster analysis, germination timing for Lerdogi was distinctly different from Ler when both were matured and dispersed (early) under cool conditions. Interestingly, in Durham, Lerdogi was the only genotype that resulted in late
a. Step: 1

Durham, NC---April Dispersal
14°C Seed Maturation

b. Step: 2

Durham, NC---May Dispersal
14°C Seed Maturation

Genotype
- Ler
- Ler\_FLC
- Col
- Ler\_DOGI

Germination Proportion

Germination Temperature

Avg. Temp

Step: 3

Durham, NC
Month

Halle, Germany---May Dispersal
14°C Seed Maturation
d.

Halle, Germany---June Dispersal
20°C Seed Maturation
e.
Figure 6: Results of germination time projections in seasonal environments. 

The steps involved in creating the germination projections. 1) To generate a predicted germination probability at every temperature, we interpolated and extrapolated temperature envelopes from data points collected. 2) To generate germination predictions across all seed ages up to a year, we interpolated and extrapolated across germination envelopes that were collected at each time point. 3) Use monthly temperatures averages in combination with the inferred germination probability surface to project germination percentages of two cohorts of fresh seeds dispersed at different times, into two different seasonal environments (D1 and D2). 

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b-e, Projections with matched pre- and post- dispersal environments: For Durham (b,c), cool-matured seeds of each genotype dispersed in April and warm-matured seeds dispersed in May; for Halle (d,e), cool-matured seeds dispersed in May and warm-matured seeds dispersed in June. Projections were truncated 1 year after dispersal.

fall germination typical of winter annuals (Figure 6c). In Halle, due to lower temperatures, most cool-matured seeds of Ler

However, when matured under warm conditions, they were far less differentiated from the other genotypes and were predicted to germinate in late summer (Figure 6d,e).

Thus, in Halle, the seed-maturation temperature determined whether this genotype exhibited autumn or spring germination.

Effects of seed-maturation temperature on dormancy compensate for differences in dispersal timing

The above results suggested that germination timing across the dispersal season was synchronous because the maternal environment allowed later dispersed seeds to germinate faster. To test if seed-maturation temperature indeed causes this pattern, we projected what the behavior would be of genotypes whose germination behavior was insensitive to seed-maturation environment by visualizing mismatched combinations of responses that do not occur in nature (schematic Figure 7a). By comparing the two blue
projections in Figure 7b,e one can visualize germination timing differences of a genotype that always behaves as if it were matured in cool conditions. Similarly, the yellow lines visualize a genotype that always behaves as if it were matured in warm conditions—these are mismatched combinations. By comparing the bold lines across colors one can see the germination synchrony of a genotype that alters its germination behavior in response to temperature—this is the matched combination that is observed in nature.

A genotype that shifted germination behavior in response to the maternal environment (Figure 7) had more synchronous germination than either genotype that was un-responsive to seed-maturation temperature. Therefore, responsiveness to seed-maturation temperature increased the similarity in germination time of seeds dispersed at different times. This was true to varying degrees for all genotypes except for LerDOG1 in both Halle, Germany (Figure 7b-e) and Durham, NC, USA (Figure 19a-d). For LerDOG1, the effect of seed-maturation temperature was much stronger than the effect of dispersal time. In Halle, cool seed-maturation temperature overcompensated for the effect of early dispersal on germination time and caused spring germination; however, in Durham, seed-maturation temperature mitigated the effect of dispersal time, as it did with the other genotypes, though the timing of seed dispersal only influenced germination of warm-matured seeds. Overall, these projections suggest that plasticity to seed-maturation temperature can reduce variation in germination that would otherwise be caused by differences in dispersal timing.
Early seed cohort

Late seed cohort

Cool-matured dormancy

Warm-matured dormancy

Favorable germination window

Natural Combination

MISMATCH

Cool-matured dormancy

Warm-matured dormancy

Natural Combination

MISMATCH

Time

Germination (Prop.)

Maturation Env.

Col

14°C

20°C

Dispersal time

May

June

a. b. c. d. e.
Figure 7: Projections of germination timing of all combinations of seed maturation and dispersal timing. a, Schematic illustrating the hypothesis that seed-maturation effects compensate for differences in dispersal timing to optimize germination timing. b, Germination projections of all combinations of seed-maturation and post-dispersal environments for each genotype in Halle, Germany. Boldface combinations are the matched combinations of seed-maturation temperature and dispersal time that occur in nature, while narrow lines are the mismatched combinations. Solid and dashed lines refer to May and June dispersal respectively while navy and gold refer to cool and warm seed-maturation temperatures. Cartoon plants courtesy of Amity Wilczek. For results based on climatic conditions for Durham, NC, see Figure 19.

DISCUSSION

We found that germination temperature envelopes changed over time with after-ripening, and these germination trajectories depended on seed-maturation temperature and genotype. Different combinations of genotype, seed-maturation temperature, and after-ripening can produce similar germination envelopes, and different genotype and seed-maturation temperatures can combine to produce similar germination trajectories over time. Thus, in combination and when exposed to seasonal temperature changes these interactions: 1) result in genetic differences in germination being masked in some environmental conditions, but revealed in others and 2) allow seed-maturation effects to mitigate effects on germination time caused by differences in the timing of dispersal.

Dual effects of temperature on germination behavior

Recent work has provided mechanistic insights into how seed-maturation and post-dispersal temperature influence germination. MacGregor et al. (2015) showed that cool maturation temperatures cause changes in the seed coat (phenylpropanoid gene
expression and procyanidin content) that reduce permeability and decrease germination.

Graeber et al. (2015) demonstrated that DOG1 regulates temperature-dependent endosperm weakening of imbibed seeds through changing gibberellic acid metabolism; however, the mechanistic interaction between these temperature effects and the potential role of DOG1 in regulating them remains to be elucidated.

Our results show that these dual temperature inputs do not have additive effects on germination behavior and are influenced strongly by allelic variation. In general, cool seed-maturation temperature reduced the temperatures under which germination occurred, and this effect persisted for the duration of after-ripening assessed here. But, as has been described across accessions (Penfield and Springthorpe 2012), genotypes differed in the magnitude of their phenotypic response to seed-maturation temperature as well as in their pattern of response to post-dispersal temperatures.

Dependence of genetic variation on environmental factors

The environmental context into which seeds are dispersed will determine the extent to which genetic differences in germination alleles will manifest as phenotypic variation within a seasonal environment. QTL and GWAS mapping in A. thaliana and other species have continually emphasized the importance of the environment in shaping which loci are associated with phenotypes and the strength of their effects (see El-Soda et al. 2014 for review). Gene network structures that lead to environment-dependent allelic effects have been elucidated for flowering time in A. thaliana and a few
other species (Amasino 2010; Pajoro et al. 2014; Shrestha et al. 2014) allowing prediction in some cases of environment-specific phenotypes (Wilczek et al. 2009). Interestingly, seed-maturation environments have recently been shown to influence the expression of genetic variation in progeny as well (Postma and Ågren in press). Because the mechanistic basis of dormancy and germination are becoming clearer (Graeber et al. 2012; Nonogaki 2014), interpreting those gene-networks in an environmental context is a rapidly-developing and exciting field (Graeber et al. 2014; MacGregor et al. 2015).

Using genotypes known to differ in germination behavior, we found that genetic differences in germination behavior between Col and Ler and Lerice and Ler are most likely to be expressed when seeds experience high or low, but not intermediate, temperatures. This implies that selection can only distinguish genotypes at temperature extremes, as would occur early and late in the growing season or at the northern or southern edges of the range. In contrast, the DOG1 allele from Cvi reduced germination proportion compared to the Ler allele at nearly every temperature and for all maturation combinations tested and would therefore have a phenotypic effect in most seasonal environments. This allele comes from the Cape Verde Islands but there are areas in the native range, particularly in Southern Europe, where populations also display strong dormancy (Kronholm et al. 2012). It would be intriguing to compare the temperature-dependent effects of these unique DOG1 haplotypes.
The environmental dependence of genetic effects was also revealed in projections of germination timing using natural seasonal variation. The hotter conditions of Durham, NC were predicted to reveal dormancy differences between the two accessions (Col and Ler) that were invisible under the cooler conditions of Halle, Germany, suggesting that warming climates may reveal currently unexpressed genetic variation. Further, the expression of genetic variation depended on dispersal timing, as it was not visible in the early Durham dispersal cohort, but was in the later Durham dispersal cohort. Similarly, we found hints that the effect of seed maturation can influence whether allelic variation is adaptive in a given location or not. In Halle, our results suggested that selection on DOG1 is likely to depend on flowering time—warm-matured seeds germinated in the fall, whereas cool-matured seeds were too dormant to germinate during the fall. Thus, genetic variation in the timing of flowering could have an indirect effect on germination timing because of its influence on dispersal timing (Chiang et al. 2013; Donohue et al. 2005c).

**Potential effects of seed-maturation temperature on germination timing**

Strong selection for germination timing within a season has been documented for many species (Donohue et al. 2010). If a single optimal window for germination exists, then our results suggest that maturation effects could canalize germination time such that two dispersal cohorts germinate at more similar times than they would without plasticity to seed-maturation temperature. Specifically, seeds that are dispersed earlier in
the spring are also matured in cooler conditions and, thus, have higher dormancy levels, whereas seeds dispersed later in the spring are matured under warmer conditions and have lower dormancy. This response would be adaptive if it caused germination to occur closer to the optimal germination time. Since the optimum time for germination is seldom known, whether such maternal compensatory effects are adaptive remains to be tested empirically.

Germination has been documented to occur in autumn, spring, and summer, and some populations exhibit more than one germination cohort (L. Burghardt personal observation; Wilczek et al. 2010). If the observed variation in germination time is actually adaptive, this suggests that the optimum timing of germination varies geographically and possibly within or between years (Pico 2012). If more than one favorable window of germination exists, maternal effects could either canalize germination to occur during the soonest favorable window or, alternatively, could spread germination over multiple suitable germination windows (Montesinos-Navarro et al., 2011).

The first scenario is supported by our projections for summer germination times of low-dormancy genotypes—we predicted that they would germinate in the summer regardless of seed-maturation condition and dispersal time. The weaker maternal effects in these genotypes did improve germination synchrony within the summer, but different dispersal dates still resulted in germination timing differences of about two
weeks. This difference could still have strong fitness consequences (Donohue et al., 2010).

In contrast, maternal temperature effects on the Ler <i>DOG1</i> genotype determined which of three germination windows were utilized—warm maternal environments led to summer germination and cooler environments led to late fall/spring germination. Thus, maternal effects could cause diversification in germination time. In fact, the <i>DOG1</i> allele from Cape Verdi Islands may induce a dormancy level that is too strong to produce fall germinants in central Europe (Figure 7). However, in Durham (Figure 19), the <i>DOG1</i> genotype matured in cool conditions resulted in both fall and spring germinants as is observed in populations near Durham (L. Burghardt, personal observation) and in populations in Spain (Pico 2012). More knowledge of the demography of <i>A. thaliana</i> will be necessary to clarify which of these processes is operating in populations across its range.

Caveats and Future Directions

The simple projections implemented here are limited in several important ways. First, they ignore the potential effect of stratification cues on dormancy levels (Auge et al. in press; Footitt et al. 2014; Footitt et al. 2011). Second, these projections do not account for differences in germination speeds across environments. Furthermore, they do not account for diurnal changes in temperature throughout the day or moisture fluctuations and photoperiod changes throughout the year. The data necessary to
address several of these additional effects have been or are being collected and will be analyzed elsewhere. The coarse-grain of the maternal temperature effects implemented here could also limit the accuracy of the projections.

Lastly, our projections were geographically limited by a lack of knowledge of *A. thaliana* phenology in natural populations. For these particular projections, it was essential to know dispersal timing and the average temperature experienced during reproduction. It is also vital, but logistically challenging, to know when germination timing occurs in these populations. Field experiments that measure germination timing of seeds of known genotypes matured in known environments will be necessary to validate the hypotheses proposed here.

**Conclusions**

As the climate changes, there are multiple scenarios for how pre-and post-dispersal environments could alter germination phenology. As the length of the unfavorable season for growth (summer) is projected to lengthen, higher dormancy levels may be essential to prevent seeds from germinating at the wrong time. How climate change shifts the conditions experienced during seed maturation then becomes critical: if the temperature during seed maturation is increased, dormancy levels will decrease—the opposite of what is advantageous given longer unfavorable seasons. However, it is not necessarily the case that increasing overall temperatures will result in high temperatures during seed maturation. Flowering time of *A. thaliana* is also
extremely responsive to environmental conditions (Wilczek et al. 2010) and so by shifting flowering time temperatures during seed maturation may stay constant or even decrease with climate change. Such hypotheses require further testing both empirically and via modeling.

Natural variation in both flowering time (which determines the environment during seed maturation) and germination time exist in this species. As climates change, the expression of genetic variation for both of these traits will also change. For instance, if warming temperatures lead to less winter chilling, flowering times may become more variable for genotypes with a chilling requirement. For germination, our results suggest that warmer temperatures during seed maturation and after dispersal will have a stronger effect on germination timing for some genotypes than others. These results suggest that maturing and germinating plants in conditions that are similar to the seasonal conditions to which they’ve adapted may be important for discovering ecologically relevant natural variation in traits across the life cycle. Continued exploration of how multiple environmental factors shape the expression of allelic variation, as we have done here, is fundamental to understanding the capacity of organisms to evolve in response to changing climates.
2. Modeling the Influence of Genetic and Environmental Variation on the Expression of Plant Life Cycles across Landscapes

INTRODUCTION

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, and 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 year (Roff 1980; Roff 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 out-crossing 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 time scales (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 for plasticity of life-stage transitions can either magnify or reduce life-cycle variation across heterogeneous environments. Genetic variation in the environmental sensitivity of life-stage transitions 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 counter-gradient 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. 2005c; 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) genetic variation that could either augment or mask the sensitivity of a transition to those environmental factors; and 3) 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, *Arabidopsis 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 paper 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 phenological traits: 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 above ground 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 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 (Deren Eaton, personal communication) and flowering occurred in the 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 UK 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 21 for cartoon summaries of these observed life cycles.

Allelic variation occurs in genes that influence the timing of flowering and germination. Increasing 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 (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 (after-ripen), 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. At present, these models investigate the effects of climatic factors but not biotic factors such as inter/intra-specific 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 (Figure 8). We linked these sub-models 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 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.
Figure 8: 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.

**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. The simulation begins with a cohort of 1000 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 Online Appendix B: *Germination sub-model*). As the simulation proceeds, the rate of progression through each life stage depends on environmental inputs each hour (Figure 9a).

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; Online Appendix B: *Germination sub-model*). Seeds with different dormancy levels progress at different developmental rates towards 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 (Figure 9b; grey 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 (Figure 9c; for details, see Online Appendix B: *Flowering sub-model*). Once flowering occurs, reproductive plants
Figure 9: 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.

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accumulate progress toward seed dispersal at a rate influenced by temperature (Figure 9d; for details, see Online Appendix B: Seed dispersal sub-model). As soon as the seeds disperse, they are assigned a dormancy level based on the maternal parameters and begin progressing toward germination (Figure 9e). 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 i.e. 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 (Figure 9a): 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 to 10 years (for most genotypes), and also that results are robust to the date of initial seed dispersal.

The present 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 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 amplitude, but temperature is milder in Norwich due to proximity to the ocean (See Figure 22 in Appendix C for example of environmental inputs for each location and Appendix B for details on climate data sources and methods). We chose these locations not only because of the breadth of environmental conditions, 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 paper have been deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.nv0p1.
Parameterization

All sub-models were fit to the common laboratory genotype (Columbia) via chamber experiments or using estimates from the literature (see Table 2 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 sub-models were reasonable approximations, we tested each model on an independent dataset generated in chambers (Burghardt, unpublished data; see Appendix B for details). Because the germination model and after-ripening 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 Figure 23 and Figure 24 in Appendix C for results).

Parameter Levels that Re semble 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 ($F_i=.737$) and null ($F_i=.598$)
alleles of the *FLC* activator, *FRI*, expressed in the Col background in field conditions. We also explored an extremely high *F* 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 ($\Psi_{\text{mean}}$) 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 Figure 10-Figure 12 have been deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.nv0p1.
repression started to diverge, and flowering times spread notype than in the high-dormancy genotype. Variation in germination timing in the low-dormancy ge-
variation in dormancy within a single parameterization apart in fall (fig. 2

Flowering:

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| Model parameter descrip

Note: Parameter levels resembling the Columbia ecotype were used for the submodels of life-stage transitions. Mean dormancy level (mean) and initial floral repression (Fi) 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.
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 9 results from a single model run for four different parameterizations/genotypes in Valencia, Spain as they respond to the environment after being dispersed on March 10th. Immediately, the two low-dormancy genotypes started accumulating progress toward germination while the high dormancy ones did not (solid vs. dotted black line, respectively). However, because very little rain occurred in the summer, germination progress of non-dormant 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 apart in fall (Figure 9b). 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 three months (Figure 9c). 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 flowering individuals to “catch-up” in calendar time (Figure 9d). 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 nine 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 bi-model 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 2 for
parameters describing this genotype) produced remarkable life-cycle variation across European environments (Figure 10; see Figure 26 for life-cycle timing of all low floral repression genotypes). In Oulu, Finland, seeds germinated in late summer, initiated flowering either in the early fall or late spring (see Figure 27 for distribution of flowering times in all sites), and matured seeds in the summer, creating a life cycle with aboveground stages present most of the year. In contrast, in Valencia, overwintering plants spent a large portion of their lives as seeds; they germinated in late fall and flowered in early spring (Figure 10a). In Valencia there were also summer- and fall-flowering cohorts (Figure 27) leading to wide variation in life-cycle length (Figure 10b). In central Europe (Halle and Norwich), our model predicted this genotype would have flowering bouts in spring, summer, and fall (Figure 10a).

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 than 200 days at both of these sites (Figure 10b). This suggests the multiple flowering bouts were created via overlapping generations, not 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
Figure 10: 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 25 for a color version.

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 (Figure 10b). A diversity of phenological patterns can lead to similar life-cycle lengths although all do not occur in all locations (Figure 10c 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 Figure 28-Figure 29; for genotypes expected to occur in each location see Figure 30-Figure 33).

*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 *A. 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 (Figure 11a) 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 vs. long life cycle (Figure 36 and Figure 12b, 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 (Figure 11d), and a smaller effect on the reproductive interval (Figure 11e; see Figure 35-Figure 37 for frequency distributions for the durations of all component life stages). These results were robust to changes in three important germination model parameters (see Figure 23-Figure 24).

Genotypes with higher initial floral repression produced slightly more individuals expressing a winter-annual life cycle as compared to a spring- or summer-annual life cycle but this effect was highly dependent on dormancy level (Figure 38). We also tested whether a very high \( F_{i} = .88 \) 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
Figure 11: 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 Figure 34–Figure 37 for color versions of graphs and frequency distributions of c–e.
led to high proportions of winter rosettes, this was not always the case (see Figure 38, 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 (Figure 11). Overall, dormancy level strongly influenced 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 one year only occurred at the lowest dormancy level, despite the observation that multiple flowering bouts in a year can occur at many dormancy levels (Figure 12). 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 (Figure 11c,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 grey), mid dormancy in Halle and Norwich (dark grey), and high dormancy in Valencia (black) (Figure 11a). 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; Figure 12). In Oulu, a winter-annual life cycle occurred only at extremely low dormancy levels and was more pronounced with high floral repression levels (Figure 26, Figure 38). In Valencia, winter annuals occurred across all dormancy and floral repression levels (Figure 12, Figure 38), but were only limited to that life cycle at higher dormancy levels.

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 (Figure 12, Figure 38). 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 (Figure 33; this phenological instability also occurred at a few other location/parameter combinations). Lowering the dormancy level from $\Psi_{\text{mean}}$ 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 this parameterization in Oulu, Finland would result in a rosette-dominated, winter-annual life cycle (Figure 12, 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 (Figure 12, 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 (Figure 26).

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 cycles can occur in a calendar year even at high dormancy levels (Figure 12). Thus we predict both winter annual and multiple flowering bout life cycles are possible at middle latitudes and dormancy parameters will be key determinants of life-cycle expression.
Figure 12: 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 26. See Figure 39 for a color version.
DISCUSSION

Despite extensive knowledge of genetic variation in *A. 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 only tested one parameter related to winter chilling: initial floral repression. It is possible that other parameters such as time to winter chilling saturation (Shindo 2005) or temporal dynamics of vernalization that are not included in the model (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 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 one year despite the fact that we are likely underestimating life-cycle length by focusing on seed dynamics on the soil surface (i.e. we only model physiologically dormant seeds, not seeds that are dormant because of burial).

Lastly, 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 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 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 QTLs are environment-specific both in controlled environment (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 southern sites. By predicting multiple phenotypes, this approach could also aid in understanding geographic patterns of co-variation 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).

Lastly, 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 2007). 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 NILs in the Columbia background with alleles introgressed that alter flowering or germination timing. 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. Lastly, 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 intra-specific 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 which 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. Lastly, 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.
INTRODUCTION TO SENECEENCE

Senescence, at the demographic level, is measured as a decline in survival (or fertility) with age in a given environment and is observed in many species across the tree of life (Jones et al. 2014; Nussey et al. 2013) although it is certainly not ubiquitous particularly in plants (Baudisch et al. 2013). This at first seems surprising from an evolutionary perspective because we might expect strong selection for organisms to live and reproduce for longer and longer time periods. But as initially noted by Medawar, the size of a birth cohort inevitably declines with age (Medawar 1952), an observation which provides the basis for classical understanding of the evolution of senescence (reviewed in Flatt and Schmidt 2009; Roach 2003). The rarity of older individuals means that by chance, deleterious mutations that increase mortality or decrease fecundity in older individuals can go to fixation (‘mutation accumulation’) in a population. Similarly, genetic changes that favour survival or reproduction at younger ages at the expense of later survival or reproduction (‘antagonistic pleiotropy’) will be able to spread in the population because selection is not as strong in later cohorts (Williams 1957). In addition, recent models by Baudisch highlight the importance of trade-offs between survival and fecundity in determining the diversity of senescence patterns observed.
(Baudisch 2008). In this chapter we outline how seasonal environments and these trade-offs may influence patterns of survival with age.

Semelparous plants, where reproduction is fatal, are a useful system for exploring the potential importance of trade-offs in plant senescence because, in some sense, they reflect the extreme of the trade-off continuum: very high returns of fertility are thought to result in lack of investment in survival and therefore for extraordinarily high rates of senescence (Young 1990). However, recent research suggests that the timing of the onset and rate of senescence can vary even for semelparous plants. For instance, some species do not stop photosynthesizing when they begin reproducing, as would be expected if plants allocated all resources toward reproduction upon flowering. In fact, some semelparous plants gain the majority of their carbon while they are reproducing (Aschan and Pfanz 2003; Earley et al. 2009; Gammelvind et al. 1996). Therefore, even within a semelparous life cycle, continued ability to survive (delayed onset of senescence) will ultimately create more resources for reproduction and thus greater fertility. In sum, despite the fact that reproduction is fatal in semelparous organisms, there is still the potential for selection for later onset of senescence or changes in senescence progression.

Seasonal environmental variation can counter selection for longer reproduction and delayed senescence. Environmental conditions are not static in natural environments, and conditions that lead to low survival of reproducing plants (e.g. frosts
in winter, drought conditions in summer) occur with high predictability in some habitats. These changes in the environment can shift mortality and fecundity rates, potentially creating enormous selection to complete reproduction within a given time horizon. Therefore the environment can counteract selection for longer reproductive periods.

The length of time a plant has to reproduce is strongly influenced by the timing of flowering. Thus flowering time could also shape the conditions during which reproduction (and senescence) occurs (Burghardt et al. 2015). Intriguingly, genetic cross-talk has been found between genes that influence flowering time and those implicated in senescence at the whole rosette level (Wingler 2011). Because flowering time strongly depends on germination time for many annual species, genetic or environmental variation that influences germination time could also influence senescence dynamics. To our knowledge no one has yet explored this possibility. Here, we suggest that 1) the environmental context of reproduction and 2) the phenology of previous life stages may contribute to observed senescence patterns in semelparous, annual plants.

Due to their genetic and experimental tractability, plants provide an unparalleled opportunity to tease apart the complex factors that determine the relationship between environmental context and senescence. In this chapter, we use an integrated life-cycle model for A. thaliana to illustrate how seasonal and geographic variation and phenology shape the size of the window of opportunity for reproduction. These results suggest the
potential for the environment to mediate mechanisms underlying senescence. Next we report on recent research into mechanisms of semelparity and tissue-specific senescence processes, highlighting the environmental dependency of these mechanisms. We close by outlining next steps in evaluating the mechanisms by which the environment influences the evolution of senescence.

**SEMELPAROUS PLANTS AS A MODEL FOR THE ENVIRONMENTAL DEPENDENCE OF SENESCENCE**

Semelparity (also known as monocarpy in plants), or a life history where reproduction is fatal, generates a classic life-history paradox—Lamont Cole first suggested in 1954 that semelparity, rather than iteroparity (multiple reproductive bouts), should be the norm: a semelparous plant only needs to produce one more seed than an iteroparous plant to match its reproductive output (Cole 1954). Yet, both life histories can be observed in nature. Charnov and Schaeffer resolved the paradox, pointing out that survival of adults in many species is higher than survival of juveniles; consequently, returns from retaining adults are greater than producing one extra seed (1973). This theory suggests that the environment in which a species is found could influence whether it has a semelparous or iteroparous life cycle. As predicted, semelparous organisms are often found in contexts where survival of adults is low such as in dry lowlands (Kim and Donohue 2011) or in life cycles with risky long-distance migration as is the case in salmonid fishes (Crespi and Teo 2002). Additionally, evidence
has been found of repeated transitions to the semelparous habit in association with season-specific climate variation (Evans et al. 2005).

Beyond the intriguing evolutionary fact of their existence, the simplicity of the semelparous life cycle makes them a unique resource for studies of life-history evolution (Jong et al. 2000; Klinkhamer et al. 1996). Much of this research has been on the evolution of the timing of flowering. The length of time spent in the vegetative stage before flowering determines whether a plant is a semelparous annual (< 1 year) or a semelparous perennial (> 1 year). Because flowering is fatal, flowering time is both under strong selection and generally the result of a relatively straightforward trade-off. The longer individuals live before flowering, the larger they grow, and the more seeds they produce, due to an allometric relationship between size and seed output (Klinkhamer et al. 1992). However, if the delay before flowering is too long, there is a risk that individuals will die without ever having reproduced (Metcalf et al. 2003). Thus, given sufficient demographic data on a particular species, the size at flowering can be predicted with considerable accuracy (Rees et al. 2006).

As a semelparous plant, and a uniquely genetically well-characterized species, Arabidopsis thaliana provides the tantalizing possibility of linking these evolutionary predictions (Rees et al. 2006) to the underlying genetic mechanisms that mediate these fundamental trade-offs (Metcalf and Mitchell-Olds 2009). However, even for a species as well understood as A. thaliana, the complexity of the map linking genotype to phenotype...
complicates this effort. For instance, one allele does not result in one particular flowering phenotype; the current environment, the sequence of past environments, and the genetic context of the allele (i.e. epistasis) all shape the timing of flowering. Recently, a large body of experimental data, encompassing many genotypes and environments has been combined into mathematical models that allow accurate prediction of flowering time for a particular genotype (Wilczek et al. 2009). Similar methods have been used to predict flowering in other species (Satake et al. 2013) and other phenological transitions such as germination (Alvarado and Bradford 2002) and budburst (Chuine and Beaubien 2001). Due to these advances, *A. thaliana* is an ideal system for examining how phenological traits function together to influence the time available for a plant to reproduce.

**AN INTEGRATED LIFE-CYCLE MODEL TO EXPLORE PHENOLOGY**

To describe the link between phenology and the environmental context shaping the evolution of senescence, we developed an integrated life-cycle model that predicts phenology across generations by linking mathematical descriptions of how germination, flowering, and seed dispersal progress as a function of the environment (Burghardt et al. 2015). These phenology models are inspired by models originally used to aid crop production (Wang 1960). Mathematical functions are parameterized for a particular genotype and are used to calculate development based on the environment. Life-stage transitions occur when the organism accumulates enough progress to cross the developmental threshold.
We linked these individual sub-models, parameterized for A. thaliana such that the timing of seed dispersal determines the environment seeds experience and thus germination time (Figure 13a, third panel from top); germination time determines the environment rosettes experience and therefore flowering time (Figure 13a, second panel from top); flowering time determines the environment in which plants reproduce which determines dispersal timing (Figure 13a, top panel); and so on for many generations. These models are driven by hourly environmental inputs to capture how diurnal variation and extremes influence developmental rates (see Burghardt et al. 2015 for model details).

Genetic variation can be incorporated into these models by changing the parameters governing how a life stage responds to the environment. For instance, by changing the parameter that describes seed dormancy, we can mimic known natural variation among populations. Figure 13a shows three different dormancy genotypes all occurring in the same environment of Norwich, England. These changes in dormancy can cause large changes in life cycle: the low dormancy genotype ‘rapid cycles’ through multiple generations in a year, while a mid dormancy genotype displays a classic winter-annual life cycle by germinating in the fall and flowering in the spring. The most dormant genotype germinates and flowers in the spring as a spring annual.
Figure 13: Summary of integrated life-cycle model structure and results for genotypes simulated in Norwich, England.  

a, Lines in each panel track progress towards germination, flowering, and seed dispersal for genotypes reflecting three different levels of dormancy (low-solid: light green, medium-dashed: dark cyan, high-double dashed: navy) that were dispersed Apr. 1; symbols in each panel show relevant environmental drivers for each life stage; the bottom panel shows the underlying environmental conditions that drive the developmental progress.

b, Corresponding flowering times across a cohort of 1000 individuals averaged across the last 25 years of a 40 year simulation.

c, Life-cycle graphs showing the proportion of individuals in each of three key life stages averaged across the same 25 years. The distance from the centre of the circle indicates the proportion of individuals in the rosette (green), reproductive (gold), and seed (brown) stages each day of the year (January is at the 3 o’clock position and the year progresses clockwise). All plots are scaled so the outermost circle reflects 100% of individuals.

Using the model to simulate environmental variation and genetic variation that occurs across the native range of *A. thaliana*, we can derive flowering phenology of...
different *A. thaliana* genotypes in different environments (Figure 13b). The model is capable of capturing the full complexity of seasonal life histories observed in this species (Lawrence 1976; Pico 2012; Wilczek et al. 2009). This diversity of life cycles results in the occurrence of flowering at multiple times of year for some genotypes (Figure 13c) and suggests that the reproductive environment can vary between individuals of the same genotype.

We must note that the model makes no assumptions about environment-dependent resource acquisition, survival, or fertility rates of the organism or their relationship with senescence. Here our goal is to set some simple, constant rules that define how the survival of reproductive plants depends on the environment and determine how much variation in reproductive windows can occur based on the phenological variation expressed by this species.

**DEFINING SURVIVAL CRITERIA FOR REPRODUCTIVE *A. thaliana***

To explore selection on senescence, we also need to include information on seasonal drivers likely to result in mortality. These can be life-stage specific, such as freezing tolerance, which decreases after the transition to reproduction in *A. thaliana* (Richter et al. 2013; Seo et al. 2009). Based on an array of empirical evidence and experience in field cultivation of *A. thaliana*, we set post-flowering mortality to occur if the minimum daily temperature fell below -5°C; if the average daily temperature fell
below 0°C or exceeded 35°C; and if conditions were dry. Because drought is a chronic stressor that occurs over multiple days, progress towards death accumulated each day that moisture conditions were below a moisture threshold (-175 MPa) and reset to zero whenever moisture was above that threshold. Progress was also temperature-dependent and scaled via the function 0.00555×T˚C such that higher temperatures in dry conditions lead to more progress toward death. Plants died if the cumulative sum reached 1. We cut off assessment of reproductive interval at 150 days post-flowering, because five months of reproduction is unlikely in this species.

In reality, of course, these tolerances may not be exact. Furthermore, they are likely to vary among populations adapted to different locations. For instance, natural populations from Northern climes exhibit increased rosette cold tolerance in common garden experiments (Ågren and Schemske 2012). Ideally, we would develop a set of mortality conditions fully parameterized from field data for each of the locations and life stages, but datasets reflecting the natural tolerances of A. thaliana remain rare (Metcalf and Mitchell-Olds 2009), and this information is not available. However, because our purpose is to qualitatively assess (rather than quantify) how extreme seasonal conditions translate into selection on senescence in vastly different environments and for very different genotypes, these rules should provide some discriminatory power.
‘WINDOWS’ FOR REPRODUCTION ACROSS THE NATIVE RANGE

Environmental conditions relevant to the *A. thaliana* life cycle differ across both spatial and temporal scales (Burghardt et al. 2015). To visualize how much time is available for reproduction without incorporating the influence of phenology across generations, we used our mortality rules (see above) to determine the mortality dates for plants bolting on each day of the year. We did this for 40-year environmental replicates extracted from a global climate model for each of four locations across the European range of the species. We used climate data spanning the years 2000-2020 from the A1B scenario in IPCC fourth assessment (NOAA-GFDL 2004). See Burghardt et al. (2015) for details on environmental series. We expressed the results in terms of both days and thermal time available for reproduction. Thermal time was calculated as a linear increase in development above the base temperature of 3°C. Thermal time may be an important measure because the rate of seed development often depends on temperature (Ainsworth and Ort 2010), but the measures yield qualitatively similar patterns so we present results only in terms of days.

The timing of drought, frost, and extreme heat events that killed reproductive plants varied considerably across years (Figure 14; grey traces; mean across years black line), but in all sites, a range of flowering times existed that would permit the maturation of one or more fruits (which contains approximately 15 seeds) to mature (Figure 14; grey curves above the dashed horizontal line). Within this range, *A. thaliana* plants in
Norwich, England and Halle, Germany were predicted have the longest (in terms of days) opportunity for reproduction (Figure 14); Norwich had a broader range of dates with maximal reproduction than Halle due to milder winters and less summer drought. In Oulu, Finland, frosts restricted successful reproduction to late spring and summer. In Valencia, Spain, the longest opportunities for reproduction occurred early in the spring, as plants flowering in late spring and summer died quickly due to drought and heat. There also seems to be a window of opportunity for reproducing in the fall in Valencia; however this requires germination during the summer or surviving the summer as a rosette which is not observed (Pico 2012).

**LIFE-CYCLE PHENOLOGY DETERMINES WHAT PORTION OF ‘WINDOW’ IS USED**

These calculated seasonal windows for reproduction alone will not determine selection on senescence dynamics for A. thaliana in a particular environment – flowering and germination phenology will also be important, because they determine when during the window the plant will actually begin to reproduce. We used the integrated model outlined above to simulate how environmental context and genetic variation in seed dormancy combine to determine this phenology.

We simulated full life cycles for 40 years to obtain flowering times for each individual each year in each location. We omitted the first 15 years of simulations to allow populations to stabilize. We then projected the amount of time each individual
had available for reproduction (and thus senescence) given its predicted flowering time and our mortality rules. An array of evidence indicates that dormancy is variable even for identical genotypes and the model reflects this reality (Donohue et al. 2005c) so that each genotype is potentially reflected by a distribution of days of flowering; and we thus explore the distribution of times available for reproduction for each dormancy genotype.

To capture the full life cycle across generations, the model uses a temperature-dependent seed-maturation function to predict the timing of the release of the first 10% of seeds (Burghardt et al. 2015). This ends the reproductive phase, and initiates the next generation. Here, we are interested in the amount of time available for reproduction (and thus senescence) given the timing of flowering and the environmental context, and not the seed-maturation function that is currently implemented. Because the seasonal context of the life cycle canalizes some of the variation in germination and flowering phenology, these small changes in seed-dispersal time should not too greatly influence the full life cycle, as evidenced by matching between simulated life cycles and observed life cycles (Burghardt et al. 2015).

Environmental variation between sites had a large influence on time available for reproduction, and thus the optimal senescence strategy. Figure 15a shows the distribution of times available for reproduction for a low dormancy genotype simulated at all four sites. For this genotype, Valencia provided the fewest days for reproduction whereas Norwich provided the most. Both Halle and Oulu had bimodal distributions of
Figure 14: Environmentally determined “reproductive windows”, i.e., days available for reproduction if an individual were to flower on each day, given the assumptions about fatal environmental conditions described in the text, and using environments in each of the four locations extracted from a global climate model. Each grey line shows a different year (out of a total of 40 years) and the solid line indicates the mean. The dashed horizontal line indicates the amount of time needed for at least one fruit to mature (one A. thaliana fruit contains 10-20 seeds).
time available for reproduction. This bimodal pattern can occur for two reasons: 1) some portion of a spring flowering cohort may flower too early and be killed by a late season frost; surviving rosettes that have not yet flowered create the second hump; or 2) plants could be flowering at different times of year and therefore experiencing disparate seasons while reproducing (Figure 13 shows how multiple generations could occur in one year). Both dynamics have been observed in natural populations (Ågren et al. 2013; Pico 2012).

To address the importance of genetic variation in determining the reproductive environment, we predicted the life cycles of three different genotypes that varied in dormancy level—seeds with higher dormancy take longer to germinate (the same genotypes used in Figure 13a). Genetic variation was captured by a parameter based on empirical data: higher values increase germination repression until after-ripening, a moisture and temperature-dependent process, releases the repression (Bradford 2002).

Changing dormancy levels can also shift the amount of time available for reproduction and thus senescence (Figure 15b) (Gremmer, unpublished). At the lowest dormancy level in Valencia, Spain, all individuals had a small window for reproduction, but as dormancy level increased, germination occurred later in the fall, shifting flowering to later in the spring and increasing the time available for reproduction. At a medium dormancy level, most individuals flowered at a time corresponding to a very short window for reproduction; but there are some peaks of individuals with a longer
window corresponding to specific years where late frosts did not happen. These exceptions illustrate the importance of year-to-year environmental variation. As dormancy increased further, more individuals flowered at a time corresponding to a long window of reproduction.

Figure 15: The influence of phenology on “time available for reproduction”. a, Density plots of the estimated days remaining from when flowering occurs to a mortality event for every individual in 25 simulated years in each of four locations. All graphs show a low dormancy genotype. b, Genetic variation that influences germination timing also influences the “time available for reproduction”. Results are shown for three genotypes that vary in dormancy level in a single location: Valencia, Spain.
POTENTIAL FOR PLASTICITY OF SENESCENCE

In addition to its selective role in determining the time in which reproduction is possible, the environment has also been shown to influence the allocation trade-off between survival and reproduction (Earley et al. 2009; Remington et al. 2013). This is perhaps not surprising given that the age and size at flowering (Wilczek et al. 2009), photosynthetic rate and therefore resource acquisition (Gammelvind et al. 1996), seed production rate (Bannayan et al. 2003; Poggio et al. 2005), and survival (Ågren et al. 2013; Reinsdorf et al. 2013) all can depend on the environment. This research suggests that the existence of the trade-off between survival and reproduction may even be environmentally dependent within seasons, such that no trade-off exists if conditions are favourable and nothing limits development but, as conditions worsen, the trade-off emerges (Stearns 1992, see pp 84-89). Senescence will only be favoured in contexts where a trade-off exists.

Year-to-year variation in time available for reproduction (Figure 14; grey lines) suggests that if the environment occurring prior to or during reproduction is predictive of the length of the remaining reproductive window, we would expect the evolution of plasticity of senescence (Roff 2003). For instance, if long photoperiods are associated with increased probability of drought, plants that accelerate investment in reproduction (and thus decrease survival and increase senescence) in long photoperiods might have higher fitness. *A. thaliana* can continue to photosynthesize and gather resources long
after the transition to flowering (Earley et al. 2009), but this may divert resources from the reproductive process. Mechanisms that help ‘tune’ the allocation of resources to continued growth versus reproduction based on the length of the reproductive window could considerably increase fitness.

In addition to year-to-year variability, another perhaps more intriguing, selection pressure for plasticity in senescence also emerges here; because a single genotype can reproduce at multiple times of year (Figure 13; also reported empirically (Lawrence 1976; Wilczek et al. 2010)), individuals of a given genotype may experience very different conditions during reproduction even in the same year. For example, cohorts that flower in the late spring in Halle have more time for reproduction than those that flower in late summer (Figure 14b). Consequently, populations that flower at multiple times of year may be under selection for increased environmental sensitivity of senescence. In contrast, those populations that reproduce in only one season would be expected to optimize senescence dynamics to a single context. Experimental research could test whether plants from locations where reproduction occurs in multiple seasons senesce differently in response to environmental cues compared to those that reproduce in a single season.

MECHANISMS OF SEMELPAROUS SENESCENCE IN A. THALIANA

In the future, it will be extremely interesting to extend the modelling results above to incorporate explicit molecular mechanisms that lead to senescence in A.
*thaliana*, but unfortunately the mechanisms that lead to whole organism death are just beginning to be established in plants (Davies and Gan 2012). Below we highlight progress to date.

Recently, it has been shown that semelparity in *A. thaliana* is attributable to the combination of three major developmental processes (Davies and Gan 2012). The first component is the death of somatic organs and tissues (*e.g.* leaves) and remobilization of those resources to new organs, reproductive structures, and storage tissues (Barth et al. 2006). This decline in function of individual tissues or organs rather than that of the entire organism is often referred to as ‘senescence’ in the physiological and molecular literature (Thomas 2013), an inconsistency in terminology across disciplines that can be confusing (Nooden, unpublished). This tissue ‘senescence’ of leaves and rosettes has been extensively studied – yielding evidence for variation among natural populations (Balazadeh et al. 2008a) and determination by numerous highly regulated genes, controlled at the chromatin and transcription as well as the post-transcriptional, translational, and post-translational levels (Lim et al. 2007; Woo et al. 2013). In addition, microarray studies have identified hundreds of genes associated with tissue senescence (*e.g.* SAG12 Grbic 2003; Hensel et al. 1993) and commensurate shifts in metabolism (Watanabe et al. 2013). While these processes may well contribute to, or be correlated with, the decline in whole organism survival with age (senescence in the evolutionary sense), they are clearly only part of the story.
In addition, semelparity in *A. thaliana* requires both the suppression of axillary meristems to prevent the formation of new shoots and the arrest of reproductive meristems. As plants grow they leave behind axillary meristems that generally remain inactive unless the primary meristem is damaged or the shoot apical meristem is too far away spatially to repress differentiation. In *A. thaliana*, the gene *AtMYB2* has been shown to actively suppress auxiliary branches specifically late in development (Guo and Gan 2011). When this gene is mutated, plants continually generate new branches and this extends the reproductive life span.

Ultimately these individual processes combine across multiple levels of organization to lead to the complex trait of whole organism senescence (Davies and Gan 2012). The precise manner in which this integration occurs is an active area of research.

**EVIDENCE FOR ENVIRONMENTAL SENSITIVITY OF TISSUE SENESCENCE**

A theme that emerges from the research highlighted above is that tissue senescence is highly dependent on the environment (reviewed in Lim et al. 2007; Quirino et al. 2000; Thomas 2013; Woo et al. 2013). For example, 30% of transcription factors associated with leaf senescence were differentially expressed in response to environmental cues (Balazadeh et al. 2008b). See Figure 16 for a summary of environmental factors known to be relevant for various tissues in *A. thaliana*. Environmental dependency of tissue senescence has also been found at the level of
individual organs in numerous other plants (Gombert et al. 2006; Li et al. 2000; Meng et al. 2013).

Figure 16: Genetic evidence for the environmental dependence of the senescence process. a, Schematic of known connections between the environment tissue senescence. The environment can influence senescence directly or indirectly through altering other developmental processes such as flowering time that have cascading effects on rosette senescence. While the senescence of individual tissues clearly contribute to whole-plant senescence, the exact relationship between this process and the other major components of whole-plant senescence remains murky as well as, how and if environmental factors influence these processes. b, Table of environmental cues and examples of the downstream genes they influence in *A. thaliana*. Examples are only provided for leaf and rosette senescence, as those tissues are the most extensively studied.

### PROGRESS ON MECHANISMS IN ITEROPAROUS SPECIES

The same seasonal environmental scenario described for semelparous plants also plays out for iteroparous plants each year when they reproduce. Progress is being made on the mechanisms underlying iteroparous repeated flowering. Most species (e.g. *Lenolium* or *Malus*), retain vegetative or quiescent axillary meristems during...
reproduction (Foster et al. 2003; Onishi et al. 2003) while a few species such as *Impatiens balsamina*, have reproductive meristems that can revert back to vegetative growth (Albani and Coupland 2010).

Comparing iteroparous mechanisms to those known in semelparous life cycles has proven enlightening (Albani and Coupland 2010; Andres and Coupland 2012). For instance, in an iteroparous species related to *A. thaliana*, a portion of meristems each year does not transition to flowering, allowing growth in the subsequent year. The genes *PEP1* and *AhFLC* that are orthologs of genes important to the flowering transition in the annual *A. thaliana* are key to this process (Aikawa et al. 2010; Wang et al. 2009). These similarities in the context of such a fundamentally different life cycle may provide leverage to separate the effects of selection for maximizing reproduction during a particular time interval from the effects of mutation accumulation that reduce function as an organism ages (Brommer 2014; Pujol et al. 2014).

CONCLUSIONS

We find that under reasonable assumptions about seasonal drivers of mortality, the window for reproduction (and therefore senescence) in a model semelparous species is location dependent. This finding suggests that there is the opportunity for selection to shape how fast *A. thaliana* diverts resources from maintenance and survival to seeds after the initiation of reproduction. Additionaly, we find that genetic variation that influences the timing of an early life-stage transition (germination timing) can have
ramifying effects on the time available for reproduction within each environment. This suggests that the optimal pattern of senescence will depend on phenology, and further, that genetic variation in early life-stage traits may influence selection on senescence or vice versa. Our model analysis therefore suggests the testable hypothesis that germination alleles may have correlated effects on senescence.

Molecular evidence in *A. thaliana* emphasizes the environmental dependence of many of the sub-processes that ultimately lead to senescence. This research also points to high level of regulation and control of these systems. Experiments that focus on how tissue-specific senescence contributes to whole-plant senescence, as well as the molecular basis and environment dependence of trade-offs between survival and fecundity, will be particularly enlightening. In sum, both molecular and modelling work suggest that the environmental dependency of trade-offs may play an important, but under-explored role in determining observed senescence patterns in semelparous, annual plants.

Both the modelling and our review of molecular mechanisms suggest the potential for environmental sensitivity (plasticity) of the allocation of resources to survival vs. reproduction (with its accompanying effects on senescence). In animals, plasticity of senescence is commonly reported (Libert et al. 2007; Mair et al. 2003) and elucidating the genetic basis of these processes was recently identified as a major direction for future work (Flatt and Schmidt 2009). In plants there is also evidence for
plasticity in longevity (Borges 2009), and there is empirical support for drought-dependent senescence in three semelparous species. Desert plant populations accelerated senescence in response to drought whereas Mediterranean populations did not (Aronson et al. 1992). Of course, environmental dependence of senescence may not be adaptive as it may also reflect non-adaptive plasticity (developmental constraints). For example, in red deer exposure to harsh early life conditions constrained development and exacerbated the aging process (Nussey et al. 2007).

**FUTURE DIRECTIONS**

There are a number of challenges in exploiting the wealth of knowledge on *A. thaliana* to understand life-history evolution. In particular, its ecology in natural settings is relatively poorly known, there are few demographic datasets in natural habitats (but see: (Pico 2012)), and knowledge of the behaviour of below-ground seed and germination dynamics is especially rare. Because our results indicate that genetic changes that underlie life-stage transitions occurring much earlier than senescence can have a large influence on time available for reproduction, more detail on this part of the life history would be of great value.

Many intriguing avenues for investigating the factors that influence the evolution of semelparity and inevitable senescence remain to be explored. As data sets become available on phenology and demography of natural populations of this species, the predictions of our model can be tested. Further, our modelling approach could be
modified to explicitly reflect potential environment-specific trade-offs and
commensurate changes in senescence to test adaptive hypotheses. Exploration of the
genetic and developmental basis of the survival/reproduction trade-off in this species
would greatly aid this endeavour. Lastly, another key direction includes selection
experiments that test the response to selection for both late life survival and
reproductive rate simultaneously.
Conclusion

OVERALL SUMMARY

Throughout the research that comprises my dissertation, I have sought to understand the processes that generate phenotypic variation. I looked at these processes through the lens of life cycles where transitions between life stages are often influenced substantially by both environmental and allelic variation. In the previous chapters, I explicitly considered how pleiotropy across life stages within and between generations, environment-dependent allelic effects, and epistasis generate patterns of life-cycle variation across ranges.

My first chapter showed that phenotypic variation is the outcome of interactions among genotypes and environments experienced in more than one generation. Specifically focusing on how flowering time and dispersal time of a previous generation can influence germination behavior, I conducted an experiment that showed that many combinations of genetic, environmental, and developmental factors can create similar germination phenotypes, and that maternal effects can have a larger influence than genotype on phenotype differences. Lastly, these maternal effects can reduce variation in germination timing, even when there is variation in flowering and dispersal time.

When the entire life cycle is considered, I find that environmental variation is a major driver of phenotypic variation across the range, and incorporating known geographic patterns of allelic variation improves the match of life cycles to those
observed across the range. Additionally, I show that variation in dormancy generated in the previous generation causes both winter-annual and summer-annual life cycles to be expressed by the same genotype in some locations. Lastly, I find evidence that counter-gradient variation may explain an observed latitudinal cline in seed dormancy levels. Thus, genetic variation in one life-stage transition is necessary to maintain phenotypic stasis of life-cycle length given environmental variation across the range. To my knowledge, this is the first comprehensive examination of how allelic variation shapes geographic variation in the expression of life cycles in this species.

Finally, I consider how the timing of germination determines the environment in which a plant reproduces and senesces and thus the time available for reproduction. I find that the relationship between germination time and duration of reproduction varies across the range. Low dormancy genotypes at mid-latitudes have more time for reproduction than those at either high or low latitudes, and in southern locations increasing dormancy level increases the duration of reproduction. Further, within a location I show that there can also be wide variation in reproductive lengths caused by differences in germination timing. If trade-offs between survival and fecundity depend on the environment, we might expect rates of senescence to also be environmentally dependent—a hypothesis bolstered by the observation of environment-dependent senescence in this species.
In sum, I have advanced our understanding of how life cycles operate in seasonal environments by demonstrating empirically the importance of pleiotropy and environment-dependent allelic effects. In tandem, I have created a modeling framework that allows the prediction of the complex life cycles these processes generate across a species range. The development of this framework is a big step forward because it allows prediction of the environments that each particular life stage experiences across ranges and how genetic variation alters those environments (Appendix D). Lastly, my research generated testable hypotheses concerning pleiotropic effects between life stages both within and between generations and laid the groundwork for future explorations of how life cycles evolve.

FUTURE DIRECTIONS

There are numerous ways to move forward in our understanding of life-cycles. On a practical level, much additional information about *A. thaliana* life cycles and fitness in natural environments will be important. In terms of the larger questions, particularly important avenues for further research include: the sources of life-cycle variation within populations, the evolution of distinct life-cycle strategies, and resilience of life cycles to environmental perturbations. Ultimately, incorporating environment-dependent demography and projecting responses to climate change will allow projections of future genotype-specific growth rates across the range. Each of the areas of future development is highlighted in turn below.
First, numerous hypotheses suggested by the model can be validated via field and chamber experiments, increased knowledge of *A. thaliana* phenology in natural populations, or population cage experiments that allow the tracking of known genotypes in seasonal environments. There are also additional dynamics that can be incorporated in the life-cycle model. Good candidates include nutrient availability (Guilband et al. 2015), temperature-dependent dormancy (Chapter 1, this dissertation), secondary dormancy dynamics (Auge et al. in press, Springthorpe in review) and biotic interactions (Metcalf et al. in press).

Second, extensive life-cycle variation is often observed within populations as well as between populations, but little is known about what generates this variation in a given population. One obvious source can be genetic variation within a population, but there are also additional mechanisms such as micro-environmental variation across a location, bet-hedging, and developmental stochasticity (Childs et al 2010; Fehrmann et al. 2013; Simons and Johnston 2006). At the extreme end of the spectrum, seasonal variation alone (Kivela et al. 2013) can lead to polyphenisms, where one genotype can create multiple discrete life-history strategies over the year (as has occurred with the dry and wet season morphs observed in butterflies; Brakefield and Reitsma 1991; Prudic et al. 2015). A general understanding of how this life-cycle variation is initiated and perpetuated across life stages is in its infancy.
In this dissertation, I have contributed to the conversation about the sources of life-cycle variation by showing that once a timing difference between two individuals is expressed, that variation can be augmented or reduced across subsequent life-stage transitions depending on the seasonal environment. For example, spring flowering is often far more synchronized than fall germination. In this process, plants can germinate many months apart, but still flower at almost the same time in the spring. This occurs because development occurs so slowly in the late fall and winter due to strong floral repression that once that repression is lifted by cold temperatures and as warm temperatures and long photoperiods arrive in the spring, the initial developmental differences are dwarfed by the speed of spring development (Burghardt et al. 2015; Springthorpe and Penfield, in review). This process can also be observed in the lab: phenotypic variances are higher when development occurs more slowly (see Suter et al. 2014 for example with winter chilling).

Similarly, some environmental contexts can magnify phenotypic differences that initially are quite small. This often occurs when certain cues are absolutely required for a transition to occur (as in the obligate winter chilling requirement in *Campanula*) or when developmental rates are slowing due to seasonal changes. For instance, in the fall a nine-day difference in germination time determines whether plants flower in the fall or wait until the spring (Wilczek et al. 2009). These findings provide a starting point for future examinations of the processes that generate life-cycle variation.
For example, little is currently known about how populations evolve from exhibiting one life cycle to another (e.g. from a winter-annual life cycle to a summer-annual life cycle). Are allelic changes with big effect sizes required for such a transition or can a small change lead to a massive life-cycle shift? Does the size of the change necessary to generate the shift vary across locations? And lastly, if the required change is large, is the life cycle destabilized by allelic changes with smaller effect sizes? Recently, interest and progress toward understanding transitions between annuals and perennials and iteroparity and semelparity (see Chapter 3) suggest that this is a field ripe for future development.

Similarly, little is known about the resiliency of life cycles to environmental perturbations. I have shown that the life cycle expressed during one year can influence the life cycles of subsequent generations. A reasonable question is to ask how long those effects last. Years with extreme environmental variations can potentially have cascading effects for many generations. Due to the difficulty of experimentation, however, longer-term effects of environmental perturbations on life cycles have rarely been studied, making this a perfect question to be addressed with a model. Understanding year-to-year environmental variation has important implications because inter annual environmental variation is predicted to increase due to climate change.

Obviously, incorporating more sophisticated environment-dependent demographic processes in the model such as stage-specific survival and fecundity is an
important next step. The biggest hindrance to this goal is parameterizing the model with realistic fitness functions. Once this is accomplished, we can ask how important survival and fertility processes are to shaping expressed life cycles. We can also describe genotype-specific fitness across the range and ask if genotype combinations known to exist in certain areas of Europe have the highest fitness according to the model. Using these results, we can also inquire which demographic processes and environmental tolerances have the largest effect on population growth rates.

Finally, another long-term goal is to project *A. thaliana* life cycles in future climates. Methods for considering how species will respond to changing climates are continually getting more sophisticated with models now considering plasticity and local adaptation (Fitzpatrick and Keller 2015), metabolic theory (Kearney et al. 2009), and process-based models (Morin et al. 2007). Running the ILC model in future climates will contribute to this increase in sophistication and allow predictions of whether phenology, pleiotropy, and stage-specific environmental exposures will change with climate. Due to the extreme plasticity of the *A. thaliana* life cycle, it is possible that, while the timing of life stages will change, the environments experienced by each life stage will remain constant. When combined with knowledge of demographic processes described above, it may be possible to predict how much evolutionary change will be necessary for populations to remain in the same locations as climates shift.
Appendix A: Additional Figures and Tables Chapter One

Figure 17: Summary boxplots describing how experimental factors influenced germination proportion, pooled over other treatments. Factors include genotype (a), seed age (b), maturation temperature conditions (c), and germination temperature (d). Boldface lines indicate the median, boxes encompass the 1st-3rd quartiles, and points denote outliers that were more than 1.5 times the 1st and 3rd quartiles.
Figure 18: Boxplots summarizing the extent to which the behavior of *LerDOG1*, *Col*, and *LerFLC* diverged from *Ler* at each of the germination temperatures. Effect size differences between *Ler* and each genotypic contrast were extracted from a bias-reduced glm (proportion data) model run at each germination temperature on maturation condition and after-ripening subsets. Numbers indicate the number of treatments (out of 12 total) in which genotypic differences were significant (for likelihood ratio tests regular glms were used). Significance levels were corrected for false discovery rate to the P = 0.05 level.
Figure 19: Germination projections of all combinations of seed-maturation and post-dispersal environments for each genotype in Durham, NC, USA. Boldface combinations are the matched combinations of seed-maturation temperature and dispersal time that occur in nature, while narrow lines are the mismatched combinations. Solid and dashed lines refer to April and May dispersal respectively while navy and gold refer to cool and warm seed-maturation temperatures.
Table 3: Likelihood ratio tests for the significance of the Temp x AR term for each combination of genotype and seed-maturation temperature. Sequential Bonferroni was used to correct p-values for multiple tests (12). Temperature envelopes for germination changed with after-ripening for every combination of genotype and seed-maturation temperature.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Maturation temperature</th>
<th>DF</th>
<th>LR. Chisq</th>
<th>P-value</th>
<th>Corrected P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>Hot</td>
<td>9</td>
<td>102</td>
<td>5.33E-18</td>
<td>2.13E-17</td>
</tr>
<tr>
<td>Ler</td>
<td>Warm</td>
<td>9</td>
<td>295</td>
<td>2.37E-58</td>
<td>2.13E-57</td>
</tr>
<tr>
<td>Ler</td>
<td>Cold</td>
<td>9</td>
<td>430</td>
<td>6.34E-87</td>
<td>7.61E-86</td>
</tr>
<tr>
<td>LerDOG1</td>
<td>Hot</td>
<td>9</td>
<td>314</td>
<td>2.46E-62</td>
<td>2.71E-61</td>
</tr>
<tr>
<td>LerDOG1</td>
<td>Warm</td>
<td>9</td>
<td>174</td>
<td>6.82E-33</td>
<td>4.09E-32</td>
</tr>
<tr>
<td>LerDOG1</td>
<td>Cold</td>
<td>9</td>
<td>61</td>
<td>8.85E-10</td>
<td>1.47E-09</td>
</tr>
<tr>
<td>LerFLC</td>
<td>Hot</td>
<td>9</td>
<td>129</td>
<td>2.34E-23</td>
<td>1.17E-22</td>
</tr>
<tr>
<td>LerFLC</td>
<td>Warm</td>
<td>9</td>
<td>95</td>
<td>1.46E-16</td>
<td>4.37E-16</td>
</tr>
<tr>
<td>LerFLC</td>
<td>Cold</td>
<td>9</td>
<td>306</td>
<td>1.78E-60</td>
<td>1.78E-59</td>
</tr>
<tr>
<td>Col</td>
<td>Hot</td>
<td>9</td>
<td>202</td>
<td>1.58E-38</td>
<td>1.11E-37</td>
</tr>
<tr>
<td>Col</td>
<td>Warm</td>
<td>9</td>
<td>268</td>
<td>1.71E-52</td>
<td>1.37E-51</td>
</tr>
<tr>
<td>Col</td>
<td>Cold</td>
<td>9</td>
<td>61</td>
<td>7.33E-10</td>
<td>1.47E-09</td>
</tr>
</tbody>
</table>
Table 4: Likelihood ratio tests and AIC differences testing for significant Genotype x Maturation x Temperature x After-Ripening interactions for germination probability. A model with the interaction was compared to a model without the interaction but with all other factors and interactions. Tests were conducted on the full dataset, and then for each genotype compared to Ler.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>DF</th>
<th>AICc</th>
<th>LR Chisq</th>
<th>Pr&gt;Chisq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>54</td>
<td>-16</td>
<td>142</td>
<td>&gt;.000001</td>
</tr>
<tr>
<td>Ler vs Col</td>
<td>18</td>
<td>18</td>
<td>23.8</td>
<td>0.16</td>
</tr>
<tr>
<td>Ler vs. Ler&lt;sub&gt;FLC&lt;/sub&gt;</td>
<td>18</td>
<td>12</td>
<td>64.3</td>
<td>0.034</td>
</tr>
<tr>
<td>Ler vs Ler&lt;sub&gt;Dog1&lt;/sub&gt;</td>
<td>18</td>
<td>-22</td>
<td>64.3</td>
<td>&gt;.000001</td>
</tr>
</tbody>
</table>

Table 5: Likelihood ratio tests and AIC differences testing for significant Mat x Temp x AR interactions for germination probability of each genotype. Results are shown for models with and without the Mat x Temp x AR interaction. Seed-maturation temperature modulated how temperature envelopes changed with after-ripening for every genotype.

<table>
<thead>
<tr>
<th>Subset</th>
<th>DF</th>
<th>AICc</th>
<th>LR Chisq</th>
<th>Pr&gt;Chisq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>18</td>
<td>-81</td>
<td>122</td>
<td>&gt;.000001</td>
</tr>
<tr>
<td>Ler&lt;sub&gt;FLC&lt;/sub&gt;</td>
<td>18</td>
<td>-63</td>
<td>104</td>
<td>&gt;.000001</td>
</tr>
<tr>
<td>Ler&lt;sub&gt;Dog1&lt;/sub&gt;</td>
<td>18</td>
<td>-58</td>
<td>100.25</td>
<td>&gt;.000001</td>
</tr>
<tr>
<td>Col</td>
<td>18</td>
<td>-35</td>
<td>76</td>
<td>&gt;.000001</td>
</tr>
</tbody>
</table>

Table 6: Likelihood ratio tests and AIC differences testing for significant Geno x Temp x AR interactions for germination probability of each seed maturation subset. Results are shown for models with and without the Geno x Temp x AR interaction. Genotype modulated how temperature envelopes changed with after-ripening for every seed-maturation subset.

<table>
<thead>
<tr>
<th>Subset</th>
<th>DF</th>
<th>AICc</th>
<th>LR Chisq</th>
<th>Pr&gt;Chisq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cool</td>
<td>27</td>
<td>-37</td>
<td>99</td>
<td>&gt;.000001</td>
</tr>
<tr>
<td>Warm</td>
<td>27</td>
<td>-128</td>
<td>190</td>
<td>&gt;.000001</td>
</tr>
<tr>
<td>Hot</td>
<td>18</td>
<td>-48</td>
<td>110</td>
<td>&gt;.000001</td>
</tr>
</tbody>
</table>
Appendix B: Phenology and Environmental Model Details

GERMINATION SUB-MODEL

Temperature and moisture are major seasonal factors that determine germination timing in combination with the depth of primary dormancy (Gutierrez et al. 2007; Holdsworth et al. 2008; Graeber et al. 2012). To project the timing of germination we used a hydrothermal germination sub-model that incorporates both dormancy variation within cohorts (Alvarado and Bradford 2002) and a function describing the rate of primary dormancy loss (Bair et al. 2006). In the hydrothermal sub-model, progress towards germination is a function of hourly soil temperature, moisture level, and dormancy level which reduces germination progress (Finch-Savage and Leubner-Metzger 2006; Batlla and Benech-Arnold 2010). Each hour, cumulative progress towards germination is summed until the germination threshold is reached. In general, germination progress is faster at higher temperatures and higher moisture levels (up to the optimum temperature and saturation point, respectively). Hydrothermal models perform well in field conditions, particularly for the earliest 70% of germinants (Hardegree and Van Vactor 1999; Meyer and Allen 2009).

A standard feature of many germination models, strongly supported by empirical studies of A. thaliana, is that genetically identical offspring start life with a distribution of initial dormancy levels (Bradford 2002; Finch-Savage and Leubner-Metzger 2006; Hardegree 2006). We model the primary dormancy distribution of
offspring as being determined by maternal parameters (genotype) and insensitive to maternal environment. By explicitly modeling this variation, we incorporate a ubiquitous phenomenon known to occur in *A. thaliana* and many other species. Traditional germination models use populations of seeds as the unit of analysis. Our approach differs in that we track the behavior of individual seeds. This formulation does little to change the quantitative results, but has many practical advantages. For instance, variation in initial primary seed dormancy may be defined according to any state distribution. Most importantly, it allows us to keep track of individual seed fates across generations.

Hydrothermal models are based on empirical observations that hold across many species: 1) In response to lower moisture levels during imbibition, fewer seeds germinate and they do so at a slower rate. 2) Seeds within a population do not all respond to moisture conditions in the same way. 3) Germination rates increase up to an optimum temperature and subsequently decline. These observations suggest that each seed has a base water potential ($\Psi_b$), analogous to a base temperature, below which it cannot make progress toward germination. During germination at sub-optimal temperatures, seeds generally maintain the same base moisture for germination ($\Psi_b$) regardless of temperature, but at supra-optimal germination conditions, the base moisture level ($\Psi_b$) increases with increasing temperature, slowing germination rate. The following equation formalizes these observations allowing computation of the
hydrothermal units accrued toward germination each hour \( \text{HTU}_{\text{Germ}}(\tau) \) (adapted from Alvarado and Bradford 2002):

\[
\text{HTU}_{\text{Germ}}(\tau) = \begin{cases} 
\left(\Psi(\tau) - \Psi_b\right) \times \left( T(\tau) - T_{b,g} \right) & \text{when } T_{b,g} < T(\tau) \leq T_0 \text{ and } \Psi_b < \Psi(\tau) \\
\left(\Psi(\tau) - m \Psi_b\right) \times \left( T_0 - T_{b,g} \right) & \text{when } T(\tau) > T_0 \text{ and } m \Psi_b < \Psi(\tau) \\
0 & \text{otherwise}
\end{cases}
\]

The first function is used at sub-optimal temperatures and the second function is used at supra-optimal temperatures. \( T \) and \( \Psi \) are the temperature (°C) and moisture (in MPa) during a given hour. \( T_o \) is the optimal temperature for germination (that which maximizes the germination rate). \( T_{b,g} \) is a constant parameter indicating the temperature below which no progress toward germination occurs. Analogously, \( \Psi_b \) is the base moisture level of a particular seed below which no progress toward germination occurs.

At supra-optimal temperatures, the \( \Psi_b \) of a seed is modified based on how much the current temperature is above the optimum temperature, according to the following equation:

\[
m \Psi_b(\tau) = \Psi_b + k_T (T(\tau) - T_0)
\]

\( k_T \) is an empirically derived scalar that defines the shift in \( \Psi_b \) for each degree the current temperature is above the optimal temperature. We estimated \( k_T \) from a batch of after-ripened Columbia seeds (Burghardt, unpublished data) by measuring germination across a range of high temperatures.

Germination progress is summed each hour until the germination threshold \( (\Theta_{\text{Germ}}) \) is reached and the germination transition occurs.
We compared the predictions of the standard hydrothermal germination model with parameters derived from the *A. thaliana* literature to a batch of Columbia seeds that were 19 weeks old matured in 20°C (Figure 20). At this age, seeds have lost most of their primary dormancy. In our model, there is a minimum dormancy level ($\Psi_{\text{min}}$, set to -1) and therefore as primary dormancy is lost, $\Psi_b$ values in a seed cohort start to pile up at -1 leading to a skewed dormancy distribution. Therefore we used a Poisson distribution as an estimate of the dormancy distribution at this age (Fig B1a). Fig B1b shows our predictions superimposed on the experimentally measured germination times. Note that the current model consistently overestimates time to germination for Columbia seeds at cool temperatures, but the standard model is not flexible enough to accommodate this pattern. Interestingly, these predictions at cold temperatures are a much better match to the germination behavior of another German ecotype, Landsberg.

In *A. thaliana* and many other species, dormancy levels change with age via a process called after-ripening (Carrera et al. 2007; Finch-Savage et al. 2007; Iglesias-Fernández et al. 2011). This process is rarely incorporated in germination models (for exceptions see Batlla and Benech-Arnold 2003; Chantre et al. 2010). We combined the germination model above with a primary dormancy loss function originally developed for *Bromus tectorum* (Bair et al. 2006; Meyer and Allen 2009). In the hydrothermal germination model outlined above, the base water potential ($\Psi_b$) of the seed modifies the
developmental rate and is therefore considered a measure of the dormancy level of the seed (Bradford 2002; Batlla and Benech-Arnold 2010). If $\Psi_b$ is above 0, no progress towards germination can be made in any environmental condition. Empirical data demonstrate that $\Psi_b$ values for a population of seeds decreases as primary dormancy is lost and this is also true in A. thaliana (Footitt et al. 2011; Burghardt, unpublished data).

After-ripening models describe mathematically how $\Psi_b$ changes over time after seeds are shed. We model the process of after-ripening (primary dormancy loss) as a gradual, unidirectional loss of dormancy, with the rate of dormancy loss dependent on hourly soil surface temperature $T(\tau)$ and moisture $\Psi(\tau)$ conditions. This model was created for Bromus tectorum because there are little data available for A. thaliana. As described in the following equation, each hour the amount of after-ripening time accrued is calculated and added to the running sum of the after-ripening ($AR_{\text{Cum}}$) experienced since seed dispersal.

$$AR_{\text{Cum}}(\tau + 1) = \begin{cases} AR_{\text{Cum}}(\tau) + T(\tau) - T_{b,ar} & \text{when } \Psi_{\text{max}} \geq \Psi(\tau) \geq \Psi_u \\ AR_{\text{Cum}}(\tau) + \frac{\Psi - \Psi(\tau)}{(\Psi - \Psi_u)}(T(\tau) - T_{b,ar}) & \text{when } \Psi_i < \Psi(\tau) < \Psi_u \\ AR_{\text{Cum}}(\tau) & \text{when } \Psi(\tau) \leq \Psi_{\text{max}} \text{ or } \Psi(\tau) \leq \Psi_i \end{cases}$$

$\Psi_i$ is the lower moisture limit below which no after-ripening occurs; $\Psi_u$ is the moisture level at which after-ripening effectiveness levels off. In response to temperatures rising above the base temperature for after-ripening ($T_{b,ar}$), after-ripening rate increases linearly. In response to moisture, after-ripening rate follows a broken stick model, such that at extremely low moisture, no after-ripening occurs; above that, the
rate increases linearly up until a saturation point ($\Psi_u$). High temperatures in moist conditions are the most effective at promoting after-ripening. After-ripening does not occur in extremely moist conditions ($>\Psi_{max}$). This model is taken directly from Bair et al. (2006) and has not been fit directly for *A. thaliana*.

Next, we used the number of days ($d_{sat}$) it takes seeds to go from 0 $\Psi_b$ to -1 $\Psi_b$ under lab storage conditions—room temperature (20°C) and dry (<-200 MPa) conditions and the equation above to calculate the cumulative after-ripening that occurred over that time period. Because the model works in hours not days, $d_{sat}$ is multiplied by 24.

To determine the proportion of after-ripening completed and the current seed dormancy level ($\Psi_b$), the current cumulative after-ripening sum is divided by a cumulative after-ripening sum that leads to a known amount of dormancy loss in the lab.

$$\Psi_b(\tau) = \begin{cases} \max(\Psi_i - \Psi_{scale} \frac{AR_{sum}(\tau)}{AR_{sum}(lab ~ conditions) \times d_{sat} \times 24}, \Psi_{min}) & \text{if } \Psi_b(\tau) > \Psi_{min} \\ \Psi_{min} & \text{if } \Psi_b(\tau) \leq \Psi_{min} \end{cases}$$

Seed dormancy $\Psi_b$ at time ($\tau$) is calculated by subtracting the amount of accumulated after-ripening from the initial dormancy level ($\Psi_i$) of the seed and is incrementally modified hourly until the seed reaches an empirically approximated “minimum” dormant state ($\Psi_{min}$). We assume that all seeds can reach this minimum dormancy level after enough time. $\Psi_{min}$ was estimated for Columbia seeds stored in lab...
conditions by determining how long it took for most seeds to germinate uniformly at a

$\Psi_s$ near the estimated $\Psi_{min}$.

Figure 20: Germination model parameterization a, Poisson distribution of
dormancy levels ($\Psi_b$ used for germination model predictions). b, Germination model
predictions superimposed over 12 replicated experimental time courses at each of four
germation temperatures (8°C, 16°C, 22°C, and 31°C). Circles show all replicates (note that
these values are jittered on both the X- and Y-axes for visualization purposes). Solid lines
show median germination at each time point, and dashed lines show model predictions.
These seeds were 19 weeks old and represent 12 maternal replicate plants matured at
20°C in 12-h days.

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FLOWERING SUB-MODEL

In *A. thaliana*, high temperature and long photoperiods are the major environmental factors that promote flowering (Mitchell-Olds and Schmitt 2006; Kobayashi and Weigel 2007; Michaels 2009) and the expression of floral repressors dampens responses to those cues delaying flowering. We use a previously developed flowering-time model that predicted flowering behavior of multiple genotypes with 92% accuracy in diverse field conditions (Wilczek et al. 2009; Wilczek et al. 2010; Chew et al. 2012). We briefly outline this model and the equations below, but please see the above references for a more in-depth treatment.

Developmental progress toward flowering (*MPTU*flowering) each hour (*τ*) is a multiplicative function of three factors: *Thermal*(*τ*), *Photoperiod*(*τ*), and *Floral repression*(*τ*). Warm temperatures and long photoperiod increase development whereas high floral repression levels reduce the number of modified photothermal units that accrue toward flowering.

The thermal component is given by

\[
\text{Thermal}(\tau) = \begin{cases} 
(T(\tau) - T_{b.f}) \cdot d_{\text{hours}} & \text{when } T(\tau) > T_{b.f} \\
0 & \text{otherwise}
\end{cases}
\]

where \( T \) is temperature that hour, \( T_{b.f} \) is the base temperature for flowering, and \( d_{\text{hours}} \) is a filter so that only thermal time that occurs during the day time is included. \( d_{\text{hours}} \)
ranges in value from 0 (no light experienced that hour) to 1 (it is light for the whole hour).

The photoperiod component describes a broken stick model where developmental rate is divided into three sections. Rate is at a minimum ($p_s$) when the daylength ($D$) is less than the critical short daylength ($d_s$) and at a maximum ($p_l$) when $D$ is above the critical long daylength ($d_l$). In between $d_s$ and $d_l$, developmental rate increases linearly from $p_s$ to $p_l$.

$$\text{photoperiod}(\tau) = \begin{cases} p_s & \text{when } D(\tau) \leq d_s \\ p_s + \frac{(p_l - p_s)(D(\tau) - d_s)}{d_l - d_s} & \text{when } d_s < D(\tau) < d_l \\ p_l & \text{when } D(\tau) \geq d_l \end{cases}$$

The floral repression component is given by a pair of nested equations. The first describes how winter chilling accumulates ($WC_{Cum}$). Each hour ($\tau$) cold temperatures experienced are translated into winter chilling units via a beta function and are added to winter chilling accumulated in previous hours $WC_{Cum}(\tau)$ until the requirement for winter chilling units is satisfied ($WC_{sat}$).

$$WC_{Cum}(\tau + 1) = \begin{cases} \min(WC_{Cum}(\tau) + e^\kappa(T(\tau) - T_{v.min})^\omega e^\kappa(T(\tau) - T_{v.max})^\xi, WC_{sat}) & \text{when } T_{v.min} \leq T \leq T_{v.max} \\ WC_{Cum}(\tau) & \text{otherwise} \end{cases}$$

Where $\kappa$, $\omega$, and $\xi$ are fitted parameters determining the shape of the winter chilling effectiveness function and $T_{v.min}$ and $T_{v.max}$ are the minimum and maximum temperatures for winter chilling.
The second equation describes how the $WC_{Cum}$ state each hour is then used to determine floral repression levels. $F_i$ is the initial floral repression level and $F_u$ is the ultimate level of repression after the winter chilling requirement ($WC_{sat}$) is satisfied.

$$Floral\ repression(\tau) = \begin{cases} 1 - F_i + (F_i - F_u) \cdot \left( \frac{WC_{Cum}(\tau)}{WC_{sat}} \right) & \text{when } WC_{Cum} < WC_{sat} \\ 1 - F_u & \text{otherwise} \end{cases}$$

These equations combine to determine the modified photothermal units for flowering ($MPTU_{Flowering}$) that accrue each hour.

$$MPTU_{Flowering}(\tau) = Thermal(\tau) \cdot Photoperiod(\tau) \cdot Floral\ repression(\tau)$$

Ultimately, development is summed up ($Flowering_{Cum}$) from the time of germination until the flowering threshold ($\Theta_{Flowering}$) is reached. The flowering threshold is determined using methods in Wilczek et al. (2009).

$$Flowering_{Cum} = \sum_{\tau=0}^{Flowering_{Cum}(\tau)=\Theta_{Flowering}} MPTU_{Flowering}(\tau)$$

**SEED DISPERSAL SUB-MODEL**

As there were no published data on *A. thaliana* reproduction dynamics, we developed a simple model of the progress toward seed dispersal as a function of temperature. We chose a thermal function because the rate of grain production is often related to temperature in crop studies (Lawlor and Mitchell 2000; Ainsworth and Ort 2010). Seeds are released when they reach a developmental threshold that reflects the time it takes the first 10% of seeds to mature ($\Theta_{Dispersal}$). We use this time point because
measurements of complete plant senescence in chambers likely overestimate
reproductive period based on our experience with *A. thaliana* in the field. In chambers,
plants experience moderate temperatures and permissive moisture conditions unlike
plants in the field. In practice seasonal changes such as the dryness of summer will
interrupt reproduction.

The rate of progress towards seed dispersal was modeled as function of
temperature, such that the rate of progress is proportional to the difference between the
soil surface temperature (*T*) and a base temperature for dispersal (*T*<sub>b.d</sub>). Rate of thermal
progress toward seed dispersal (*T*<sub>SD</sub>) was modeled as a thermally dependent
developmental accumulation above a base temperature (*T*<sub>b</sub>).

\[
T_{SeedDispersal}(\tau) = \begin{cases} 
  T(\tau) - T_{b,d} & \text{when } T(\tau) > T_{b,d} \\
  0 & \text{otherwise}
\end{cases}
\]

The higher temperatures are above *T*<sub>b.d</sub> the faster development occurs.

Development is summed each hour from flowering until plants reach the threshold
(*Ω<sub>Dispersal</sub>*).

\[
Seed Dispersal_{sum}(\tau) = \sum_{\tau=0}^{\Omega_{Dispersal}} T_{SeedDispersal}(\tau)
\]

Our range of simulated average maturation lengths (Fig C17) correspond well to
the range of average reproduction lengths reported by Chiang et al. (2012) in a field
experiment. Model parameters and threshold were determined based on fit to seed
dispersal times measured in chamber conditions at 14°C, 20°C, and 25°C with the Columbia genotype at a neutral photoperiod (Burghardt, unpublished data).

CREATION OF ENVIRONMENTAL INPUTS:

Because the phenology models use hourly environmental inputs, we converted the available environmental data to that scale. We used temperature predictions from 20 years of a climate model to create temperature replicates that encapsulated year-to-year climate variability. For moisture data, we used monthly contemporary moisture statistics from WorldClim and created stochastic moisture profiles driven by monthly precipitation amount and number of precipitation events. We also calculated latitude-specific photoperiods. Replicates of these environmental series were generated by (1) randomly drawing temperature from the sample of years, (2) pairing those profiles with site-specific photoperiod, and (3) combining those with stochastic moisture profiles.

Photoperiod: Equations were used to calculate the time of dawn and dusk at each latitude and photoperiod length (Ham 2005).

Temperature: 20 years (from 2001-2020) of daily maximum and minimum temperature data were extracted from the NOAA GFDL CM2.1 A1B_X1 climate scenario from grid cells over each of the four focal sites (NOAA GFDL 2004; Delworth et al. 2006). We then interpolated those maximum and minimum daily temperatures to hourly measures and subsequently translated them from air to surface temperatures as outlined below. Further details can be found in Wilczek et al. (2010). These 20 years of hourly
data are assembled at random with replacement when we create environments for model runs at each location.

Converting daily maxima and minima to hourly temperatures: Conversions were modified from Cesaraccio et al. (2001).

\[
T(\tau) = \begin{cases} 
  c + \frac{a}{2} \cos \left( \pi + \frac{\tau - H_n}{H_m - H_n} \pi \right), & H_n \leq \tau \leq H_m \\
  T_s + k \log_j L, & H_m \leq \tau \leq H_s \\
  T_s + b \sqrt{\tau - H_s}, & H_s \leq \tau \leq H_p
\end{cases}
\]

where \( t \) is the current hour and \( H_n, H_m \) and \( H_s \) are the time of dawn, daily maximum, and dusk of that day. \( T_n \) and \( T_m \) are the day’s minimum and maximum temperature, respectively. \( T_p \) is the next day’s minimum temperature.

\[
H_m = H_n + x \sin \left( \frac{2\pi (w - y)}{365} \right) + z \quad \text{and} \quad b = \frac{T_p - T_s}{\sqrt{H_p - H_s}}
\]

Additional intermediates included: temperature at sunset \( (T_s) \) estimated as \( T_s = T_m - s(T_m - T_p) \); \( c \) is the arithmetic mean of \( T_m \) and \( T_n \); \( a \) is the amplitude of increase \( T_m - T_n \); \( k \) is \( T_m - T_s \); and the logarithmic base \( j \) is \( 1 + H_s - H_m \); and \( L = j - (\tau - H_m) \). Final parameter values were set to \( x = 2.036391 \), \( y = 79.22015 \), \( z = 9.285504 \) and \( s = 0.227538 \) based on fitting to hourly temperatures gathered at all four sites modeled here plus Cologne, Germany.

Air to ground conversion: Surface temperature \( (T_s) \) in kelvins was simulated for each hour based on Kelvin air temperature \( (T_a) \) according to the equation

\[
T_s(\tau) = aW + cT_a + e \sin \left( \frac{2\pi t}{365} + f \right) + d
\]
where \( a, c, d, e, \) and \( f \) are parameters fit empirically using ground level data from European weather stations at each of the four sites studied here plus Cologne, Germany \((a = 0.004099, c = 0.920493, d = 22.466179, e = 21.861643, \) and \( f = 1.549941\)). \( W \) (hour, day of year) is clear sky irradiance as calculated in Ham (2004) and \( t \) is (fractional) time since midnight on 1 January in days.

**Moisture:** Stochastic moisture profiles were generated for each site. We obtained site-specific monthly precipitation totals and rainy day totals derived from the Climate Research Unit in East Anglia. Each hour, the chance of a rain event occurring was based on the number of rainy days given the site and month of the year. After each rain event, the soil dried hourly according to the equation \( \Psi(\tau) = \Psi(\tau - 1) + (\Psi(\tau - 1)(d)/p_{(m,s)}) \), where decay \( d \) is constant parameter and cumulative precipitation \( p \) is dependent on both site \( s \) and month \( m \). Therefore the soil drying is scaled such that it occurs more slowly after rain events in months and locations where the total amount of precipitation in a month was higher.

While these precipitation inputs are qualitatively different between the sites in an arguably valid way, they are unlikely to be quantitatively correct. For instance, to maintain generality, we held \( d \) constant across sites despite potential differences in soil that may influence soil-drying rates. While we sacrifice some accuracy with this simple method, it will be nearly impossible to get accurate, long-term surface water potential levels across Europe, particularly as such parameters are soil-type specific. In any event
we are primarily interested in how landscape differences in moisture, temperature, and photoperiod inputs interact. A sensitivity analysis of the decay parameter indicates that we get similar site-specific model results up to a point as the absolute moistness of the environment changes. Overall germination takes longer to occur in drier soils and therefore the time in which seeds germinate given a dispersal day is pushed later in the season as the decay parameter increases.
Appendix C: Additional Figures for Chapter Two

Figure 21: Illustrations of observed life cycles from four locations in Europe. Colored blocks indicate different seasons (red = fall, blue = winter, green = spring, and orange = summer), and the width of blocks.
Figure 22: Example of 4 years of environmental inputs for each site. Yellow lines indicate day length, black lines indicate hourly temperatures, and gray lines show precipitation events.
Figure 23: Effects of variation in germination parameters on life-cycle length in Oulu, Finland (a), Halle, Germany (b), and Valencia, Spain (c), for three dormancy/floral repression genotypes. Variable parameters were $T_0$ (temperature optimum for germination: 16°, 22°, and 28°C), $T_b$ (base temperature for afterripening: high = 3, low = −3), and $\Psi_u$ (upper limit of moisture or maximal afterripening: high = −50, low = −200). Asterisks denote the default parameter combination used in all simulations presented in the main text. Lowering the afterripening parameters increased afterripening rate, which reduced the magnitude of the dormancy effect (visible when comparing the blue circles across high/low combinations). However, except in Valencia with $T_0 = 16$, floral repression had a much smaller effect on life-cycle length than did dormancy (difference between light green and dark green circles vs. difference between light green and blue circles).
Figure 24: Effects of variation in germination parameters on the length of the vegetative stage in Oulu, Finland (a), Halle, Germany (b), and Valencia, Spain (c). Variable parameters were $T_o$ (temperature optimum for germination: 16°C, 22°C, and 28°C), $T_{b.ar}$ (base temperature for afterripening: high = 3, low = −3), and $\Psi_u$ (upper limit of moisture or maximal afterripening: high = −50, low = −200). Asterisks denote the default parameter combination used in all simulations in the article. Floral repression level consistently increased the vegetative-stage length in all parameter combinations (difference between light green and dark green circles). The magnitude of the difference did change across parameters, particularly in Oulu due to fluctuating proportions of winter annuals. The effect of primary dormancy level on vegetative-stage length varied (difference between light green and blue circles). This is perhaps to be expected because many of the germination parameters varied also reduced dormancy levels.
Figure 25: Color version of Figure 10 in the main text.
Figure 26: Effect on life-stage phenology of altering dormancy level in a low-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. Model results were averaged over the last 45 years of a 60-year simulation.
Figure 27: Predicted frequency distribution of flowering times in a population for each genotype in each of the four locations studied. Model results were averaged over the last 45 years of a 60-year simulation.
Figure 28: Proportion of individuals in the rosette stage over the year for a low-dormancy and low–floral repression genotype in Halle, Germany. Individual lines represent 10 distinct, consecutive years color coded in rainbow order (dark red = year 1). The black line indicates the average proportion over the course of the last 45 years of a 60-year simulation. Some years, the summer cohort happens in early summer; in other years, it happens in late summer. The timing of fall germination also varies yearly.
Figure 29: Proportion of individuals in the rosette stage over the year for a low-dormancy and low-floral repression genotype in Norwich, England. Individual lines represent 10 distinct, consecutive years color coded in rainbow order (dark red = year 1). The black line indicates the average proportion over the course of the last 45 years of a 60-year simulation. Late-summer cohorts are more frequent than early ones. The timing of fall germination also varies yearly.
Figure 30: Proportion of individuals in the rosette stage over the year for a high-dormancy and high–floral repression genotype predicted to occur in Valencia, Spain. Individual lines represent 10 distinct, consecutive years color coded in rainbow order (dark red = year 1). The black line indicates the average proportion over the course of the last 45 years of a 60-year simulation.
Figure 31: Proportion of individuals in the rosette stage over the year for a low-dormancy and high–floral repression genotype predicted to occur in Oulu, Finland. Individual lines represent 10 distinct, consecutive years color coded in rainbow order (dark red = year 1). The black line indicates the average proportion over the course of the last 45 years of a 60-year simulation.
Figure 32: Proportion of individuals in the rosette stage over the year for a mid-dormancy and low-floral repression genotype predicted to occur in Norwich, England. Individual lines represent 10 distinct, consecutive years color coded in rainbow order (dark red = year 1). The black line indicates the average proportion over the course of the last 45 years of a 60-year simulation.
**Figure 33:** Proportion of individuals in the rosette stage over the year for a mid-dormancy and low-floral repression genotype predicted to occur in Halle, Germany. Individual lines represent 10 distinct, consecutive years color coded in rainbow order (dark red = year 1). The black line indicates the average proportion over the course of the last 45 years of a 60-year simulation. A different environmental replicate is used from the circle graphs in figures 3 and 5 in the main text. The graph demonstrates life-cycle expression drifting across years due to dormancy/environment mismatch.
Figure 34: Color version of Figure 11 in the main text.
Figure 35: Time spent as a seed across sites. 

a. Reaction norm of different genotypes in response to the four sites for the average time spent as a seed. 

b–e, Density distribution of the duration of the seed stage interval for each genotype in each location. Model results were averaged over the last 45 years of a 60-year simulation.
Figure 36: Time spent as a rosette across sites. 

- **a.** Reaction norm of different genotypes in response to the four sites for the average time spent as a vegetative rosette.
- **b–e.** Density distribution of the duration of the seed stage interval for each genotype in each location. Model results were averaged over the last 45 years of a 60-year simulation. Color coding is the same as in Figure 35.
Figure 37: Time spent reproducing across sites. a, Reaction norm of different genotypes in response to the four sites for the average time spent as a reproductive plant. b–e, Density distribution of the duration of the seed stage interval for each genotype in each location. Model results were averaged over the last 45 years of a 60-year simulation. Color coding is the same as in Figure 35.
Figure 38: Summary of life-cycle phenology (proportion of life cycles with winter rosettes) and life-cycle length (in years) for each combination of parameters in each of the four locations in the study. Floral repression ($F_i$): low = 0.598, high = 0.737, and very high = 0.88. Primary dormancy ($\Psi_{mean}$): low = 0, mid = 1.25, and very high = 2.5. Model results were averaged over the last 45 years of a 60-year simulation.
Figure 39: Color version of Figure 12 in the main text.
Appendix D: Effects of Life-Cycle Phenology on the Environment Experienced during Reproduction

The environment during reproduction is important because it determines seed dormancy levels and because it defines how much time is available for reproduction before the environment becomes unfavorable. Figure 40 shows that there is variation between locations in the average temperature during reproduction. Counter-intuitively, plants reproducing in more southern and oceanic sites are predicted to produce seeds in cooler conditions than those in the north. Because cool seed-maturation temperature induces stronger dormancy, this would result in more dormant seeds at lower latitudes. This pattern reinforces the observed genetic cline in dormancy, in which more dormant genotypes are located at lower latitudes. Also note that in some locations there is a bimodal distribution of seed-maturation environments, indicating that the same genotype is maturing seeds in divergent environmental conditions.
Figure 40: Bean plots of average temperature experienced during reproduction for low dormancy/low floral repression genotypes at four sites across Europe. Black bars indicate the median temperature experienced and mirrored density distributions indicate the number of individuals that experienced each average temperature. Wider areas indicate more individuals.

Changes in genotype can have a strong influence on the average temperature of seed maturation. For instance, low and high dormancy levels in Norwich lead to both winter and summer-annual life cycles, whereas medium dormancy levels lead to the canalization of a winter-annual life cycle (Figure 41—circlegraphs). These life-cycle differences lead to both warm and cool temperatures being experienced during seed maturation for low and high dormancy genotypes, but uniform low temperatures being experienced during seed maturation by medium dormancy genotypes (Figure 41).
Because dormancy levels are temperature-dependent, these differences could have large effects on the life-cycle phenotypes of offspring.

Figure 41: Average temperature experienced during reproduction for three dormancy genotypes in Norwich, England. Black lines indicate median temperature during reproduction and mirrored density plots show the distribution of individuals. Wider areas indicate more individuals experienced that reproductive environment.

Insets of circle graphs show the life cycle expressed in each location that leads to differences in the reproductive environment—multiple flowering bouts at low and high dormancy levels and only one flowering bout in the late spring for medium dormancy levels. January starts at the the 3’oclock position and the months move clockwise through the year. All genotypes shown have a low floral repression level.
Figure 42 shows the amount of thermal time plants are predicted to have available in which to reproduce in each of the four sites across Europe. The combinations of dormancy and flowering alleles known to occur in each location across the range are frequently those that exhibit the longest (thermal) time available for reproduction in their home location. I show thermal time because it more highly correlated with reproductive output than time as measured in days. These calculations differ from the ones shown in Chapter 3 because they use a less stringent criteria for cold tolerance of flowering plants. New observations of natural populations have led to this revision of cold tolerance parameters (L. Burghardt, personal obs.). Graphs generated using the old criteria show the same general trends but the strength of allelic effects is less prominent.
Figure 42: Density plots of thermal time available for reproduction for six genotypes across the range of A. thaliana. Thermal time is essentially a measure of accrued warmth. In Oulu, Finland low dormancy/high floral repression genotypes are expected to occur and in Valencia, Spain higher dormancy genotypes are expected to occur regardless of floral repression level. In the central European sites many different combinations of dormancy and floral repression are known to occur.
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Biography

Liana Twardosz Burghardt and her twin sister Karin were born in Knoxville, Tennessee on July 29, 1985 to Sandra L. Twardosz and Gordon M. Burghardt. After graduating from South-Doyle High School, she attended Carleton College in Northfield Minnesota where she graduated *magna cum laude* with a Biology Major in May 2007. After completing a two-year post-baccalaureate position in the lab of Johanna Schmitt at Brown University (now at UC Davis), she pursued a doctorate degree at Duke University in the lab of Kathleen Donohue. While at Duke, she was a James B. Duke Fellow, a NSF Pre-Doctoral Fellow, and received a dissertation improvement grant from the NSF. She has also received multiple fellowships to develop her teaching skills including being a Preparing Future Faculty Fellow, a BioBass Teaching Fellow, and a Pathfinder Fellow. She capped off her graduate career by teaching her own upper-level evolutionary ecology seminar on “Extreme Life Styles.” During her tenure at Duke she has published seven peer-reviewed articles and a book chapter: 1) “Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes”, 2) “Applying developmental threshold models to evolutionary ecology”, 3) “Secondary dormancy induction depends on primary dormancy status in *Arabidopsis thaliana*”, 4) “The evolution of senescence in annual plants: the importance of phenology and the potential for plasticity”, 5) “Germination, post-germination adaptation, and species ecological ranges”, 6) “Avoiding the crowds: the evolution of
plastic responses to seasonal cues in a density dependent world”, 7) “Genetic and physiological bases for phenological responses to current and predicted climates”, and 8) “The community-level effect of light on germination timing in relation to seed mass: a source of regeneration niche differentiation”. She is moving on to a Post-doc in the Lab of Peter Tiffin at the University of Minnesota where she will examine the genomic and transcriptomic basis of environmental sensitivity in *Medicago truncatula*. 