Evolution of *Arabidopsis thaliana* Flowering Time in Response to Water Availability Post-Introduction

by

Amanda Joan Stock

A thesis submitted in conformity with the requirements for the degree of Master's of Science
Department of Ecology & Evolutionary Biology
University of Toronto

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Abstract

Flowering is one of the most influential events in the life history of a plant – its beginning and duration are two main determinants of reproductive investment and lifetime fitness. Understanding the selective pressures influencing time to flowering and being able to reliably predict how it will evolve in novel environments is an unsolved challenge for plant evolutionary geneticists. Using naturalized lines of *Arabidopsis thaliana* from across the eastern North American range, I examined the impact of simulated winter precipitation levels on flowering time and the fitness consequences of early versus late flowering. Flowering time was significantly genetically correlated across two environments – in common gardens outdoors and in environmental chambers set to mimic mid-range photoperiod and temperature conditions – suggesting that flowering time rank between lines remains consistent. The interaction between flowering time and water treatment for fitness indicates that water availability contributes to differential selection on flowering time in introduced populations.
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Chapter 1

General Introduction

Background and context

The necessity of understanding adaptations from multiple angles

One of the central goals of evolutionary ecology is to explain how genetic variation – the raw material which responds to selection – produces adaptive differences between populations. Linking the genetic architecture of traits to ecological processes that create selective pressures and the resulting evolutionary consequences has rarely been accomplished, but can provide a complete description of how populations adapt to their environments over time in the natural world (Metcalf & Mitchell-Olds, 2009). There are hundreds, even thousands of studies which have contributed to an understanding of adaptations, whether via quantitative estimates of selection, identification of candidate QTL, or ecological studies of fitness differences. Yet to accurately describe an allele as adaptive, a connection must be observed between all three components: genotype, phenotype and fitness (Barrett & Hoekstra, 2011). Aside from a few well-studied examples where the phenotype is caused primarily by one or a few large-effect loci (i.e. pigmentation in mice, armour plating in sticklebacks – for a review see Barrett & Hoekstra, 2011), we lack a complete understanding of how adaptive differentiation is created and selected for from the genetic level to the phenotypic level. Describing more completely other examples of adaptive evolution – especially complex, quantitative traits which are more representative of the rest of adaptive phenotypes – will help determine how general the patterns we have already characterized are, and lend support to or call into question our theoretical understanding of how evolution by natural selection operates at the level of favourable alleles. The major objective for future studies in evolutionary genetics should be to design complementary lab and field projects, or stand-alone experiments which will illuminate these connections (Anderson, Willis & Mitchell-Olds, 2011).
Since the genomics revolution, attempts to understand adaptation have frequently focused on detecting the loci underlying trait differentiation; next-gen sequencing, genome-wide association studies, and QTL mapping approaches have all promised to give us a complete picture of all the genes involved (e.g., Fournier-Level et al., 2011; McKay et al., 2008). Yet the limitations of this type of approach have been given little consideration as enthusiasm for rapidly advancing genomics technologies spurred a deluge of such studies. Issues of resolution, linkage disequilibrium, and epistatic interactions restrict the set of questions we can answer using these techniques (Phillips, 2005). A recent review of the problems with the “QTN program” by Rockman (2012) summarizes these issues and places them within a larger framework, suggesting that without knowing the effect size distribution of alleles underlying adaptive variation, we cannot rely solely on a molecular approach to understanding evolution. Ultimately, we can learn more from connecting modern molecular genetics techniques with the tried-and-true mathematical and statistical approaches of quantitative genetics understood since the early twentieth century.

Combining genomics data with quantitative genetics approaches is one of the most promising routes to understanding adaptive evolution as a complete process rather than just a piecemeal story cobbled together from information at different levels (Stinchcombe & Hoekstra, 2008). There has, of course, been some progress towards synthesizing data on molecular variation with phenotypic observations of populations. For example, a recent genome-wide association study utilizing SNPs and climate data was able to predict a substantial amount of the variation in fitness of Arabidopsis thaliana when grown in a common garden (Hancock et al., 2011). Combined approaches promise to elucidate the connections between different components of adaptation—and are made especially powerful when paired with model systems.

**The utility of Arabidopsis thaliana**

The model plant species *Arabidopsis thaliana* has an interesting history of introduction
across the world (Jørgensen & Mauricio, 2004) and has extensive genomic, seed accession, and inbred line resources available. (Mitchell-Olds & Schmitt, 2006; Gan et al., 2011). Along with the wealth of information from previous studies which is readily available, A. thaliana makes an ideal study system for working towards the goal of connecting DNA sequence variation to the evolution of life history traits (Koornneef, Alonso-Blanco & Vreugdenhil, 2004; Keller & Taylor, 2008; Metcalf & Mitchell-Olds, 2009). Current research suggests that naturalized A. thaliana have and are continuing to experience rapid adaptation in response to selection in North America (Mitchell-Olds & Schmitt, 2006; Huang et al., 2010). Precisely because a good understanding of the major effect genes and candidate SNPs affecting adaptation in life history traits is already available, A. thaliana makes an excellent work-horse to determine how evolution of complex traits occurs in response to novel selective pressures. The pre-existing resources allow new experiments to focus on connecting known molecular sequence variation to quantitative measures of evolutionary change and the ecological processes driving these changes (Mitchell-Olds & Schmitt, 2006). If the "holy grail" of modern ecological genetics is to measure selection on genes with well characterized phenotypic effects in natural populations or conditions, Arabidopsis is the species to use.

Recent studies have begun to utilize the extensive genetic resources available for Arabidopsis to address questions about the relative importance of genetic and environmental factors. For example, Hancock et al. identified adaptive SNP differences associated with a number of different climate variables, including multiple measures of precipitation (2011). Knowing the SNP genotypes of their maternal lines allowed them to successfully predict relative fitness among a set of geographically diverse European A. thaliana accessions, grown together in a common garden located in France. More work connecting genetic and phenotypic differences between populations is needed to understand how selection is acting on wild plants, especially those which inhabit introduced ranges.
Understanding how North American A. thaliana lines have rapidly adapted to the novel conditions they have experienced on this continent has wide-ranging implications for the biology of introduced species and studies of life history evolution. My thesis contributes to a growing number of studies which are elucidating the details of how A. thaliana, so well-studied in the lab, actually responds to environmental pressures (Donohue et al., 2005; Murren, Denning & Pigliucci, 2005; Malmberg et al., 2005; Korves et al., 2007).

**The importance of life history traits**

Life history traits (e.g., characteristics related to the timing and duration of life stages – such as early growth or reproduction – and the transitions between them) are some of the most important characteristics determining adaptation. They are complex – polygenic, hierarchically related, and affected by the environment – which also makes them difficult to characterize from a molecular to phenotypic level (Bergelson & Roux, 2010). Additionally, understanding how genetic variation influences life history evolution in introduced plants is integral to predicting the impacts of rapid climate change and invasive species on native plant communities – two biological issues of great economic and conservation importance (Bossdorf *et al.*, 2005; Gordo & Sanz, 2009; Lavergne *et al.*, 2010). For my thesis I focused on rapid adaptation of flowering time, one of the most prominent of these ecologically relevant suites of traits which have been identified as most likely to experience change upon introduction to new habitats (Hodgins & Rieseberg, 2011). Utilizing a model system like A. thaliana to investigate adaptive variation in life history traits is currently the best chance we have at connecting “black-box” quantitative genetic approaches to the underlying molecular sequence variation (Metcalf & Mitchell-Olds, 2009), as well as to the selective pressures imposed by the environment -- thereby providing a complete view of adaptation.

Latitudinal and longitudinal clines in important morphological and life history traits –
including flowering time, germination success, parental investment, plant size, and growth – along environmental gradients have been repeatedly recognized as signs of adaptation to differing environmental conditions across the cosmopolitan range of *A. thaliana* (Li, Suzuki & Hara, 1998; Stinchcombe et al., 2004, 2005; Boyd et al., 2007; Samis, Heath & Stinchcombe, 2008). In *Arabidopsis*, many of the candidate loci for one life history trait have also been shown to affect others, so further investigation of the pleiotropic effects is possible (Stinchcombe et al., 2005; Balasubramanian et al., 2006; Chiang et al., 2009). My work has focused on flowering time because it is a characteristic known to show adaptive differentiation to different environmental conditions and has been previously identified as having significant influence on the performance and fitness of introduced plants (Simpson & Dean, 2002; McKay, Richards & Mitchell-Olds, 2003; Korves et al., 2007).

**The experimental approach**

The overall goal of my thesis is to elucidate the effects of a major agent of selection acting on flowering time in introduced populations of *Arabidopsis thaliana*. By linking a putative agent of selection to adaptive changes of a life history trait in populations with known genetic variation for major genes underlying that trait, we will be able to understand how rapid adaptation takes place on the levels of genotype, phenotype, and their interaction with the environment. To achieve this broader goal, I completed two major projects for my MSc. thesis: (1) Manipulating a proposed selective agent, to verify the ecological causes of selection (Chapter 2), and (2) Generating a population of *A. thaliana* that is polymorphic for known, epistatically interacting flowering time genes, for use in experimental evolution studies in the field (see Chapter 3). Collectively, my work also provides a jumping off point for future experiments which can more closely examine the generation-to-generation genetic changes in a field population, and the selective agents determining that change. By using seed accessions from naturalized North American populations to address this gap in understanding, this work and the work which may follow it contribute to a greater understanding of how a model species
usually studied from lab-grown lines actually responds to novel environments in a natural context.

The general question posed by this thesis is: how do differing environmental conditions affect the expression of life history traits and the selection acting on them? Using a specific case to shed light on that question is the purpose of the experiment documented in the following chapter. Our goal was to confirm a major selective agent exerting divergent selective pressure on flowering time differences between sites in the introduced North American range of *A. thaliana*, specifically the level of winter precipitation, which had previously been recognized as a correlate of fitness in a common garden experiment (Samis *et al.*, 2012).

Unlike most existing studies of *A. thaliana*, which have focused on associations with environmental variables and can therefore only suggest potential causes of local adaptation (*e.g.*, Fournier-Level *et al.*, 2011; Hancock *et al.*, 2011), our project was designed to manipulate a putative selective agent on flowering time differences between sites in the introduced range. We addressed two main questions: 1) Is flowering time variation predominantly genetic? and 2) Do early flowering lines have higher fitness in dry winters? We were particularly interested in determining whether conclusions about agents of selection drawn in environmental chamber experiments could be applicable to plants growing in field conditions, and whether there was a G x E effect on fitness mediated by precipitation and flowering time.
Chapter 2

Water Availability as an Agent of Selection in Introduced Populations of *Arabidopsis thaliana*: Impacts on Flowering Time Evolution

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Amanda J. Stock¹,†, Brechann V. McGoey¹,†, and John R. Stinchcombe¹,²

(1) Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada.
(2) Centre for the Analysis of Genome Evolution and Function, University of Toronto, Toronto, ON Canada.
(†) Authors contributed equally.

Corresponding author
John R. Stinchcombe,
john.stinchcombe@utoronto.ca

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Abstract

Flowering is one of the most influential events in the life history of a plant and one of the main determinants of reproductive investment and lifetime fitness. It is also a highly complex trait controlled by dozens of genes. Understanding the selective pressures influencing time to flowering, and being able to reliably predict how it will evolve in novel environments, are unsolved challenges for plant evolutionary geneticists. Using the model plant species, *Arabidopsis thaliana*, we examined the impact of simulated high and low winter precipitation levels on the flowering time of naturalized lines from across the eastern portion of the introduced North American range, and the fitness consequences of early versus late flowering. Flowering time order was significantly correlated across two environments—in a previous common garden experiment and in environmental chambers set to mimic mid-range photoperiod and temperature conditions. Plants in low water flowered earlier, had fewer basal branches and produced fewer fruits. Selection in both treatments favored earlier flowering and more basal branches. Our analyses revealed an interaction between flowering time and water treatment for fitness, where flowering later was more deleterious for fitness in the low water treatment. Our results are consistent with the hypothesis that differences in winter precipitation levels are one of the selective agents underlying a flowering time cline in introduced *A. thaliana* populations.

Subjects  Ecology, Evolutionary Studies, Plant Science

Keywords  Flowering time, Selective agents, Selection gradients, Water relations, Life history, Introduced species, Evolution in introduced species

Introduction

When plants are introduced to new habitats, they may become established, and then sometimes expand their ranges beyond the area of initial introduction. Selective pressures can then act on plants in different parts of the range, leading to local adaptation. Observing this progression in introduced species offers an excellent
opportunity to study evolutionary responses in colonizing populations. Many of the examples of rapid adaptation are from the invasive species literature (Sax et al., 2007), and adaptive evolution is increasingly recognized as an explanation for the ability of some introduced plants to persist and spread (Yoshida et al., 2007; Sax et al., 2007; Dlugosch & Parker, 2008). Selective agents may be the same as those found in the native range, or completely novel. Confirming major ecological agents of selection is still an active area of investigation, with more manipulative experiments necessary to confirm the forces leading to adaptation in introduced species. Here we experimentally evaluate a potential selective agent responsible for adaptive evolution of life history in introduced populations of the model plant, *A. thaliana*.

Optimizing flowering time is critical for plant species, as it will directly affect the number of seeds they can produce, and the environmental conditions their flowers and fruits will experience at critical reproductive and developmental stages (Simpson & Dean, 2002). Population differentiation in flowering time is often correlated with climatological variables, and clinal patterns in phenology are widespread (Montague, Barrett & Eckert, 2008; Keller et al., 2009). As it is such an important life history trait, adaptation in flowering time could be crucial for the success of introduced plant populations (Barrett, Colautti & Eckert, 2008; Montague et al., 2008). An unresolved challenge for plant evolutionary geneticists is understanding the selective pressures influencing time to flowering, and being able to reliably predict how it will evolve in novel environments; a task made more difficult by the high degree of plasticity involved (Stinchcombe, Dorn & Schmitt, 2004a). Flowering is also a highly complex trait controlled by multiple physiological pathways: some internally regulated and some environmentally dependent (Salome et al., 2011). Much of the past work on the transition from vegetative growth to reproduction has been accomplished using the model plant species, *Arabidopsis thaliana* (Simpson & Dean, 2002). Recent work on *Arabidopsis* has demonstrated that the same genes that underlie flowering time also influence water resource responses (Lovell et al., 2013), and that variation in water availability can impose selection on flowering time and a host of physiological traits
(Kenney et al., 2014). Consequently, our knowledge of Arabidopsis genetics can inform ecological hypotheses and allow a more cohesive understanding of geographic variation—from the genes underlying the variation to the selection pressures behind differentiation.

In addition to its popularity as a model organism in genetics, A. thaliana is increasingly used to study topics in evolutionary ecology. The advantages of its wide geographic and environmental range, its experimental tractability, and the wide array of available genetic tools make it an excellent species for studies of local adaptation (Gaut, 2012). Research on Arabidopsis thaliana in its native European range has found clinal patterns and evidence of adaptation to climatic conditions (Stinchcombe et al., 2004b; Caicedo et al., 2004; Rutter & Fenster, 2007; Samis, Heath & Stinchcombe, 2008; Hancock et al., 2011; Fournier-Level et al., 2011). Arabidopsis thaliana was introduced to North America 150-200 years ago (Jørgensen & Mauricio, 2004). Despite this recent colonization, preliminary evidence for adaptive evolution has been found (Samis et al., 2012).

Samis and colleagues observed a longitudinal cline in flowering in introduced, North American Arabidopsis, which was parallel to that previously described in Europe and was robust to the inclusion of population structure — evidence that the cline reflects local adaptation (Samis et al., 2012). There was a correlation between a site’s longitude and the total precipitation it experienced, with eastern sites experiencing wetter conditions compared to those further west (r=0.62, p<0.0001) (see Mitchell & Jones, 2005 for climate data description; Samis et al., 2012). As in Europe, more coastal populations tended to flower later, while more central populations in lower precipitation conditions flowered earlier. In their analyses, Samis and colleagues observed that winter precipitation was the best explanatory factor for the North American cline. They thus hypothesized that precipitation levels through the winter are the selective agent behind population differentiation in flowering times.
Fully confirming the role of precipitation in leading to adaptive differentiation in flowering time, however, requires experimental manipulation of water availability to confirm its role as a selective agent (Wade & Kalisz, 1990). While Kenney et al. (2014) provide evidence that water availability can impose selection on flowering time using Eurasian accessions, we have no experimental data on whether it also acts on a selective agent in the introduced range. Previous studies of Arabidopsis flowering time clines and genetic associations have suggested that the genetic and geographic composition of the sample can have strong effects on the findings of a study, with variation in the strength of genetic associations or observed clines depending on what lines are included (Samis et al., 2008), presumably because different samples contain different genetic variation.

Because *A. thaliana* was introduced to North America only a few hundred years ago, it is possible to examine patterns of local adaptation to the new continent since then. We examined the effects of winter precipitation on flowering time and addressed two related questions: (1) Is there support for the Samis et al. hypothesis that winter precipitation could be a selective agent on flowering time variation in the introduced *Arabidopsis* range and (2) Do Kenney et al.’s findings of water availability imposing selection on life history hold for a different sample of lines and genotypes, and with water restrictions imposed during simulated winter conditions rather than warm greenhouse conditions?

**Materials and methods**

**Study species**

We chose to use natural accessions of *A. thaliana* (common name: thale or mouse-ear cress) from populations in the Eastern half of the introduced North American range. We selected a subset of 199 lines used in a previous study of parallel adaptation in flowering time (Samis et al., 2012). Seeds came from accessions which had been field collected or ordered from the *Arabidopsis* stock center (*Arabidopsis* Biological Resource...
Center [ABRC]) and previously bulked in an environmental chamber with a vernalization period to ensure flowering.

We randomly chose 25 lines from each of the two extreme flowering time quintiles as defined by Samis et al. (2012) experiment, representing the beginning and end of the flowering time distribution (early and late flowering lines). In total, lines were drawn from 25 geographically distinct populations. The origin sites of the accessions ranged from 33.38°N to 43.27°N in latitude, and from -70.67°W to -86.62°W in longitude (Figure 1).
**Figure 1:** Map of origin sites of selected *A. thaliana* lines. Lines selected from the earliest flowering quintile are shown as grey circles, while lines selected from the latest flowering quintile are dark triangles.
Chamber common garden

The goal of the experiment was to determine the effect of winter water levels on flowering in the introduced range. We used a 12 mL ("low") treatment and a 24 mL ("high") treatment administered to individual plants every other day. These parameters are similar to those used in past studies manipulating water availability (e.g. Pigliucci, Whitton & Schlichting, 1995). We applied the treatment in a split plot design because, although water was applied to individual plants, it drained down into the closed bottom of the trays where it could be absorbed by other plants in that tray. We chose to designate half the trays as "low" and half as "high" treatment level trays to prevent standing water from potentially eliminating the treatment effect.

To synchronize germination, we stratified seeds in a low concentration agar solution (0.15 mg/100 mL) for four days at 4 °C and then planted them into 4 x 8 cell trays filled with Sunshine Mix #1 soil (Sun Gro Horticulture). There were ten replicate cells for each line, divided evenly between treatments: five in low water conditions and five in high. Within a treatment, lines were randomly assigned to cells in one of two blocks --which corresponded to the top shelf and bottom shelf of the chamber -- to reduce microenvironmental differences. There were a total of 16 trays, eight for each treatment, and a total of 500 experimental plants plus 12 randomly chosen individuals to avoid empty cells.

The plants were germinated and grown in an environmental chamber at the Earth Sciences Centre, University of Toronto. Initially, we set the chamber to 22°C to encourage germination, with 14 hour days. Once seeds had germinated, we began the simulation of seasonally appropriate conditions (e.g. Li et al., 2006), starting with the month of October. We used the average high temperature across sites as our daytime temperature, and average low temperature as our night temperature. We set the day length as the mid-month value at the mid-longitude and latitude point for all accession sites, using standard illumination intensity (Samis et al. 2012). We adjusted the day
length and temperature settings every two weeks, compressing the growing season by setting the chamber to mimic average monthly conditions from October to July. When individual plants bolted, we recorded bolting date along with rosette diameter and the number of rosette leaves. We also noted the emergence of the first flower as flowering date. These data were collected on a daily basis, save for the earliest eight plants to bolt and flower, which were missed initially. We narrowed down the window between when they were last observed and when they were observed bolting or flowering to a period of ten days. Taking a conservative approach, we assigned all plants the last day of that window as their bolting date, or if flowering, their flowering date. When plants senesced, we collected them and recorded harvest date. Dried plants were later scored for final height, number of basal branches, and the total number of fruits -- a proxy for fitness.

Data analysis

All analyses were carried out using SAS v. 9.3 (SAS Institute Inc. 2008), with figures created in R (2014).

Correlations with previous flowering time data

We evaluated whether flowering times in the chamber was correlated with flowering time in the Samis et al. (2012) study using Spearman rank correlations. The significance of our results was assessed using a randomization test, described below. We also evaluated whether there was a correlation between fruit set in the chamber and precipitation at the site of origin. We included fruit set in the high and water treatments, January precipitation at the site of origin, and overall winter precipitation in these analyses.

Main effects of water treatment on traits and fitness

We used mixed-model ANOVA to determine the main effects of the water treatment on traits. Briefly, for each trait as a response variable, we included block and treatment as fixed effects. Block was designated as a fixed effect because our two blocks were two
shelves in the chamber and did not represent random samples of spatial variation. Random effects in each model were line, line * treatment, and tray nested within treatment; the latter term was included to account for the fact that the water treatment was applied to whole trays at a time. We tested the significance of random effects using 1-tailed likelihood ratio tests (because variances cannot be less than zero), comparing -2 log likelihoods of models with and without the random effect of interest. In these models, the main effects of treatment indicate the plastic, environmental response of the trait to our experimental manipulation. The line * treatment interaction term indicates whether there is genetic variation in these plastic responses.

We estimated the denominator degrees of freedom for fixed effects using the Kenward Roger approximation (SAS, Proc Mixed). For any traits that showed differences in their means between treatments, we performed follow-up tests to determine if their relationship with fruit number differed between treatments. To do so, we modified our standard mixed model to include fixed effects of the traits and the trait by treatment interaction term.

**Selection gradients**

We estimated selection gradients for two traits, basal branch number and flowering time, in each treatment separately. We included basal branch number in our selection models because the Lande-Arnold (1983) method is sensitive to the omission of correlated traits from selection models, and several previous studies have detected selection on basal branch production (Weinig, Stinchcombe & Schmitt, 2003; Griffith, Kim & Donohue, 2004; Kelley et al., 2005; Stinchcombe et al., 2009). We estimated relative fitness by dividing individual fruit number by the mean fruit number in that treatment. We then used a mixed model with block, days to flowering, and basal branch number as fixed effects; we included line and tray as random effects. We present the fixed effect solutions for the traits as estimates of the selection gradients, as well as P-values from the mixed model. While the mixed model is necessary for our data to obtain proper hypothesis tests and degrees of freedom for our selection gradients, the
results from a simple multiple linear regression are identical in direction and statistical significance, and are quantitatively extremely similar (parameter estimates differ by less than 1 s.e.). We also present the arithmetic mean and the standard deviation of the traits to facilitate the calculation of mean-standardized (Hereford, Hansen & Houle, 2004) and standard deviation standardized gradients (Lande & Arnold, 1983) for any subsequent meta-analyses.

**Randomization testing**

Because some of our response variables were not normally distributed, we verified the P-values for our hypothesis tests with randomization tests using modified versions macros written by Cassell (2002). For the correlation between flowering time in the chamber and the rooftop common garden used by Samis et al. (2012), we examined whether the observed correlation coefficients fell into the upper or lower 2.5\textsuperscript{th} percentile of the randomized distribution, which would indicate that it was more extreme than expected by chance alone. Because our goal was to compare the flowering characteristics of lines, we used inbred line means for these analyses. For the mixed models, we compared the p-values corresponding to the treatment, trait, or treatment*trait interaction terms to the distribution of p-values obtained from randomized data. These analyses were done using individual-level data. For all cases, we observed identical patterns of statistical significance using randomization, and as such we present P-values from standard hypothesis tests.

**Results and Discussion**

**Correlations with previous flowering time data**

Plants in the growth chamber flowered over 115 days, with the first plant flowering on the 37\textsuperscript{th} day. Compared to previously published data on these lines, the flowering phenology observed in the growth chamber was compressed—Samis et al. (2012) report flowering time means in excess of 200 days. We had expected that plants grown in the chamber would flower earlier than the plants grown in the outdoor common
garden experiment conducted by Samis et al. (2012), based on the latter having overwintered in colder conditions for much longer. Some of the plants in our experiment were able to flower during "winter" conditions because of the above 0 degree temperatures, and also experienced seasons that were artificially sped up by changing temperature and day length every two weeks.

Despite the differences in growth conditions, and mean flowering times, we observed moderate but significant rank correlations between the flowering time phenotypes observed in the growth chamber and those in the common garden (Figure 2). Days to flower in high water and in low water were correlated with flowering time in the common garden ($\rho_s = 0.2966$, $P=0.037$ and $\rho_s = 0.3163$, $P=0.025$, for high and low respectively). Correlations across experiments were higher for bolting time ($\rho_s = 0.4272$, $P=0.002$ and $\rho_s = 0.3874$, $P=0.0054$). Comparisons between the common garden and the mean phenotypes of the chamber experiment (i.e., averaging both treatments) are of similar magnitude and significance ($\rho_s = 0.306$, $P=0.03$ for flowering time; $\rho_s = 0.417$, $P=0.002$ for bolting time). These data indicate that early and late flowering or bolting genotypes from the common garden experiment were also early and late flowering (bolting) in the growth chamber. We can conclude that there is some common underlying genetic basis for the pattern of flowering observed both outdoors and in the chamber. However, because the correlation is still significantly lower than $r=1$, GxE interactions play a large role in determining flowering time of the lines under different conditions (Lynch & Walsh, 1998). Genotype by environment effects for flowering time in Arabidopsis are widely known (e.g. Jansen et al., 1995; Alonso-Blanco et al., 1998; El-Assal et al., 2001; Weinig et al., 2002; Ungerer et al., 2003; Juenger et al., 2005; Giakountis et al., 2010).
Figure 2: Correlation between flowering times of lines grown in our chamber experiment, and the same lines grown for a study by Samis et al. (2012) in an outdoor roof setting. For clarity, we portray the mean phenotype of lines in the chamber experiment, including both high and low water treatments.
We failed to observe any correlation between fruit set in the chamber and precipitation levels at the site of origin (r < -0.109, P > 0.45 for all correlations). One possible explanation for this is that our chambers did not accurately mimic natural conditions (e.g., light conditions, soil availability and pot size, our use of mid-point day lengths and temperatures, etc.). Thus while generalizing from fitness measures in the chambers to those under field conditions requires caution, comparisons between the experimental treatments within the chamber still allow a strong test of the hypothesis that precipitation levels could in principle impose selection on flowering time.

**Main effects of water treatment on traits and fitness**

Our next goal was to evaluate the effects of the water treatment on plant size, life history, and fitness (Table 1). We found consistent effects of the water treatment on life history and morphology (least-square means ± 1 standard error): plants in the low water treatment took marginally less time to flower (97.87 ±3.91 –vs- 100.49 ± 3.9 days, P = 0.062), had significantly fewer basal (rosette) branches (2.32 ± 0.22 –vs- 3.69 ± 0.22), and set significantly fewer fruits (216.52 ± 11.23 -vs- 299.17 ± 11.39) (Figure 3). For flowering time, our ability to resolve main effects of the treatments with the present sample sizes may be obscured by the substantial genetic variation in the plastic response to the treatment (Table 1). While on average plants accelerated their flowering under low water conditions, some lines delayed it appreciably (Supplemental Figure 1). Because of our a priori interest in flowering time, and the significant differences between treatments in basal branch number, we focus on these traits for subsequent analyses.
Figure 3: Trait and fitness differences between the high and low water treatments. 

(A) Flowering time (B) Number of basal branches (C) Fruit number (a proxy for fitness)
<table>
<thead>
<tr>
<th>Trait</th>
<th>Block</th>
<th>Treatment</th>
<th>Line</th>
<th>Line * Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to Flowering</td>
<td>$F_{1, 13.4} = 0.39, $ $P = 0.54$</td>
<td>$F_{1, 15.5} = 4.04, P = 0.062$</td>
<td>$\chi^2 = 763.9, P &lt; 0.0001$</td>
<td>$\chi^2 = 3.6, P = 0.028$</td>
</tr>
<tr>
<td>Basal Branches</td>
<td>$F_{1, 13.1} = 0.08, P = 0.78$</td>
<td>$F_{1, 14.4} = 26.9, P &lt; 0.0001$</td>
<td>$\chi^2 = 67.1, P &lt; 0.0001$</td>
<td>$\chi^2 = 1.7, P = 0.09$</td>
</tr>
<tr>
<td>Fruit Number</td>
<td>$F_{1, 13} = 0.65, P = 0.44$</td>
<td>$F_{1, 12.9} = 36.7, P &lt; 0.0001$</td>
<td>$\chi^2 = 43.3, P &lt; 0.0001$</td>
<td>$\chi^2 = 0.7, P = 0.2$</td>
</tr>
<tr>
<td>Days to bolting</td>
<td>$F_{1, 13.3} = 0.01, P = 0.93$</td>
<td>$F_{1, 11.2} = 0.88, P = 0.37$</td>
<td>$\chi^2 = 790.3, P &lt; 0.0001$</td>
<td>$\chi^2 = 0.2, P = 0.33$</td>
</tr>
<tr>
<td>Rosette Leaf Number</td>
<td>$F_{1, 13.4} = 0.33, P = 0.57$</td>
<td>$F_{1, 17} = 1.42, P = 0.25$</td>
<td>$\chi^2 = 333.2, P &lt; 0.0001$</td>
<td>$\chi^2 = 5.4, P = 0.01$</td>
</tr>
<tr>
<td>Rosette Diameter</td>
<td>$F_{1, 12.9} = 3.67, P = 0.08$</td>
<td>$F_{1, 14.9} = 1.26, P = 0.28$</td>
<td>$\chi^2 = 107.8, P &lt; 0.0001$</td>
<td>$\chi^2 = 9.4, P = 0.001$</td>
</tr>
</tbody>
</table>

**Table 1:** Main effects of the water manipulation on life history and morphological traits. Results of mixed model ANOVAs for the six measured traits. For fixed effects, $F$ statistics and $p$-values are reported. For random effects, likelihood ratio statistics and $p$ values are reported.
**Relationships between traits and fitness**

We next examined whether the traits that showed significant differences between treatments also had different relationships with fitness in the two treatments. The relationship between flowering time and fitness differed significantly between treatments (Figure 4; $F_{1,420} = 5.43$, $P = 0.02$). Early flowering always led to higher fruit set, but late flowering was significantly more costly in the dry treatment. In contrast to flowering time, we observed a similar relationship between basal branch number and fruit number in both treatments ($F_{1,437} = 0.72$, $P = 0.40$). In both treatments, greater fruit number was associated with greater numbers of basal branches.

Plants can experience trade-offs in terms of investment in growth versus reproduction (Obeso, 2002). In this scenario, delayed flowering will correlate with an increased ability to accumulate resources, which can then be converted into reproductive structures and lead to high fitness. In *Arabidopsis* however, the reproductive structures can contribute significantly more than rosettes to carbon acquisition (Earley et al., 2009), suggesting that there might not be an advantage to delaying flowering in terms of acquiring carbon. Given this, it may be advantageous for *Arabidopsis* to flower early, and have extended reproductive duration, which could explain why, in both treatments, plants that flowered earlier produced more fruits.
Figure 4: The total fruits produced across first flowering dates in the low (grey) and high (black) water treatments.
The more severe consequences of flowering later in the lower water treatment correspond with the phenotypic and climate characteristics of the cline that Samis et al. (2012) examined. In their study, accessions from inland sites, which are drier, tended to flower earlier, which is consistent with our findings that selection acts more strongly against late flowering under drier conditions. Harsher end of season conditions in dry sites may be the major selective pressure for earlier flowering inland. Precipitation differences could therefore lead to earlier flowering in drier inland sites in the introduced range.

**Selection gradients**

Our final goal was to estimate selection gradients for flowering time and basal branch number, and thus evaluate their direct effects on fitness while accounting for their phenotypic correlation ($r = 0.21$, $P = 0.0018$ in the high water treatment; $r = -0.028$, $P = 0.67$ in the low water treatment). Under high water conditions, we found significant selection for earlier flowering, and for greater basal branch number (Table 2). Under low water conditions, we found the same pattern: highest relative fitness was associated with early flowering and more basal branches. For both traits, selection was of much greater magnitude under low water conditions than under high water conditions: selection gradients were ~1.7 to 1.9-fold higher under low water conditions. These results are qualitatively similar to a field study of selection *Arabidopsis thaliana*, where Kelley et al. (2005) found stronger selection on herbivore resistance traits in low-fitness environments.
<table>
<thead>
<tr>
<th>Trait</th>
<th>High water</th>
<th>Low water</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (s.e.)</td>
<td>P</td>
<td>β (s.e.)</td>
</tr>
<tr>
<td>Days to Flower</td>
<td>-0.0030 (0.0007)</td>
<td>&lt;0.0001</td>
<td>-0.0053 (0.0013)</td>
</tr>
<tr>
<td>Basal Branch Number</td>
<td>0.0414 (0.0078)</td>
<td>&lt;0.0001</td>
<td>0.0775 (0.0221)</td>
</tr>
</tbody>
</table>

Table 2: Unstandardized selection gradients for flowering time and basal branch number in the two watering treatments. The arithmetic mean and standard deviation of each trait in each environment are also given, to aid calculation of standardized selection gradients (see methods).
In both our high and low water treatments, selection favored earlier flowering times, which raises the question of why so much variation in flowering time was seen among our lines. Selection for early flowering seems ubiquitous; a recent meta-analysis has suggested earlier flowering is generally favoured by selection, and that other constraining forces act to prevent all plants from flowering early (Munguia-Rosas et al., 2011). If flowering time really is one of the most important traits in determining fitness of a plant, why do we see so much variation among individuals from the same population experiencing the same environmental conditions? Among our populations, there were many which were polymorphic for a designation of "early" or "late" flowering, as designated by Samis et al. (2012). The cline is by no means perfect -- many earlier flowering lines occur closer to the East coast, and many later flowering lines were collected from sites farther west. The possible reasons behind this within population variation include: insufficient time since colonization to respond to selection, migration from other populations, selection on correlated traits which constrains responses in flowering times, and temporally variable selection. Selection on flowering time can also be seasonally and epistatically variable in the field (Korves et al., 2007), both of which could possibly maintain variation within populations in flowering time. In addition, the timing of germination dramatically influences the fitness consequences of flowering time variation and variation at flowering time genes (Wilczek et al., 2009), suggesting that germination and seed bank dynamics could contribute to the maintenance of variation in flowering time. Selective agents outside of climatic factors may be acting on Arabidopsis populations, including herbivory, competition, or disturbance; different selective forces operating in opposing directions could maintain variation in flowering traits. Brachi et al. (2011) describe, in detail, how broad scale climatic variation can lead to the evolution of clines in phenotypes, but micro-environmental variation in factors such as soil quality, competition, or biotic factors could lead to the maintenance of variation within populations arrayed along a cline.

Future research could focus on distinguishing among these, and potentially other, reasons why we still see such a range of flowering times maintained within populations.
Conclusions

Understanding why plant populations evolve divergent trait values across their ranges is an important component of explaining and predicting the spread of introduced species. We have characterized one selective agent capable of acting on North American populations of A. thaliana in chamber conditions—winter precipitation level—as a potential cause of longitudinal variation in flowering time. Precipitation accumulated during the winter determines how moisture-limited plants will be during the spring growing season when they make the transition to reproduction and begin flowering and producing fruit. We suggest that varying patterns of precipitation across the Eastern United States could be an important selective agent acting differentially on populations across the longitudinal range of A. thaliana, contributing to the observed cline in flowering time.

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Additional information and declarations

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**Author contributions**
Amanda Stock and Brechann McGoey performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
John Stinchcombe conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

**Data Deposition**
The following information was supplied regarding the deposition of related data: Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2jk12.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.898#supplemental-information. (This information is also included in the Appendix of this thesis).
Chapter 3
Conclusions and Future Directions

Significance of findings

One of the central goals of evolutionary ecology is to explain how genetic variation responds to selective pressures in the environment to produce adaptive differences between populations. However, many existing studies have focused only on associations with environmental variables, and can therefore only suggest potential causes of local adaptation based on correlations. Confirming ecological agents of selection is still an active area of investigation, with more manipulative experiments necessary to determine the relative contributions of genetic and environmental factors, as well as to identify relevant selective forces. It is challenging to link the genetic architecture of traits to ecological processes that create selective pressures and quantifying the resulting evolutionary consequences, but doing so can illuminate the way in which populations evolve to deal with new environments (Metcalf & Mitchell-Olds, 2009).

The work presented in this thesis demonstrates one means of confirming the action of putative agents of selection. Our findings related to winter precipitation are an excellent example of how environmental conditions affect life history traits and the selective forces acting on them. The comparison between outdoor common garden and environmental chamber data demonstrates that life history evolution is, to some extent, predictable, though context-dependent. It also provides another piece of the puzzle in the story of local adaptation of *A. thaliana* in North America, but doesn't elucidate how long these sorts of changes take to happen. Nor does it provide the exact underlying genetic basis for these changes, though the genetic variation present in the lines used has been studied by others and can be used to further clarify the genetics of adaptation in future.
Our experiment confirms that winter water levels differentially influence the fitness of early and late flowering plants in the chamber, implicating it as a potentially important selective agent in the field. Plants which flower later have much lower fitness in dry conditions than wet ones, and plants flowering early have similar fitness regardless of water availability. In the introduced North American range, winter precipitation levels may be an important selective agent acting to determine flowering time, with wetter, more Eastern sites under less selective pressure, consequently flowering later than their centrally-located counterparts. Considering that earlier flowering time was favoured in both treatments in our experiment, and a recent meta-analysis has suggested that this is a general trend (Munguía-Rosas et al., 2011), future research needs to investigate the reason why we see maintenance of genetic variation for flowering times. Maintenance of variation is especially important to explore among lines within the same population, which are experiencing the same environmental conditions, suggesting other factors may be at play in determining why multiple alleles are maintained at loci of major effect.

**Future avenues for research**

My thesis ties into a framework upon which to base future questions about life history evolution and local adaptation, not only in *A. thaliana*, but for introduced species in general. To understand how a variety of ecological factors influence the resulting evolutionary trajectories of introduced populations, future related work must address two overarching questions: 1) How do life history traits respond to selection at the genetic and phenotypic level in populations over successive generations? and 2) How do differing environmental conditions affect the expression of these traits and type of selection acting on them? These questions should be approached using the techniques of experimental evolution and studies of selection in the field, thereby connecting genotype, phenotype, and fitness to determine evolutionary dynamics in populations over multiple generations (Barrett & Hoekstra, 2011).
To understand what is occurring during adaptation to novel conditions post-introduction, we need to test hypotheses about selective pressures associated with climate and seasonality. Future experiments should focus on elucidating how both long- and short-term environmental pressures interact with the molecular sequence variation underlying important life history traits, thereby driving rapid adaptation to novel conditions. To have the widest applicability, these questions need to be approached from a number of angles; though field work on both experimental and natural populations should be a central focus, allowing actual environmental selective pressure to act on the genetic variation underlying important life history traits (eg. Donohue et al., 2005; Malmberg et al., 2005; Korves et al., 2007). The following step would be to investigate some of the environmental factors discovered to be potentially important agents of selection, and thereby determine the predictability of life history evolution in a given selective regime.

**Experiments based on current work**

As mentioned above, bringing studies of evolutionary genetics outside of the lab and determining the impacts of natural environmental selection pressures is one of the most important challenges in understanding rapid adaptation post-introduction. Conducting experimental evolution in the field and being able to track allele frequency changes year-to-year at loci whose phenotypic effects have been previously characterized, though complex and a bit risky, is also likely to yield very novel information about how rapid adaptation proceeds under natural conditions (Collins, 2010; Barrett & Hoekstra, 2011). By elucidating the molecular patterns underlying ecologically important traits of a model species, many related areas of research – which currently provide only a piecemeal understanding of life history evolution – will be linked, contributing to a synthesis of our understanding of the genetics of adaptation in complex traits. The genes which influence life history traits in *A. thaliana* are also common to many other species – including non-model study systems and other introduced plants – making them generally important to an understanding of
local adaptation (Metcalf & Mitchell-Olds, 2009).

In addition to the water availability experiment presented here, I have been laying the groundwork for a field study of changes in allele frequency for genes of major effect in a number of life history traits in North American *A. thaliana*. The goal of this future work is to separate year-to-year differences in selection pressures from overall trends and patterns of adaptation (Korves *et al.*, 2007; Lee & Gelembiuk, 2008). I have developed recombinant lines from crosses between individuals with differing *FRI* and *FLC* alleles collected from a single polymorphic population of North American *A. thaliana*. The progeny, which show all combinations of both alleles for the two loci, – which have been shown to be major flowering time regulators interact epistatically (Caicedo *et al.*, 2004) could be planted in multiple experimental field populations. These populations would be left to grow naturally for three to four years, and assigned to either a fall or spring tilling schedule to examine the impacts of seasonality on fitness. Each generation, a representative sample of plants could be genotyped at the *FRI* and *FLC* loci, and potentially at other candidate life history loci (*e.g.*, *DOG* - delay of germination (Chiang *et al.*, 2011)). Plot-level traits such as peak flowering, germination and duration of flowering/fruiting could be tracked to determine if any trend in timing of life history events can be seen in a quantitative sense. Tracking multi-generational change in *FRI* or *FLC* allele frequencies would connect the quantitative genetics of complex life history traits with allele frequencies from individual loci of major effect. The G × E interactions which affect life history phenotypes also mean laboratory-based studies of the genetics of adaptation may not reflect the biological reality, so field studies become especially necessary to determine how candidate alleles influence adaptive phenotypes (Anderson, Willis & Mitchell-Olds, 2011; Barrett & Hoekstra, 2011).

A large-scale, multi-year field experiment such as the one I propose here would elucidate the mode and tempo of evolution in populations of introduced *A. thaliana* in ways that previous experiments, mostly lab-based, have been unable to do. Following multi-generational change in candidate genes which interact epistatically may
illuminate the influence of seasonality on life history strategies (Wilczek et al., 2009; Scarcelli & Kover, 2009; Brachi et al., 2010) and help to determine whether seasonality or specific biotic/abiotic selective agents act to maintain one or multiple life history strategies in a population (see Pigliucci, 2002).

This sort of experiment could also give some indication of how quickly shifts in life history can occur in natural populations. The level of standing variation present in the cross-derived lines, while not yet quantified in any way, may be similar enough to actual founding populations in novel habitats to draw strong conclusions about how the ephemeral and fluctuating population dynamics of *A. thaliana* impact its evolution in a natural situation. Whether the epistatic effects of *FRI* and *FLC* on flowering time and fitness are of sufficient magnitude (relative to stochastic factors) to cause rapid evolutionary change in allele frequencies and phenotypes is one of the more specific yet broadly applicable potential findings of such an experiment. Finally, connecting the quantitative genetics of complex traits with allele frequencies from individual loci of major effect is another important and as of yet relatively unexplored territory (Liedvogel, Cornwallis & Sheldon, 2012). Studies which are able to reveal the relative importance of large effect candidate genes and smaller effect quantitative or genome-wide changes are necessary, and a current issue of some debate (Rockman, 2012).

Another option for extending the work on life history evolution in introduced *A. thaliana* would be to do a large-scale reciprocal transplant experiment with lines from eastern and western North American populations. A transplant experiment could substantiate preliminary observations of local adaptation in ecologically important traits – such as vernalization requirements, germination success, and flowering time – by confirming the causal ecological processes. Lab-germinated seedlings from the 213 accessions of *A. thaliana* used in both this work and previous experiments (Samis *et al.*, 2012) could be planted into two field sites: one in the Eastern portion of the introduced range and one in the Western portion. Important life history traits could be measured and compared in "home" and "away" environments, and the effects of biotic
components of the environment on local adaptation could be quantified by measuring damage from herbivores and pathogens (Bossdorf et al., 2005). Testing for associations between life history traits and fitness differences in Eastern and Western populations, we could determine if there is a “home-site advantage” (Hereford, 2009) for populations which have adapted locally since A. thaliana was introduced to North America.

**Alternative approaches to investigating questions of life history evolution post-introduction**

There are also some alternative ways of investigating the questions which extend from the work presented here on flowering time evolution. Descriptive observational study of western and eastern portions of the North American range would be one way of investigating local differences in traits and allele frequencies. However, this method cannot determine if putative adaptive differences are actually selected for or beneficial in any way -- they may be due to other factors such as founder effects and genetic drift. Non-manipulative studies also do not allow for controlling the effects of genetic background, which can have a major impact on complex life history traits due to their polygenic nature. A theoretical modelling approach to explore potential evolutionary dynamics could also be taken. This would require estimates of response to environmental selection pressures from previous experimental studies and, ideally, some initial allele frequencies for candidate loci of interest. The advantages would be numerous: hundreds of generations with little time and effort expended, macro-scale predictions made possible, and the ability to test multiple environments and selective pressures at once. However, all such simulations are simplified – they may not capture natural variability in selective regimes and make many assumptions regarding differences between plant lineages and genetic control of traits – and therefore may not reflect biological reality. These approaches certainly provide their own value, and would complement further experimental studies of life history evolution in response to novel environmental pressures.
Final thoughts and perspectives

As the global climate continues to change, comprehending how rapid adaptation to environmental conditions occurs will be vital for both conservation efforts and agriculture (Lavergne et al., 2010). The genetics of invasiveness and how plants become naturalized to novel environments will also be illuminated by this work (Bossdorf et al., 2005; Keller & Taylor, 2008; Hereford, 2009). For these reasons alone, the pursuit of deeper understanding of adaptation post-introduction is a worthy one. Furthermore, a better understanding of the interplay between genotype, phenotype, and agents of selection will allow for new tests of theories of the genetics of adaptation, genome-environment interaction, and an examination of the relative contribution of epistatically interacting loci in natural conditions (Orr, 2005). The body of research to which this thesis belongs and the future directions I have proposed will ultimately contribute to our understanding of life history evolution at all scales, connecting molecular genetic variation to ecological selection pressures and the evolutionary outcomes which arise from them, and improving our ability to predict the effect of unfamiliar environmental conditions on plants.
References Cited


Appendix

Supplemental Information from Chapter 2

Supplemental Table 1

Winter precipitation levels of collection sites for each selected line, along with flowering phenotypes from the low water treatment, high water treatment, and rooftop common garden of Samis et al. (2012). Rows are in order from driest to wettest average January precipitation levels. Data are 30 year averages from a 0.5° grid (Mitchell & Jones, 2005).

<table>
<thead>
<tr>
<th>LineID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Average precipitation in January</th>
<th>Average total precipitation in winter months</th>
<th>Low treatment days to flower</th>
<th>High treatment days to flower</th>
<th>Rooftop experiment days to flower (Samis et al. 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>42.500</td>
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<td>39.0033</td>
<td>341.9866</td>
<td>40.80</td>
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**Supplemental Figure 1**

*Reaction norms of flowering time in response to the water treatment.*

Reaction norm plot of flowering time in the two water treatments. The symbols and lines connect the mean flowering times of the same inbred lines in the two experimental treatments.