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Phenotypic plasticity, an individual's phenotypic response to environmental change, is a fundamental characteristic of all life on earth. Phenotypic plasticity plays a central role in adaptation, phenotypic differentiation, and speciation. Temperature-sensitive phenotypic plasticity, i.e. thermal plasticity, often increases with latitude, suggesting an increasingly adaptive role of thermal plasticity in predominantly cool, thermally variable environments. Whereas the hypothesis is reasonable, it has not been thoroughly tested. Demonstrating local adaptation of thermal plasticity requires showing that: 1) thermal plasticity increases fitness in high latitude environments, 2) clinal variation arises from natural selection, and not by chance alone, 3) differences in thermal plasticity persist in the presence of gene flow, 4) thermal plasticity is inherited from parents to offspring, 5) thermal plasticity varies genetically along a latitudinal gradient, and 6) thermal plasticity is a derived phylogenetic character. Today, little is known about the genetic properties of thermal plasticity. I took advantage of natural geographic variation in a widespread perennial herb, *Plantago lanceolata* to improve our understanding of adaptation along latitudinal clines by examining the genetic features of thermal plasticity. With genetic data I address the questions: 1) Is clinal variation in thermal plasticity best explained by natural selection driven by environmental differences among populations, neutral genetic evolution, or both? 2) What is the genetic architecture of thermal plasticity and single-environment trait variation, and how are they related? 3) Do genetic properties of thermal plasticity mirror phenotypic patterns along a latitudinal gradient?

Among 14 European populations of *Plantago lanceolata*, I estimated differentiation in temperature-sensitive floral reflectance plasticity (Q_{ST}/P_{ST}), neutral genetic differentiation (F_{ST} & Jost's D) of AFLP markers, and between-population differences in aspects of the reproductive

environment. I used phenotypic Q_{ST} (P_{ST}) vs. F_{ST} comparisons to investigate the evolutionary forces responsible for geographic patterns of thermal plasticity, and to determine if differences brought about by neutral evolutionary forces are sufficient to explain these patterns. My data supported the hypothesis that natural selection, driven by environmental properties of the reproductive season, particularly the duration and proportion of time at cool temperatures, has contributed to geographic patterns of thermal plasticity. As between-population differences in these environmental variables increased, differences in thermal plasticity increased more quickly than did neutral genetic differences.

To determine the genetic architecture of thermal plasticity I produced an F_2 mapping family from parents derived from distant northern and southern European populations that exhibited high (northern parents) and low (southern parents) thermal plasticities of floral reflectance. I then grew parents and offspring in two environments (cool and warm) mimicking what plants would encounter in nature. I attained genetic markers via genotype-by-sequencing (ddRADseq), produced a recombination map and performed QTL mapping of thermal plasticity and single-environment trait values for six traits: floral reflectance, flowering time, rosette diameter, leaf length, leaf fresh mass, and leaf area. My data provide critical genetic support for the hypothesis that temperature-sensitive floral reflectance plasticity in *P. lanceolata* is adaptive in high latitude environments where growing seasons are cool and short. My data confirm that thermal plasticity in *P. lanceolata* has a genetic basis as I found one single QTL underlying the thermal plasticities of three traits, floral reflectance, flowering time and leaf length. Floral reflectance plasticity and flowering time plasticity QTLs colocalized with, and shared phenotypic effects with corresponding single environment QTLs. The leaf length plasticity QTL did not colocalize with any single-environment QTLs, and was influenced by cytoplasm. I did not find evidence that plasticity QTLs of different traits were pleiotropic. Additionally, genotypic

differences at plasticity QTLs paralleled patterns of plasticity along latitudinal clines. At plasticity QTLs, northern genotypes (Danish and Swedish) increased the magnitude of thermal plasticity, while southern genotypes (French and Italian) decreased plasticity.

THE GENETICS OF THERMAL PLASTICITY IN *PLANTAGO LANCEOLATA*

by

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DEDICATION

I dedicate my dissertation to my family and friends who have provided me with continuous love, encouragement and support, and without whom this work would not have been possible. To my beautiful wife Colleen, my parents Patty and Mike, my siblings Missy, Becca, Mike, Em, and Kait, my extended family Aram, Suz, Will, Zac, Mitch, Aaron, Patty, George, Aykan, and Julie, and my closest friends Jonny, Beizav, Parksie, Tedo, Calvin. CDS Baby! Thank you all for your love, reassurances and conversations that helped me stay motivated.

APPROVAL PAGE

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CHAPTER I

INTRODUCTION

The Response to Environment

Phenotypic Plasticity

Phenotypic plasticity, an individual's phenotypic response to environmental change, is a fundamental characteristic of all life on earth. It plays a central role in adaptation, phenotypic differentiation, and speciation (Bradshaw 1965; DeWitt & Scheiner 2004; Moran 1992). In spite of this, many questions about plasticity persist. Which traits are sensitive to environmental change? In which environments is phenotypic plasticity adaptive? What genes cause phenotypic responses to environmental change? What is the genetic architecture underlying phenotypic plasticity? Are traits that respond to the same environmental conditions genetically correlated? These are exciting questions in evolutionary ecology considering that all organisms encounter variability in their external environment. The questions address a major challenge in biology: the connections between genotype, environment, and phenotype. In this dissertation I address these questions using a widespread perennial herb, *Plantago lanceolata*. My dissertation research explores evolutionary factors that have contributed to variation in temperature-sensitive plasticity along geographic clines, and additionally, explores the inheritance, genetic architecture, and genetic correlations of thermal plasticity in fitness-related traits. My research methods were designed to identify environmental conditions that favor thermal plasticity, describe trait responses to temperature change, their genetic basis, and the correlations among thermal plasticities of different traits. My findings help illuminate the connections between quantitative

genetic variation and the environment, which together explain the spectrum of phenotypic variation we see in nature.

Thermal Plasticity

Temperature-sensitive phenotypic plasticity in many traits is likely to be adaptive (i.e. improve fitness) because environmental temperature has a strong influence on the structural and functional properties of organisms (Hazel & Williams 1990; Jockusch 1966; Marmur & Doty 1962). Ectotherms, which rely on external heat sources to mediate internal body temperature (Huey & Stevenson 1979; Wieser 1973), have evolved countless phenotypic responses that confer acclimation to a new temperature and/or avoidance of thermally stressful environments. For example, many organisms respond to temperature through adjustments in 1) behavior e.g. movements through microhabitats, solar tracking (Clench 1966; Ehleringer & Forseth 1980; Huey 1991; Webster & Weathers 1990), 2) phenology of sensitive life stages e.g. bud, flower, and fruit emergence, laying/birthing date in animals (Crick *et al.* 1997; Fitter & Fitter 2002; Visser & Holleman 2001) and 3) cellular physiology e.g. of cellular membranes and gene expression (Angilletta Jr *et al.* 2002; Hazel 1995; Huey & Bennett 1990; Huey & Stevenson 1979; Lacey & Herr 2005; Marmur & Doty 1962; Somero 1995). Some temperature-sensitive responses (e.g. behavior, movements, gene expression) can be reversible if periods of thermal variation are shorter than the life-span of the organism, while others (e.g. germination and hatching date) are developmentally fixed (Gabriel 2005; West-Eberhard 2003). Thermal responses that do not influence fitness can be neutral to selective pressures. Adaptive thermal plasticity requires both accurate phenotypic responses that confer higher fitness relative to individuals lacking the ability to respond, and early thermal cues (prior to the phenotypic response) that accurately predict future environments (Reed *et al.* 2010). Failure of accurate responses, responses that reduce fitness, or unpredictable thermal cues can result in maladaptive plasticity (Nicotra *et al.* 2010;

Visser 2008). Ultimately, the adaptive value of thermal plasticity depends upon how the thermal response affects fitness in a given environment. For example, in montane ecosystems, advancing flowering in response to early season warming can increase reproductive output (Anderson *et al.* 2012) but also increases susceptibility to frost damage (Inouye 2008). Thus, thermal plasticity of flowering time can be adaptive if environmental conditions remain favorable and allow completion of reproduction, but the same thermal plasticity becomes maladaptive if a late frost damages sensitive reproductive tissues and reduces fitness.

Importance of Researching Thermal Plasticity

Today it is clear an organism's phenotype is determined by both its genetic makeup and the environmental conditions to which they have been exposed. Investigating thermal plasticity can help assess the range and nature of plasticity that organisms display in response to environmental cues, and the standing genetic variation in plasticity they possess (Bradshaw & Holzapfel 2008; Chevin & Lande 2011). With this information we can better evaluate how variation in the natural world has contributed to phenotypic differences among individuals across the landscape.

In many species, thermal plasticity displays positive correlations with latitude, e.g. developmental rate (Laugen *et al.* 2003), body size (Liefting *et al.* 2009), thermal tolerance (Addo-Bediako *et al.* 2000; Ghalambor *et al.* 2006), leaf shape (Royer *et al.* 2009), flower/seed number (Molina-Montenegro & Naya 2012), and flower reflectance (Lacey *et al.* 2010). These latitudinal patterns of thermal plasticity are presumed to reflect local adaptation, in the sense that temperature-sensitivity is believed to be more adaptive in thermally variable environments where growing seasons are cooler and shorter (Huey & Stevenson 1979; Lacey *et al.* 2010; Wieser 1973). While the hypothesis is reasonable, it has not been thoroughly tested. Demonstrating local adaptation of thermal plasticity requires showing that: 1) thermal plasticity increases fitness in

high latitude environments, 2) clinal variation arises from natural selection, and not by chance alone, 3) differences in thermal plasticity persist in the presence of gene flow because selection counteracts the homogenizing effects of gene flow, 4) thermal plasticity is inherited from parents to offspring, 5) thermal plasticity varies genetically along a latitudinal gradient, and 6) thermal plasticity is a derived phylogenetic character (Brandon 1990, Lacey *et al.* 2010). Satisfying all of these requirements is challenging, although there is evidence that thermal plasticity of some traits meets a number of these requirements.

Ultimately, we would like to identify the genes underlying the plasticity. Doing so can help us understand the molecular mechanisms by which organisms respond to environmental changes. Such information can give us clues as to how plasticity has evolved in the past and potentially provide us with useful tools to modify crop species and improve yield in future environments.

Additionally, researching thermal plasticity has several practical applications. Contemporary climate changes characterized by increasing atmospheric and surface temperatures and rapid shifts in local weather conditions is altering ecosystems (IPCC 2014). The uncertainty about how organisms will respond to these changes gives pause to evolutionary biologists who have recently increased their research toward seeking to understand plastic and evolved responses to changing environments (Charmantier & Gienapp 2014; Chown *et al.* 2010; Hoffmann & Sgrò 2011; Méndez-Vigo *et al.* 2016; Mercer & Perales 2010). Because adaptive plasticity and evolutionary change may facilitate species persistence in changing environments (Bell & Gonzalez 2011; Ghalambor *et al.* 2007), identifying traits with thermal plasticity that improves fitness will undoubtedly improve our ability to evaluate species facing peril. Thereafter, conservation efforts aimed toward protecting threatened species, mitigating negative effects of

climate change and preserving natural genetic diversity can be developed and implemented (Mawdsley *et al.* 2009).

Crop yield is sensitive to environmental variation, and more crops of higher quality must be produced to sustain the rapidly growing human population. There are many concerns about our ability to grow, or even sustain crop yield in the face of our rapidly changing climate, particularly rising temperatures (Lobell *et al.* 2008; Tester & Langridge 2010). For example, warm temperatures above a critical threshold ~30ºC tend to decrease harvest yields of the most important cereal crops including rice, wheat, maize, barley, soy, and sorghum (Lobell & Field 2007; Peng *et al.* 2004). Phenotypic plasticity in response to many environmental factors directly influences crop adaptation and yield (Nicotra *et al.* 2010; Sadras 2007; Sadras *et al.* 2009; Sadras & Trentacoste 2011; Trentacoste *et al.* 2011; Zhu *et al.* 2010). Thus, manipulating thermal plasticity in crop species has the potential to increase crop yields. For example, increasing growth rate and decreasing development time during cool periods can allow farmers to plant crops earlier in the season when temperatures remain cool, and/or harvest prior to harmful warmer temperatures. Planting crops that increase growth rate and develop faster under cool periods in locations with long periods of favorable conditions may also allow farmers to conduct more harvests per season. Thus, crop improvement strategies can benefit from knowledge of complex traits and genetic control of trait responses to various environmental conditions, including temperature (Tester & Langridge 2010).

Studying global patterns of temperature-sensitive plasticity can contribute valuable information to aid scientists in answering many of the longstanding questions about how organisms respond to their thermal environment. The information can also help more accurately predict evolutionary trajectories in the near future (Etterson 2004; Laurie *et al.* 2004). Geographic clines in thermal plasticity suggest local adaptation to changing environmental conditions (Addo-

Bediako *et al.* 2000; Ghalambor *et al.* 2006; Lacey *et al.* 2010; Laugen *et al.* 2003; Liefting *et al.* 2009; Molina-Montenegro & Naya 2012; Royer *et al.* 2009). However, we need more information to evaluate whether environmental conditions have selected for thermal plasticity, or whether other factors neutral to selection have produced these clines. Also, we would like to identify the specific environmental parameters that have favored, or selected against plasticity. Finally, we would like to understand the genetic architecture of thermal plasticity to evaluate how phenotypic divergence came about. We still know little about the genetic architecture underlying geographic variation in temperature-sensitive traits, i.e., the number of genes, their chromosomal locations, or their phenotypic effects (Alonso-Blanco & Méndez-Vigo 2014; Des Marais *et al.* 2013; Dittmar *et al.* 2016; Gerken *et al.* 2015; Méndez-Vigo *et al.* 2016). Ultimately, we would like to know whether: 1) few or many genetic loci control adaptive traits (Fisher 1919; Fisher 1930; Orr 1998, 2005), 2) genetic loci typically exhibit small or large phenotypic effects (Remington 2015; Rockman 2012), 3) pleiotropic genes affect adaptive thermal responses (Anderson *et al.* 2011; Des Marais *et al.* 2013; Méndez-Vigo *et al.* 2016), and 4) epistatic interactions influence thermal plasticity (Gaertner *et al.* 2012; Leinonen *et al.* 2013; Taylor & Ehrenreich 2015; Zeng 1993).

I took advantage of natural geographic variation in *Plantago lanceolata* to examine these questions about thermal plasticity. In this dissertation I build upon a body of previous work showing that thermal plasticity of floral reflectance in *P. lanceolata*: 1) is genetically variable within and among populations (Lacey & Herr 2005), 2) improves seed production at cool, but not warm temperatures relative to the absence of plasticity (Lacey *et al.* 2012), and 3) is positively correlated with latitude and altitude in its native Europe (Lacey *et al.* 2010).

Study Organism: Plantago lanceolata

Plantago lanceolata is an excellent organism for genetic studies of phenotypic plasticity generally. It is an herbaceous short-lived perennial that has successfully spread from its native Eurasia to all temperate regions of the world. Genotypes can be easily cloned and grown in artificial settings, and flowering is photoperiodically controlled. Extensive research over several decades has established that many fitness-relevant traits are genetically variable, e.g., leaf length, width, and angle, rosette diameter, number, and height, scape length, spike length, flowering time, male sterility, floral reflectance, alternative oxidase content, photosynthesis and respiration rates, floral anthocyanin content, and pollen viability (e.g. Barber *et al.* 1968; Case *et al.* 1996; Covey-Crump *et al.* 2002; Herrera & Lacey In prep.; Lacey & Herr 2005; Moore *et al.* In prep.; Primack & Antonovics 1982; Teramura & Strain 1979; Van Tienderen 1990; Van Tienderen & van der Toorn 1991; Wolff 1990; Wolff & Van Delden 1987). Traits are also highly plastic and genetically variable for plasticity. Temperature-sensitive traits include leaf length, width, and angle, scape length, spike length, flowering time, male sterility, floral reflectance, alternative oxidase content, photosynthesis and respiration rates, floral anthocyanin content, and pollen viability (Lacey unpublished data). Genetic correlations have been found among leaf angle and many traits including leaf length, width and weight, scape and spike length, and flowering date in *P. lanceolata* (Wolff & Van Delden 1989). Multiple studies show evidence of evolutionary divergence of populations, likely the consequence of variation in local selective pressures. For example, individuals from Dutch hayfield habitats, characterized by intense competition for light, produce longer leaves with more erect growth, fewer daughter rosettes, and fewer but larger flowering spikes than individuals from openly grazed pastures (Van Tienderen 1990).

Previously, Lacey and colleagues examined geographic patterns of temperature-sensitive floral reflectance plasticity and environmental properties of 29 European *P. lanceolata*

populations and found positive correlations between thermal plasticity with latitude, and altitude (Lacey *et al.* 2010). Additionally, path analyses strongly suggested the geographic clines in thermal plasticity are a result of evolutionary responses to the local thermal environment experienced during the reproductive season, specifically the proportion of time at cool temperatures and season duration, but not the magnitude of thermal variation (Lacey *et al.* 2010). I begin my dissertation research by adding genetic data to a subset of these populations to determine if geographic clines in thermal plasticity show evidence of natural selection. Then I crossed individuals from distant northern and southern populations that differed in thermal plasticity, and analyzed the genetic architectures underlying thermal responses of different traits. I uses these empirical genetic data to address the following longstanding questions about the genetics of plasticity.

Dissertation Goals

Dissertation Goal 1

Determine if the positive correlation between thermal plasticity and latitude is best explained by natural selection driven by environmental differences among populations, neutral genetic evolution, or both.

Chapter 2 - Natural selection contributes to geographic patterns of thermal plasticity in *Plantago lanceolata*.

I added neutral genetic data of amplified fragment length polymorphism (AFLP) markers to data from 14 European populations that displayed a positive correlation between temperature-sensitive floral reflectance plasticity and latitude. I then used this dataset to:

1. Examine patterns of neutral genetic diversity and genetic differentiation to determine whether founder effects correlate with latitudinal patterns of thermal plasticity.

2. Test how well different genetic, geographic, and environmental factors explained patterns of phenotypic differentiation in thermal plasticity.
3. Plot phenotypic Q_{ST} (P_{ST}) against neutral genetic differentiation (as F_{ST}) to determine if values fell above, at, or below the line of equality.
4. Conduct permutation tests to determine the correlations among phenotypic differentiation (P_{ST}), neutral genetic differentiation (F_{ST} and Jost's D) and environmental distance matrices. These tests can identify variables that help explain patterns of phenotypic differentiation.
5. Regress phenotypic (P_{ST}) and neutral genetic differentiation (F_{ST} and Jost's D) against geographic distance and environmental properties of the reproductive season. The regressions were used to determine whether i) phenotypic and neutral genetic differentiation increased as environmental conditions diverged, and ii) phenotypic differentiation was greater than neutral genetic differentiation.

Dissertation Goal 2

Determine and describe the genetic architectures of thermal plasticity and single-environment trait values for two reproductive traits (floral reflectance and flowering time) and four vegetative traits (rosette diameter, leaf length, leaf fresh mass and leaf area).

Chapter 3 - The genetics of thermal plasticity in *Plantago lanceolata*: QTL mapping

I produced an F_2 mapping family from parental genotypes derived from distant northern and southern European populations that exhibited high (northern parents) and low (southern parents) thermal plasticities of floral reflectance from over 300 genotypes sampling 29 European populations previously studied by Lacey et al. (2010). I then grew parents and offspring in two controlled thermal environments (cool and warm) that mimicked what plants would encounter in

their natural environment during the reproductive season. I developed a de novo genetic recombination map from genetic markers attained via double-digest restriction associated digest sequencing (ddRADseq) and performed QTL mapping to examine the genetic architectures underlying thermal plasticity and single-environment trait values. I used the dataset produced in this experiment to address the following questions:

1. Where in the genome are plasticity QTLs located?
2. How many loci underlie thermal plasticity and do they have small or large phenotypic effects?
3. Are plasticity QTLs the same as, or different from single-environment QTLs?
4. Is there a common genetic mechanism by which different traits respond to temperature changes?
5. Do overlapping QTLs display similar additive, dominant and/or interaction effects?
6. Are interactions between genetic loci an important component of the genetic architecture of thermal plasticity?

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CHAPTER II

NATURAL SELECTION CONTRIBUTES TO GEOGRAPHIC PATTERNS OF THERMAL PLASTICITY IN *PLANTAGO LANCEOLATA*

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Abstract

In natural populations, temperature-sensitive phenotypic plasticity (i.e. an individual's phenotypic response to temperature) often increases with latitude, suggesting an increasingly adaptive role of thermal plasticity in predominantly cool, thermally variable environments. Theoretical evolutionary models suggest environmental variability is important for maintaining phenotypic plasticity, and predict thermal plasticity to increase with the magnitude of thermal variation. Alternatively, recent empirical research has found thermal plasticity to decrease with temperature range, and increase with the duration of cold temperature exposure. We used phenotypic Q_{ST} (P_{ST}) vs. F_{ST} comparisons to investigate the evolutionary forces responsible for geographic patterns of thermal plasticity, and to determine if differences brought about by neutral evolutionary forces are sufficient to explain these patterns. We estimated differentiation in temperature-sensitive floral reflectance plasticity (P_{ST}) among 14 European populations of *Plantago lanceolata*, a widespread perennial herb. Greater thermal plasticity increases a plant's ability to partially thermoregulate reproduction. We measured neutral genetic differentiation (F_{ST} & Jost's D) using AFLP markers, and between-population differences in aspects of the reproductive environment. Our data indicated divergent selection for thermal plasticity was

present between populations where $P_{ST}>F_{ST}$. Regression models supported the hypothesis that environmental properties of the reproductive season, particularly the duration and proportion of time at cool temperatures, have contributed to geographic patterns of thermal plasticity. As between-population differences in these environmental variables increased, differences in (P_{ST}) of thermal plasticity increased more quickly than did neutral genetic differences. Our data did not support the hypothesis that the magnitude of thermal variation influenced geographic patterns of thermal plasticity.

Keywords

Phenotypic plasticity, natural selection, genetic drift, geographic clines, temperature, thermal plasticity, divergent selection.

Introduction

Temperature-sensitive plasticity (i.e. an individual's phenotypic response to changes in external temperature), which can confer thermoregulatory or acclimation ability, varies predictably across large geographic regions, displaying a positive correlation with latitude (e.g., Addo-Bediako *et al.* 2000; Angilletta 2009; Ghalambor *et al.* 2006; Lacey *et al.* 2010; Laugen *et al.* 2003; Liefting *et al.* 2009; Molina-Montenegro & Naya 2012). These correlations suggest that thermal plasticity becomes increasingly adaptive in thermally variable environments where growing seasons are predominantly cool and short (Huey & Stevenson 1979; Lacey *et al.* 2010; Wieser 1973). Furthermore, theoretical evolutionary models suggest environmental variability is important for maintaining phenotypic plasticity, and predict thermal plasticity to increase with the magnitude of thermal variation (Gomulkiewicz & Kirkpatrick 1992; Moran 1992; Via 1993; Via & Lande 1985). In this study we used empirical data from natural *Plantago lanceolata*

populations to test this adaptive plasticity (i.e., thermal acclimation) hypothesis against the alternative hypothesis that patterns of plasticity can be explained by neutral genetic evolution.

In *Plantago lanceolata* L. (ribwort plantain, English plantain), a widespread perennial herb native to Eurasia, floral reflectance and color of spikes (i.e. inflorescences of tightly-packed flowers) are influenced by the ambient temperature experienced during flower development (Lacey & Herr 2005). While some genotypes display negligible thermal plasticity and produce only highly reflective/lightly colored spikes, most genotypes reduce reflectance/darken spikes in response to cool environments, but to different degrees (Lacey & Herr 2005; Lacey *et al.* 2012; Lacey *et al.* 2010; Stiles *et al.* 2007; Umbach *et al.* 2009). The internal temperature of poorly reflective spikes is consistently warmer (~1-2°C) than that of highly reflective spikes when placed in a common thermal environment (Lacey & Herr 2005). As a result, floral reflectance plasticity allows individuals to partially thermoregulate the temperature of developing gametes, embryos, and seeds, and likely improves seed production in cool temperatures (Lacey *et al.* 2012). This thermal response is genetically variable within and among natural populations of *P. lanceolata* (Lacey & Herr 2005; Umbach *et al.* 2009).

Previously, Lacey and colleagues examined geographic patterns of temperature-sensitive floral reflectance plasticity and environmental properties of 29 European *P. lanceolata* populations and found positive correlations between thermal plasticity with latitude, and altitude (Lacey *et al.* 2010). Additionally, path analyses strongly suggested the geographic clines in thermal plasticity are a result of evolutionary responses to the local thermal environment experienced during the reproductive season, specifically the proportion of time at cool temperatures and season duration, but not the magnitude of thermal variation (Lacey *et al.* 2010). In this study we added a neutral genetic data set derived from amplified fragment length polymorphism (AFLP) markers for a subset of 14 of these European populations to determine if

the positive correlation between thermal plasticity and latitude is best explained by neutral genetic evolution, environmental differences among populations, or both.

Founder effects from historical migrations coupled with limited gene flow between populations and genetic drift can produce a positive correlation between genetic and phenotypic differentiation, and geographic distance between populations, producing a pattern of isolation by distance, IBD (Hutchison & Templeton 1999; Orsini *et al.* 2013; Wright 1943). Neutral genetic differentiation is expected to be most different between distinct ancestral populations, as well as between ancestral and most recently founded populations (Fischer 1960; Hewitt 1999; Schmitt 2007). Generally, within-population genetic variability (i.e., allelic diversity) is expected to be highest in ancestral populations and lowest in recently established populations, due to loss of alleles via founder effects and genetic drift, especially in small populations (Mayr 1942). However, gene flow (i.e., admixture) between populations of different ancestries can increase within-population diversity and reduce between-population differentiation.

In addition to neutral evolution, local selection can drive adaptive divergence between habitats and lead to a positive correlation between adaptive phenotypic and neutral genetic population divergence (isolation by adaptation, IBA; Nosil *et al.* 2005). Environmental selection can generate disproportionate gene flow by selecting against immigrant alleles/phenotypes or by generating pre-mating isolation, producing a positive relationship between population genetic differentiation and environmental differences (isolation by environment, IBE; Crispo *et al.* 2006; Lee & Mitchell - Olds 2011; Nosil *et al.* 2005). As a result, the gene flow-genetic drift-selection balance can produce different patterns of genetic and phenotypic differentiation across the landscape (Conover *et al.* 2009; Gould & Johnston 1972).

Comparisons between neutral genetic differentiation (F_{ST}) and phenotypic differentiation (Q_{ST}, P_{ST}) can differentiate between the effects of selection and neutral forces on a quantitative

trait. Unfortunately, obtaining reliable estimates of the standard errors associated with Q_{ST}, and the additive genetic variances needed to calculate Q_{ST} is difficult with a small number of populations and individuals per population (Leinonen *et al.* 2008; O'Hara & Merilä 2005). Instead, Q_{ST} can be estimated by its phenotypic semblance, phenotypic differentiation (P_{ST}), using the phenotypic variances within and between populations (Leinonen *et al.* 2006). Comparisons between phenotypic and genetic differentiation can produce three outcomes: $F_{ST} = P_{ST}$, $F_{ST} > P_{ST,}$ or $F_{ST} < P_{ST}$ indicating observed patterns of differentiation are best explained by neutral genetic drift, stabilizing selection, or diversifying selection, respectively (McKay & Latta 2002; Merilä & Crnokrak 2001).

Natural selection can have a strong influence on geographic patterns of genetic differentiation. By locally eliminating deleterious alleles/phenotypes from populations, selection can cause the frequency of advantageous alleles/phenotypes to increase and counteract the homogenizing effects of gene flow (Antonovics & Bradshaw 1970). Isolation by adaptation produces a geographic pattern of genotypic variation characterized by similar neutral and non-neutral alleles, and similar phenotypes in populations experiencing similar environmental conditions (Andrew *et al.* 2012; Luo *et al.* 2015; Muir *et al.* 2014; Nosil *et al.* 2009; Orsini *et al.* 2013).

First, we examined patterns of neutral genetic diversity and genetic differentiation to determine whether founder effects correlate with latitudinal patterns of thermal plasticity. Second, we incorporated measurements of environmental variation into our investigation to test how well different environmental factors explained patterns of phenotypic differentiation in thermal plasticity. We evaluated the extent to which environmental differences and geographic distance between populations explain neutral genetic variation. Third, to differentiate between the effects of selection and neutral forces on floral reflectance plasticity, we plotted phenotypic Q_{ST}

(P_{ST}) against neutral genetic differentiation (as F_{ST}) to determine if values fell above, at, or below the line of equality (P_{ST}, Leinonen *et al.* 2006; Q_{ST}, Spitze 1993; F_{ST}, Wright 1943). Fourth we used permutation tests to determine correlations among phenotypic differentiation (P_{ST}), neutral genetic differentiation (F_{ST} and Jost's D) and environmental distance matrices to identify variables that help explain patterns of phenotypic differentiation. Fifth we regressed phenotypic (P_{ST}) and neutral genetic differentiation (F_{ST} and Jost's D) against geographic distance and environmental properties of the reproductive season to determine whether 1) phenotypic and neutral genetic differentiation increased as environmental conditions diverged, and 2) phenotypic differentiation was greater than neutral genetic differentiation. The selection hypothesis predicts phenotypic differentiation should increase at a greater rate than should neutral genetic differentiation as environmental conditions diverge.

Methods

Experimental Populations

For this study, we selected fourteen European *P. lanceolata* populations of the 29 used in (Lacey *et al.* 2010), that span a latitudinal range of 39.3-50.9°N and an altitudinal range of 1-1,886m (Table 2.1). Distance between populations was determined by uploading latitude-longitude coordinates into Google Earth (earth.google.com) as 1) minimum linear Euclidean distance in meters, and 2) minimum geographic distance over land as determined using the path tool. Analyses conducted with Euclidean distance and distance over land produced the same conclusions; those with distance over land are presented because they are the most biologically reasonable.

Phenotypic Variables

Measures of thermal plasticity in floral reflectance came from Lacey's previous study (for details see Lacey *et al.* 2010). Genotypes used were the progeny of seeds gathered from each wild population. To reduce effects of the native environment individual seeds collected from each population were grown and isolated by population for reproduction in similar environments. Offspring (i.e., genotypes used in this study) were grown and phenotyped in the same environments. One clone per genotype had been grown at each of warm (27°C, 16hr day/20°C, 8hr night) and cool (15°C, 16hr day/10°C, 8hr night) temperatures in multiple growth chambers. Floral reflectance at 850nm was measured twice on a single pre-flowering spike per clone. A genotype's plasticity was calculated by subtracting mean reflectance at cool temperature from mean reflectance at warm temperature (for complete methodology, see Lacey & Herr 2005; Lacey *et al.* 2010).

Phenotypic differentiation (P_{ST}) was calculated as a conservative proxy for quantitative genetic differentiation (Q_{ST}) associated with floral reflectance plasticity as

$$P_{ST} = \frac{\sigma_{PB}^2}{\sigma_{PB}^2 + 2(h^2\sigma_{PW}^2)}, \quad (1)$$

where σ_{PB}^2 denotes between-population phenotypic variance, σ_{PW}^2 within-population phenotypic variance, and h^2 the heritability (Leinonen *et al.* 2006; Merilä *et al.* 1997). We were unable to determine reliable estimates of heritability and calculated P_{ST} using the null assumption that h^2=1, making our measure of P_{ST} a conservative proxy for Q_{ST}. Phenotypic variance components were calculated using ANOVA tests for floral reflectance plasticity between each pair of populations in SPSS (Merilä *et al.* 1997; SPSS 18.0, 2009). Phenotypic differentiation 95% confidence intervals were determined from 200 bootstrapped P_{ST} values sampled and calculated in R (R Development Core Team 2013).

Neutral Genetic Markers

We extracted DNA from leaf tissue of 315 genotypes (n= 10-33 genotypes/population) using a modified CTAB method (Doyle & Dickson 1987) and prepared amplified fragment length polymorphism (AFLP) reaction templates following (Vos *et al.* 1995) using 500ng of DNA digested with *Eco*RI and *Mse*I. Thereafter, we completed ligation with *Eco*RI (E) and *Mse*I (M) primers and selective preamplification using standard AFLP *Eco*RI (E) and *Mse*I (M) primers containing selective nucleotides E + AC and M + CC (Remington *et al.* 1999; Vos *et al.* 1995). Selective amplification was performed using combinations of the following E primer with three selective nucleotides and M primers with four selective nucleotides (E + 3/M + 4), *Eco*RI primer E + ACC labeled with one of the fluorescent dyes FAM or TAMRA, in combination with each of the selective *Mse*I primers M + CCAA, M + CCAT, M + CCAC, M + CCAG, M + CCTA, M + CCTT, M + CCTC, M + CCTG. As such, AFLP fragments from each genotype were produced using each primer-pair combination and either the FAM or TAMRA dye. We pooled DNA samples of fragments from the same genotypes but with different dye and primer combinations into the same well for desalting and fragment detection (e.g. selective amplification products from E + ACC + FAM / M + CCTA and E + ACC + TAMRA / M + CCTC were pooled for individuals 1-48). We quantified AFLP reaction products with MegaBACE ET550-R size standards on a MegaBACE^{TM} Fragment Profiler and scored them in GeneMarker (Softgenetics).

We established consensus AFLP scoring panels for each primer pair combination using individual genotypes repeated within that primer pair combination, and all loci were repeated with at least 5 individual genotypes. All of the individuals that were genotyped with multiple dyes were used to create scoring panels. In all cases where one of the samples of repeated individuals was too poor to score, the other sample was used for scoring. In cases of disagreement, the sample with the clearest standards in that region was used. If both samples were of equal quality,

disagreements were treated as missing data. In total we scored 313 unique AFLP loci in each of 315 genotypes (Table S2.1).

Within AFLP scoring panels we determined scoring error for each dye, and between dyes (Table S2.1). We calculated scoring error in AFLP markers as percent of markers that disagreed (percent disagreement) between multiple runs of an individual genotype within and between FAM and TAMRA dyes. We calculated error within each primer pair combination as the number of markers in disagreement divided by the total number of markers able to be scored within repeated individuals. To calculate overall error, we summed the average error within all primer pairs, weighted by the number of individuals used, and divided by the total number of individuals used. The percent of AFLP markers that disagreed between multiple runs of the same individual genotype were 5.33 ± 1.78% and 5.69 ± 2.09% for FAM and TAMRA dyes, and 8.86 ± 1.67% between dyes (Table S2.1).

Once AFLP scoring panels were established they were used to score each individual genotype. In the final AFLP data set each individual was included once for each primer pair (genotypes were not repeated). Using the criteria described above, we developed a consensus score for individuals for which we had data from multiple runs.

Environmental Variables

We chose four environmental characteristics of the reproductive season to test for correlations with population differentiation in plasticity. The proportion of the reproductive season at cool temperatures (below 15°C based on monthly means) and the reproductive season duration (# months) were chosen because they had previously been found to show statistically significant and biologically meaningful relationships with geographic patterns of temperature sensitivity in floral reflectance (Lacey *et al.* 2010). The rationale is more fully explained in Lacey et al. (2010), but for more clarity here, 15°C was chosen as the upper limit because plant

physiological research has shown that below 15°C, temperature strongly controls metabolic rate Covey-Crump *et al.* 2002). The magnitude (or range) of thermal variation was calculated as the difference between the mean monthly maximum and the mean monthly minimum temperature that occurred during the reproductive season. We chose thermal magnitude to specifically test the prediction that plasticity will increase with environmental variation, as predicted by evolutionary theory. These variables, proportion of the season at cool temperature, thermal magnitude, and season duration were used in (Lacey et al. 2010). In addition, we used mean monthly total precipitation within the reproductive season as a negative control. We chose precipitation because it is a climatically relevant environmental variable that can, in general, influence plant life history and reproduction. Precipitation was a negative control in our study because it was not expected to influence thermal plasticity. Thirty-year averages (1961-1990) were extracted from the Climatic Research Unit Global Climate data set (www.ipcc-data.org). Values were estimated by interpolation of the nearest neighboring weather stations to each population (complete methodology in Lacey *et al.* 2010).

Environmental Principal Components Variables

We created three composite environmental variables by conducting principal components analyses (PCA) via the *prcomp* function in R (R Development Core Team 2013). In each case the first principal components axis explained the majority (>80%) of the variance among the variables and this axis alone was used in subsequent analyses (Table 2.2). Principal components axes were used to reduce environmental variables into a single variable. Combining multiple variables allowed us to assess the combined effect of multiple factors in our linear regression analyses. First we created the Mag_Therm_PC1 (see below) to determine if the combined effect of both thermal variables and duration would better explain patterns of thermal plasticity than either thermal variable alone. Second we created the Thermal_PC1 because both proportion of

time below 15°C and duration had shown a strong biological association with plasticity in Lacey et al. (2010). Third, we created the Magnitude_PC1 to allow for equal comparisons between the duration of cool temperature variable and the thermal magnitude throughout all analyses.

Mag_Therm_PC1

We used PCA to combine the magnitude of thermal variation, the proportion of time at cool temperature, and the duration of the reproductive season. Factor loadings indicated more positive Mag_Therm_PC1 values represent longer reproductive seasons with a greater magnitude of thermal variation and a smaller proportion of time at cool temperatures and more negative Mag_Therm_PC1 values represent shorter reproductive seasons with less thermal variation and a greater proportion of time at cool temperatures (Table 2.2).

Thermal_PC1

We used PCA to combine the proportion of time below 15°C and duration of the reproductive season. Factor loadings indicated more negative Thermal_PC1 values represent shorter reproductive seasons containing a higher proportion of time below 15°C, and more positive Thermal_PC1 values represent longer reproductive seasons with a smaller proportion of time below 15°C (Table 2.2).

Magnitude_PC1

We used PCA to combine the magnitude of thermal variation and duration of the reproductive season. Factor loadings indicated more positive Magnitude_PC1 values represent longer reproductive seasons with more thermal variation and more negative Magnitude_PC1 values represent shorter reproductive seasons with less thermal variation (Table 2.2).

Finally, we calculated absolute pairwise differences between populations for each environmental variable and for the composite principal components variables. Phenotypic, genetic, and environmental differentiation variables were then standardized to zero mean and unit

variance using the *decostand* function in R for Mantel tests and multiple regression of distance matrices analyses (R Development Core Team 2013).

Analyses

Neutral genetic population structure

Scored AFLP markers were used to estimate genetic diversity within populations and differentiation between populations, and to conduct population structure analyses. We estimated neutral genetic diversity as population mean heterozygosity for each population in Hickory v1.1 with 100,000 iterations following a burn-in of 5,000 (Holsinger *et al.* 2002). Comparative phylogeographic studies have found evidence of post-glacial migration from southern European refugia following the Pleistocene glaciation in other species (Schönswetter *et al.* 2005; Taberlet *et al.* 1998). To determine if we could identify post-glacial migration routes in *P. lanceolata*, we mapped diversity at population locations and looked for emerging patterns. Two-sided Pearson correlations between heterozygosity with latitude and altitude were calculated in R (Goslee & Urban 2007; R Development Core Team 2013).

We calculated neutral genetic differentiation and 95% confidence intervals between all population-pairs using two statistics; $F_{ST,}$ estimated as θ^{II} in Hickory v1.1 with 100,000 iterations following a burn-in of 5,000, and Jost's D, calculated in SPADE using 300 bootstraps (Chao & Schen 2010; Holsinger *et al.* 2002; Jost 2008). Q_{ST}/P_{ST} and F_{ST} statistics are equivalent measures of population phenotypic and genetic differentiation, and thus are derived from the same evolutionary history and respond similarly to the evolutionary processes that give rise to them (i.e., realized migration and genetic drift). Jost's D on the other hand is specific to the loci being measured and is more strongly affected by mutation than migration, thus Jost's D is not legitimately equivalent to Q_{ST}/P_{ST} (Whitlock 2011). However, we wanted to include Jost's D as

an alternative measure of neutral genetic diversity to evaluate how robust comparisons using F_{ST} are.

To examine patterns of genetic differentiation across the landscape we grouped populations into geographic regions based on physical location, and calculated genetic differentiation between regions in Hickory with 25,000 iterations following a burn-in of 5,000 (Holsinger *et al.* 2002). We then looked for regions separated by higher genetic differentiation that may represent ancestral populations, and regions separated by lower differentiation representing historical post-glacial migration routes (Fischer 1960; Hewitt 1999; Schmitt 2007).

To explore the genetic groups within samples and infer 1) the presence of distinct populations, 2) geographic locations of barriers to gene flow, and 3) the presence of admixture, non-hierarchical Bayesian clustering was performed in STRUCTURE 2.3.4 (Pritchard *et al.* 2000). The correlated allele frequencies model with admixture was used to test values of K. Five replicates for each K from 2-10 were run with a burn-in of 10^5, followed by 10^6 replicates, with convergence monitored for each run. We combined and interpreted all runs with Structure Harvester (Earl 2012), using the methods of (Evanno *et al.* 2005; Pritchard *et al.* 2000). We used CLUMPP to average admixture proportions over runs (Jakobsson & Rosenberg 2007), and visualized averaged runs using Distruct (Rosenberg 2004). To best resolve ancestral relatedness among populations, we visually examined average admixture plots from low to high values of K groups, with regard to the geographic location of populations.

P_{ST} vs. F_{ST}

We determined whether phenotypic (P_{ST}) and neutral genetic (F_{ST}) differentiation statistics differed, and their relationship (i.e., if $F_{ST} = P_{ST}$, $F_{ST} > P_{ST,}$ or $F_{ST} < P_{ST}$) by examining whether 95% confidence intervals for P_{ST} and for F_{ST} among population pairs overlapped the value where $F_{ST} = P_{ST}$. When 95% confidence intervals failed to overlap the value where F_{ST} =

P_{ST}, we concluded F_{ST} and P_{ST} differed. We concluded we did not have statistical support for differences between F_{ST} and P_{ST} in cases where 95% confidence intervals overlapped the value where $F_{ST} = P_{ST}$.

Isolation by Distance

To determine if isolation by distance explains a significant proportion of the variance in neutral genetic markers, we conducted Mantel tests (10^6 permutations) between neutral genetic differentiation (F_{ST} and Jost's D) on geographic distance between populations in the *ecodist* package in R (Goslee & Urban 2007; R Development Core Team 2013). Geographic patterns of floral reflectance plasticity along altitudinal and latitudinal gradients were calculated as positive one-sided Pearson correlations in R to confirm floral reflectance plasticity increased with latitude and altitude in our 14 populations (R Development Core Team 2013). To determine if isolation by distance explains a significant proportion of the variance in floral reflectance plasticity, we performed multiple regressions on distance matrices (MRM) of phenotypic differentiation (P_{ST}) on genetic differentiation (F_{ST} and Jost's D) and geographic distance over land with 10^6 permutations in *ecodist* (Goslee & Urban 2007; R Development Core Team 2013). The MRM models and regression coefficients were tested by permuting the dependent distance matrix (i.e., phenotypic differentiation of thermal plasticity) while holding the explanatory matrices constant (Lichstein 2007). All MRM analyses were conducted on differentiation variables standardized to zero mean and unit variance with the *decostand* function (R Development Core Team 2013).

Natural Selection

We determined the relationships of geographic distance and neutral genetic differentiation (F_{ST} and Jost's D) with environmental properties of the reproductive season with Mantel tests (10^6 permutations) in the *ecodist* package in R (Goslee & Urban 2007; R Development Core Team 2013). We correlated pairwise estimates of phenotypic and neutral

genetic differentiation with environmental differences between population pairs to test the natural selection hypothesis in two ways. First, we performed multiple regression of distance matrices analyses of phenotypic differentiation (P_{ST}) on genetic differentiation (F_{ST} and Jost's D) and each environmental variable independently with 10^6 permutations in *ecodist* (Goslee & Urban 2007; R Development Core Team 2013). We completed all models with MRM to allow for ease of comparison among models. Second, we regressed phenotypic differentiation (P_{ST}) and genetic differentiation (F_{ST} and Jost's D) against geographic distance and pairwise differences between environmental properties of the reproductive season (i.e., duration, proportion of cold temperature, thermal magnitude, and total precipitation) using standardized values (Prism v6.04 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com) to determine whether 1) phenotypic and genetic differentiation increased with increasing geographic distance and/or environmental differences, 2) slopes of phenotypic and genetic differentiation differed along these axes and 3) y-intercepts of phenotypic and genetic differentiation differed. Under natural selection phenotypic differentiation should increase as environments become more different between populations, and be greater (i.e., have a greater y-intercept) than neutral genetic differentiation (Leinonen *et al.* 2006). In cases where the slope of phenotypic differentiation was significantly greater than neutral genetic differentiation there was no need to test for equal y-intercepts. Unadjusted p-values are reported, and their significance was determined after controlling for the false discovery rate at α=0.05 in multiple testing using the graphically sharpened method (Benjamini & Hochberg 2000).

Results

Mean heterozygosity was not correlated with latitude (t = -0.583, p= 0.571) or altitude (t= -0.222, p=0.828). One population in northern Italy, IB, had the highest overall heterozygosity

(Table 2.1). Interestingly, IB was also genetically more similar to populations in Germany, southern Italy, and Spain than other populations ($F_{ST} \leq 0.021$, Figure 2.1, Table S2.2). Relatively low genetic differentiation was also found between regions in Spain and southern France, southern and northern France, and northern and western France ($0.10 \geq F_{ST} \geq 0.05$, Table S2.2). Neutral genetic differentiation was strongly correlated with geographic distance between populations (F_{ST}: $r=0.602$, $p<0.001$; Jost's D: $r=0.640$, $p<0.001$).

The structure analysis indicated that the best arrangement for AFLP data was for K=7 or 8 groups. The highest delta K value was observed at K=7, while K=8 showed the highest log probability and low run-to-run variability (Figures S2.2 & S2.3). As values of K increased from 2 to 8, evidence of gene flow among populations (admixture) also increased. Despite the gene flow, southern Italian populations (IA, ICa, and ICs) consistently remained different from all other populations (Figure S2.4). This pattern was noticeable at K=2 and K=8 (Figure S2.4).

Geographic distance between populations and Jost's D were not correlated with any of the environmental properties of the reproductive season we examined (Table S2.3). A marginally significant correlation was found between F_{ST} and the proportion of the reproductive season below 15°C ($r=0.20$, $p=0.079$), and a significant relationship was found when this variable was incorporated with season duration as Thermal_PC1 ($r=0.29$, $p=0.030$). F_{ST} was not correlated with any of the other environmental properties of the reproductive season we examined (Table S2.3).

For 36 of the 91 (greater than 39%) population pair-wise comparisons phenotypic differentiation P_{ST} was greater than neutral genetic differentiation (F_{ST}), i.e. 95% confidence intervals did not include values where $P_{ST} = F_{ST}$ (Figure 2.2A). In the remaining 55 comparisons 95% confidence intervals of P_{ST} or F_{ST} included values where $P_{ST} = F_{ST}$, and we did not have

statistical support that P_{ST} and F_{ST} differed (Figure 2.2B). We did not find any comparisons where $F_{ST} > P_{ST}$.

Mean thermal plasticity increased significantly with latitude (r= 0.528, one-sided p=0.026) and marginally with altitude (r=0.418, one-sided p=0.068). Multiple regression of distance matrices models indicated that when examined alone, a significant proportion of the variation in plasticity P_{ST} could be explained by distance between populations, F_{ST} and Jost's D, and the proportion of the reproductive season below 15°C (Table 2.3 A-C, G). In models that included genetic differentiation and the proportion of the reproductive season below 15°C, the effect of the environment was marginally significant with F_{ST} (Table 2.3 H), and significant with Jost's D (Table 2.3 I). The effects of the remaining environmental variables (duration, thermal magnitude, and precipitation) were insignificant (Table 2.3 D-F, J-L, V-X).

Two composite principal component axes, Thermal_PC1 and Mag_Therm_PC1, displayed the same pattern as the proportion of the reproductive season below 15°C alone. Incorporating the proportion of the reproductive season below 15°C and duration into a single Thermal_PC1 axis improved the model's explanatory power relative to the proportion of the reproductive season below 15°C alone, albeit very little (improved r^2 by 0.02 when alone (Table 2.3 G vs. M), and 0.01 when including genetic differentiation (Table 2.3 H, I vs. N, O)). However, incorporating magnitude into the composite Mag_Therm_PC1 failed to further improve explanatory power (Table 2.3 M- O vs. S-U). The composite Magnitude_PC1 axis did not explain a significant proportion of the variation in plasticity P_{ST} (Table 2.3 P-R).

Including geographic distance always improved the MRM model's power to explain the variation in plasticity P_{ST} (i.e., improved r^2; Table S2.4). When included in the same model, geographic distance was always statistically or marginally significant, and genetic differentiation became insignificant (Table S2.4). Interestingly, including geographic distance into models with

environmental variables did not influence the overall significance of environmental variables nearly as much as distance influenced genetic differentiation. As a result, models that included geographic distance produced the same conclusions as models without distance (Table S2.4).

As geographic distance between populations increased, P_{ST}, F_{ST}, and Jost's D increased significantly, and P_{ST} increased at a greater rate than did F_{ST} ($F=12.13$, $p<0.001$) and Jost's D ($F=9.39$, $p=0.003$; Figure 2.3A, Table 2.4A). The linear regression analyses using proportion of the reproductive season below 15°C as the environmental variable showed that only P_{ST} had a significantly positive slope (Figure 2.3C, Table 2.4C). In the analysis using Thermal_PC1 as the environmental variable, the slopes for both P_{ST} and F_{ST} increased significantly as the difference in Thermal_PC1 increased (Figure 2.3E, Table 2.4E), but the slope for P_{ST} was significantly greater than for F_{ST} ($F=4.62$, $p=0.033$). The analysis using Mag_Therm_PC1 as the environmental variable showed that only P_{ST} had a significantly positive slope, and the slope for P_{ST} was greater than for F_{ST} ($F=4.05$, $p=0.046$; Figure 2.3G, Table 2.4G) The slope for Jost's D did not significantly differ from zero in either analysis. In analyses with either duration, thermal magnitude, Magnitude_PC1, or precipitation the slopes for P_{ST} did not significantly differ from zero (Figure 2.3 B, D, F, H, Table 2.4 B, D, F, H). For analyses where slopes did not differ between phenotypic and neutral genetic differentiation, the y-intercepts of phenotypic differentiation were always greater than y-intercepts of neutral genetic differentiation (reproductive season duration: P_{ST} vs. F_{ST}, $F=83.90$, $p<0.001$; P_{ST} vs. Jost's D, $F=81.30$, $p<0.001$; proportion of the reproductive season below 15°C: P_{ST} vs. F_{ST}, $F=85.95$, $p<0.001$; P_{ST} vs. Jost's D, $F=83.21$, $p<0.001$; thermal magnitude: P_{ST} vs. F_{ST}, $F=83.55$, $p<0.001$; P_{ST} vs. Jost's D, $F=81.09$, $p<0.001$; Magnitude_PC1: P_{ST} vs. F_{ST}, $F=83.87$, $p<0.001$; P_{ST} vs. Jost's D, $F=81.32$, $p<0.001$; precipitation: P_{ST} vs. F_{ST}, $F=84.01$, $p<0.001$; P_{ST} vs. Jost's D, $F=82.25$, $p<0.001$; Figure 2.3 B, C, D, F, H).

Discussion

A small but growing number of studies using Q_{ST}/P_{ST} - F_{ST} comparisons of multiple populations have shown that population variation along thermal gradients (e.g., latitudinal or altitudinal) can be, at least partially attributed to selection, even when measured against contributions of neutral evolutionary factors. For example, Q_{ST}/P_{ST} is significantly greater than F_{ST} along thermal clines for coloration traits in birds (Antoniazza *et al.* 2010) and multiple traits in amphibians (Hangartner *et al.* 2012; Luquet *et al.* 2015; Muir *et al.* 2014), Drosophila (Chenoweth & Blows 2008) and plants (Frei *et al.* 2014; Luo *et al.* 2015; Savolainen *et al.* 2007; Yoshida *et al.* 2009). Our comparative study, to the best of our knowledge, is the first to have provided evidence that the geographic variation in the plasticity of a trait has resulted from contributions of adaptive divergence and neutral evolutionary forces.

Our results are consistent with the hypothesis that natural selection has helped to shape clinal variation in thermal plasticity for floral reflectance in *P. lanceolata* flowers. In our study, F_{ST} showed significant relationships with geographic distance and population differences in the environmental variable that incorporated the proportion of the reproductive season at cool temperature and reproductive season duration. These relationships are consistent with heterogeneous gene flow related to both distance (IBD) and with environmental differences (IBE) contributing to population divergence, suggesting geographic distance and environmental differences between populations have influenced gene flow among populations (Andrew *et al.* 2012; Bradburd *et al.* 2013; Sacks *et al.* 2008; Wang 2012). Overall, P_{ST} of thermal plasticity was correlated with geographic distance and neutral genetic distances indicating that neutral genetic processes have contributed to European populations. However, we found P_{ST} values were significantly greater than F_{ST} values among over 39% of the comparisons we conducted, providing evidence that differences in thermal plasticity among these populations are best

explained by divergent selection. The significant relationships between P_{ST} of thermal plasticity and differences among thermal properties of the reproductive season we identified, even while controlling for neutral genetic differences, are consistent with the prevailing hypothesis that thermal plasticity becomes increasingly adaptive with high latitude environments characterized by a cooler and shorter reproductive season (Ghalambor *et al.* 2006; Huey & Stevenson 1979; Lacey *et al.* 2010; Laugen *et al.* 2003; Liefting *et al.* 2009; Molina-Montenegro & Naya 2012; Wieser 1973).

Phenotypic differentiation (P_{ST}) increased more quickly than did neutral genetic differentiation (F_{ST} and Jost's D) as the difference in proportion of time spent at cool temperatures increased, and the P_{ST} -F_{ST}/Jost's D difference was even more striking along the composite axis of environmental differentiation that represented increasingly cool and short reproductive seasons. This finding is consistent with the selection hypothesis that predicts phenotypic differentiation should increase at a greater rate than should neutral genetic differentiation as environmental conditions diverge. Our results suggest that population divergence in thermal plasticity in *P. lanceolata* has been influenced by two environmental variables: the proportion of the reproductive season at cool temperatures and the reproductive season duration, but not the magnitude of thermal variation.

These findings are consistent with conclusions from a larger study of European *P. lanceolata* populations that inspired the incorporation of neutral genetic data into the investigation of geographic patterns of thermal plasticity, and found mean thermal plasticity of *P. lanceolata* populations increased as the reproductive season became shorter and cooler (Lacey et al. 2010). Furthermore, the implications of these findings are also consistent with fitness effects of temperature-sensitive floral reflectance plasticity identified in a common garden experiment of *P. lanceolata* genotypes from North Carolina, USA. Onset and duration of flowering were similar

between low- and high-plasticity genotypes, but directional selection favored genotypes with higher thermal plasticity early in the reproductive season when temperatures were cooler. Later in the season when temperatures warmed, stabilizing selection favored genotypes with lower plasticity (Lacey et al. 2012).

In *P. lanceolata*, genotypes with lower plasticity had a reduced ability to darken floral tissues (i.e., they produce lightly colored floral spikes independently of the thermal environment), while those with higher plasticity were able to produce lightly colored spikes in warm environments and darker spikes in cool environments. We have not yet come across any genotypes that produced constitutively dark flowers. Altogether, our data and that from previous studies suggests thermal plasticity (i.e. the ability to darken flowers) confers higher fitness in environments where the reproductive season is shorter and consists of a high frequency of intense/strong cold temperature selection.

Our P_{ST} values are likely conservative underestimates of Q_{ST} because we collected reflectance data from clones of the same genotypes grown under the same controlled temperatures and because we used a heritability value of 1.0, which makes P_{ST} a conservative estimate of $Q_{ST.}$ Also, parental environmental effects had been reduced by passing parents of our experimental genotypes through one generation in a similar environment in order to produce the genotypes used here (see Lacey et al. 2010). Therefore, we expect selection on thermal plasticity in nature to be greater than our data suggest.

More generally, our results add support for the hypothesis that variation in selection intensity across a geographical gradient can explain geographic patterns of plasticity. Selection intensity or strength, e.g., from temperature or precipitation, can change locally within a growing season for a population, and this temporal variation in intensity can change spatially, e.g., along thermal gradients. Plasticity should be favored where the frequency of intense selection favoring

plasticity is high, but not where the frequency is low (Gavrilets & Scheiner 1993; Gomulkiewicz & Kirkpatrick 1992; Levins 1968; Moran 1992). Consequently, geographic variation in plasticity will be correlated with thermal clines (e.g., latitude/altitude) when the frequency of intense selection for plasticity varies along these clines.

Our data support an alternative explanation to the prevailing one that thermal plasticity, which is greater at higher latitudes and altitudes, has evolved in response to variation in the magnitude (or range) of temperature variation, also larger at higher latitudes and altitudes (e.g., Addo-Bediako *et al.* 2000; Angilletta 2009; Ghalambor *et al.* 2006; Molina-Montenegro & Naya 2012; Ragland & Kingsolver 2008). We found genetic evidence supporting the alternative that thermal plasticity has evolved in response to variation in the frequency of intense temperature-mediated selection, which is higher at higher latitudes and altitudes.

It is important to focus on the environment during the active, or relevant, portion of the life cycle of a species in order to understand the causes of geographic variation in plasticity. One should examine the geographic variation in frequency of time strongly favoring plasticity, in addition to the magnitude of environmental variation. The former may be a more potent selective force than the range of variation experienced during the relevant portion of a life cycle.

Our data also show that isolation by distance has contributed to the geographic pattern of temperature-sensitive plasticity in *Plantago lanceolata* flowers. The admixture analysis shows gene flow among populations has occurred. In spite of this, we saw evidence of isolation by distance and divergent natural selection. Genetic drift and ecological selective agents can both underlie isolation by distance patterns (Orsini *et al.* 2013). Thus, it is possible other ecological factors may also contribute to the local adaptation in plasticity, in addition to the two we identified. The most genetically distinctive populations identified by STRUCTURE were in southern Italy. These may have been partially isolated because of the Alps, particularly during the

last glacial maximum, thus, resembling patterns in other European species (Demesure *et al.* 1996; Huntley & Birks 1983; King & Ferris 1998; Taberlet *et al.* 1998). However, even these southern Italian *P. lanceolata* populations, showed evidence of some gene flow with the other populations we sampled, and not only the closest.

Finally, phenotypic plasticity provides organisms with the potential to respond rapidly to changes in their environment and has been proposed as a mechanism for coping with contemporary climate change (Charmantier *et al.* 2008; Gienapp *et al.* 2007; Matesanz *et al.* 2010; Przybylo *et al.* 2000; Réale *et al.* 2003; Visser 2008). Our study highlights some useful points when evaluating this idea. Global climate change is occurring via widespread temperature increases, regional changes in precipitation and local land-use changes, e.g., urbanization (Pachauri *et al.* 2014). Whether or not plasticity, or the evolution of plasticity, can ameliorate the effects of these changes depends on several factors (Andrew *et al.* 2012; Munday *et al.* 2013; Parmesan 2006; Visser 2008; Walther *et al.* 2002). Among these are the range and nature of phenotypic plasticity organisms display in response to environmental cues and the standing genetic variation in plasticity they possess (Bradshaw & Holzapfel 2008; Chevin & Lande 2011).

At present clarity about the selective factors that have created current levels of plasticity is limited. Our data are consistent with other studies (mentioned above) showing geographic and genetic variation in thermal plasticity. Thus, as warming proceeds, these plastic individuals are likely to lead any pole-ward migration of a species, given dispersal capability. If southern populations are genetically variable for thermal plasticity, as is true for *P. lanceolata* flowers, the reproductive organs, then they may also survive warming, but thermal plasticity is likely to diminish, or evolve in a direction toward greater tolerance to a warm climate (Lande 2009). Given genetic recombination, populations should persist and perhaps evolve in response to some further lengthening of the reproductive season and more time at warmer temperatures. What is

unknown, however, is where the upper thermal limit lies, a parameter that will be critical when evaluating the amount of environmental change that allows for long-term persistence (Chevin *et al.* 2010). This represents a big gap in our understanding for most species.

Table 2.1 Population Locations and Characteristics: Country of Origin, Location Within Country, Population Symbol, Mean Heterozygosity, Mean Floral Reflectance Plasticity, and the Number of Genotypes Measured (N).

Source country, location in country	Symbol	Latitude (°N)	Longitude (°E)	Altitude (m)	Mean Heterozygosity (±2 SD); N	Mean Reflectance (±2 SD); N
France						
Massif de la Chartreuse	FrG	45.37	5.4	1,000	0.266 (0.01); 19	28.005 (29.30); 25
Hameau de St. Felix	FrH	43.58	3.97	35	0.267 (0.01); 23	19.346 (24.45); 27
St. Pierre, Ile d'Oléron	FrI	45.95	-1.29	10	0.284 (0.01); 21	25.878 (29.66); 22
St. Martin d'Hére	FrM	45.17	5.77	230	0.239 (0.01); 29	27.857 (22.45); 26
St. Martin d'Uriage	FrMu	45.15	5.83	684	0.274 (0.01); 14	22.045 (30.67); 13
Orsay	FrO	48.68	2.18	80	0.279 (0.01); 12	27.687 (32.19); 17
L'Alpe d'Huez	FrR	45.09	6.07	1,886	0.249 (0.01); 34	27.103 (32.27); 26
Germa						
Jena	GJ	50.93	11.58	150	0.254 (0.01); 30	17.011 (22.26); 30
Italy:						
Aprilia	IA	41.6	12.65	70	0.265 (0.01); 20	7.652 (14.75); 16
Bagni di Vinadio	IB	44.3	7.08	1,300	0.297 (0.01); 27	26.231 (26.32); 23
Castel Volturno	ICa	41.03	13.93	1	0.268 (0.01); 33	10.802 (17.86); 29
Cosenza	ICs	39.3	16.25	238	0.280 (0.01); 10	11.671 (20.82); 7
Spain:						
Cangoria	SpC	42.69	-0.52	1,080	0.265 (0.01); 22	15.138 (23.18); 24
Orbil de Villanua	SpO	42.66	-0.54	920	0.265 (0.01); 21	25.822 (31.23); 24

Table 2.2 Principal Components Analyses used to Combine Multiple Aspects of the Reproductive Season into Composite Variables. Thermal_PC1 combines proportion of the reproductive season under 15°C (DegMoB15°C) and season duration, Magnitude_PC1 combines thermal magnitude and season duration, and Mag_Therm_PC1 combines proportion of the reproductive season under 15°C, thermal magnitude, and season duration. Only primary axis was used in subsequent analyses.

			Factor Loadings		
	% Explained	Eigenvalue	DegMoB15°C	Magnitude	Duration
Thermal_PC1					
PC1	83.2%	1.66	-0.707	-	0.707
PC2	16.8%	1.34	0.707	-	0.707
Magnitude_PC1					
PC1	92.7%	1.85	-	0.707	0.707
PC2	7.3%	0.15	-	-0.707	0.707
Mag_Therm_PC1					
PC1	81.5%	2.45	-0.539	0.593	0.598
PC2	13.6%	0.41	-0.841	-0.409	-0.353
PC3	4.9%	-0.03	-0.035	0.693	-0.720

Table 2.3 Multiple Regression of Distance Matrices Test Results of the Phenotypic Differentiation (P_{ST}) in Temperature-Sensitive Floral Reflectance Plasticity Matrix on Matrices of Geographic Distance, Genetic Differentiation (F_{ST} and Jost's D), and Environmental Differences Between 14 *Plantago lanceolata* Populations. Environmental variables examined were reproductive season duration (Duration), the proportion of the reproductive season under 15°C (DegMoB15°C), the magnitude of thermal variation of the reproductive season (Magnitude), three principal components axes integrating these variables (Thermal_PC1, Magnitude_PC1, Mag_Therm_PC1, see text for details), and total reproductive season precipitation (Precipitation). Regression coefficients and p-values are obtained from permutation tests. Bold type indicates $p < 0.05$ after controlling for multiple comparisons. *Italic* type indicates $0.05 < p < 0.10$.

	coefficient	p
A. P_{ST} ~ Distance		**0.006**
$r^2 = 0.17$		
Intercept	2.35E-11	0.962
Distance	4.32E-01	**0.006**
B. P_{ST} ~ F_{ST}		**0.019**
$r^2 = 0.11$		
Intercept	3.30E-11	**0.019**
F_{ST}	3.27E-01	**0.019**
C. P_{ST} ~ Jost's D		**0.005**
$r^2 = 0.13$		
Intercept	3.70E-11	**0.005**
Jost's D	3.65E-01	**0.005**
D. P_{ST} ~ Duration		0.188
$r^2 = 0.02$		
Intercept	3.74E-11	0.115
Duration	1.34E-01	0.188
E. P_{ST} ~ F_{ST} + Duration		**0.041**
$r^2 = 0.11$		
Intercept	3.58E-11	0.122
F_{ST}	3.15E-01	**0.026**
Duration	8.51E-02	0.408
F. P_{ST} ~ Jost's D + Duration		**0.016**
$r^2 = 0.15$		
Intercept	4.08E-11	**0.028**
Jost's D	3.59E-01	**0.006**
Duration	1.17E-01	0.227
G. P_{ST} ~ DegMoB15°C		**0.033**
$r^2 = 0.06$		
Intercept	2.76E-11	0.967
DegMoB15°C	2.43E-01	**0.033**
H. P_{ST} ~ F_{ST} + DegMoB15°C		**0.022**
$r^2 = 0.14$		
Intercept	2.89E-11	0.836
F_{ST}	2.90E-01	**0.036**
DegMoB15°C	1.83E-01	0.069
I. P_{ST} ~ Jost's D + DegMoB15°C		**0.009**

$r^2 = 0.17$		
Intercept	3.23E-11	0.239
Jost's D	3.41E-01	**0.007**
DegMoB15°C	2.02E-01	**0.046**
J. P_{ST} ~ Magnitude		0.253
$r^2 = 0.02$		
Intercept	3.45E-11	0.139
Magnitude	1.35E-01	0.253
K. $P_{ST} \sim F_{ST}$ + Magnitude		**0.034**
$r^2 = 0.13$		
Intercept	3.44E-11	0.06
F_{ST}	3.28E-01	**0.018**
Magnitude	1.35E-01	0.227
L. P_{ST} ~ Jost's D + Magnitude		**0.015**
$r^2 = 0.16$		
Intercept	3.88E-11	**0.008**
Jost's D	3.73E-01	**0.005**
Magnitude	1.54E-01	0.151
M. P_{ST} ~ Thermal_PC1		**0.022**
$r^2 = 0.08$		
Intercept	2.99E-11	0.984
Thermal_PC1	2.80E-01	**0.022**
N. $P_{ST} \sim F_{ST}$ + Thermal_PC1		**0.018**
$r^2 = 0.15$		
Intercept	3.07E-11	0.568
F_{ST}	2.69E-01	0.054
Thermal_PC1	2.03E-01	0.074
O. P_{ST} ~ Jost's D + Thermal_PC1		**0.008**
$r^2 = 0.18$		
Intercept	3.41E-11	**0.032**
Jost's D	3.26E-01	**0.008**
Thermal_PC1	2.24E-01	**0.04**
P. P_{ST} ~ Magnitude_PC1		0.184
$r^2 = 0.02$		
Intercept	4.28E-11	0.114
Magnitude_PC1	1.49E-01	0.185
Q. $P_{ST} \sim F_{ST}$ + Magnitude_PC1		**0.038**
$r^2 = 0.12$		
Intercept	4.14E-11	0.109
F_{ST}	3.19E-01	**0.022**
Magnitude_PC1	1.28E-01	0.241
R. P_{ST} ~ Jost's D + Magnitude_PC1		**0.017**
$r^2 = 0.16$		
Intercept	4.71E-11	**0.045**
Jost's D	3.66E-01	**0.005**
Magnitude_PC1	1.53E-01	0.143
S. P_{ST} ~ Mag_Therm_PC1		**0.035**
$r^2 = 0.07$		
Intercept	2.73E-11	0.965

Mag_Therm_PC1	2.59E-01	**0.035**
T. $P_{ST} \sim F_{ST}$ + Mag_Therm_PC1 $r^2 = 0.15$		**0.019**
Intercept	2.85E-11	0.825
F_{ST}	2.88E-01	**0.037**
Mag_Therm_PC1	2.02E-01	0.081
U. $P_{ST} \sim$ Jost's D + $r^2 = 0.18$		**0.008**
Intercept	3.18E-11	0.305
Jost's D	3.41E-01	**0.007**
Mag_Therm_PC1	2.21E-01	**0.050**
V. $P_{ST} \sim$ Precipitation $r^2 = 0.01$		0.488
Intercept	3.43E-11	0.258
Precipitation	-1.20E-01	0.488
W. $P_{ST} \sim F_{ST}$ + Precipitation $r^2 = 0.11$		0.051
Intercept	3.37E-11	0.133
F_{ST}	3.17E-01	**0.025**
Precipitation	-6.62E-02	0.704
X. $P_{ST} \sim$ Jost's D + Precipitation $r^2 = 0.13$		**0.019**
Intercept	3.72E-11	**0.007**
Jost's D	3.58E-01	**0.006**
Precipitation	-2.39E-02	0.891

Table 2.4 Linear Regressions of Phenotypic Differentiation (P_{ST}) in Temperature-Sensitive Floral Reflectance Plasticity and Neutral Genetic Differentiation (F_{ST} and Jost's D) on an X-axis of Increasing Geographic Distance and Environmental Differences. A runs test p>0.05 indicates no deviation from linearity. Bold type indicates $p < 0.05$ after controlling for multiple comparisons. *Italic* type indicates $0.05 < p < 0.10$.

		Slope	F	p	p(runs)
A. Geographic Distance	P_{ST}	0.16	25.69	**<0.001**	0.158
	F_{ST}	0.05	49.67	**<0.001**	0.523
	Jost's D	0.06	56.58	**0.001**	0.664
B. Reproductive Season Duration	P_{ST}	0.05	1.63	0.205	**0.009**
	F_{ST}	0.01	2.19	0.143	**0.030**
	Jost's D	0.00	0.21	0.651	*0.070*
C. Proportion of Reproductive Season Under 15°C	P_{ST}	0.08	5.09	**0.027**	0.981
	F_{ST}	0.02	3.87	*0.052*	0.207
	Jost's D	0.01	1.26	0.264	0.956
D. Magnitude of Thermal Variation In Reproductive Season	P_{ST}	0.05	1.86	0.176	0.274
	F_{ST}	0.00	0.00	0.995	0.685
	Jost's D	-0.01	0.24	0.626	0.146
E. Thermal_PC1	P_{ST}	0.10	8.27	**0.005**	0.661
	F_{ST}	0.02	8.03	**0.006**	0.200
	Jost's D	0.02	2.75	0.101	0.225
F. Magnitude_PC1	P_{ST}	0.05	2.13	0.148	0.604
	F_{ST}	0.01	0.40	0.529	0.900
	Jost's D	0.00	.0.1	0.920	0.736
G. Mag_Therm_PC1	P_{ST}	0.09	6.38	**0.013**	0.254
	F_{ST}	0.02	3.53	*0.064*	0.420
	Jost's D	0.01	1.07	0.304	0.746
H. Total Precipitation in Reproductive Season	P_{ST}	-0.05	1.75	0.189	0.127
	F_{ST}	-0.01	2.67	0.106	0.861
	Jost's D	-0.03	6.92	**0.010**	0.512

Figure 2.1 Map of European *Plantago lanceolata* Populations Sampled Showing Genetic Similarity and Differentiation Calculated in Hickory. Circles represent geographic regions between-which F_{ST} (estimated as θ^{II} in Hickory) values were calculated. Lines connect genetically similar regions with $F_{ST} < 0.09$. Population symbols identified in Table 1. Pop-out boxes are zoomed 4x.

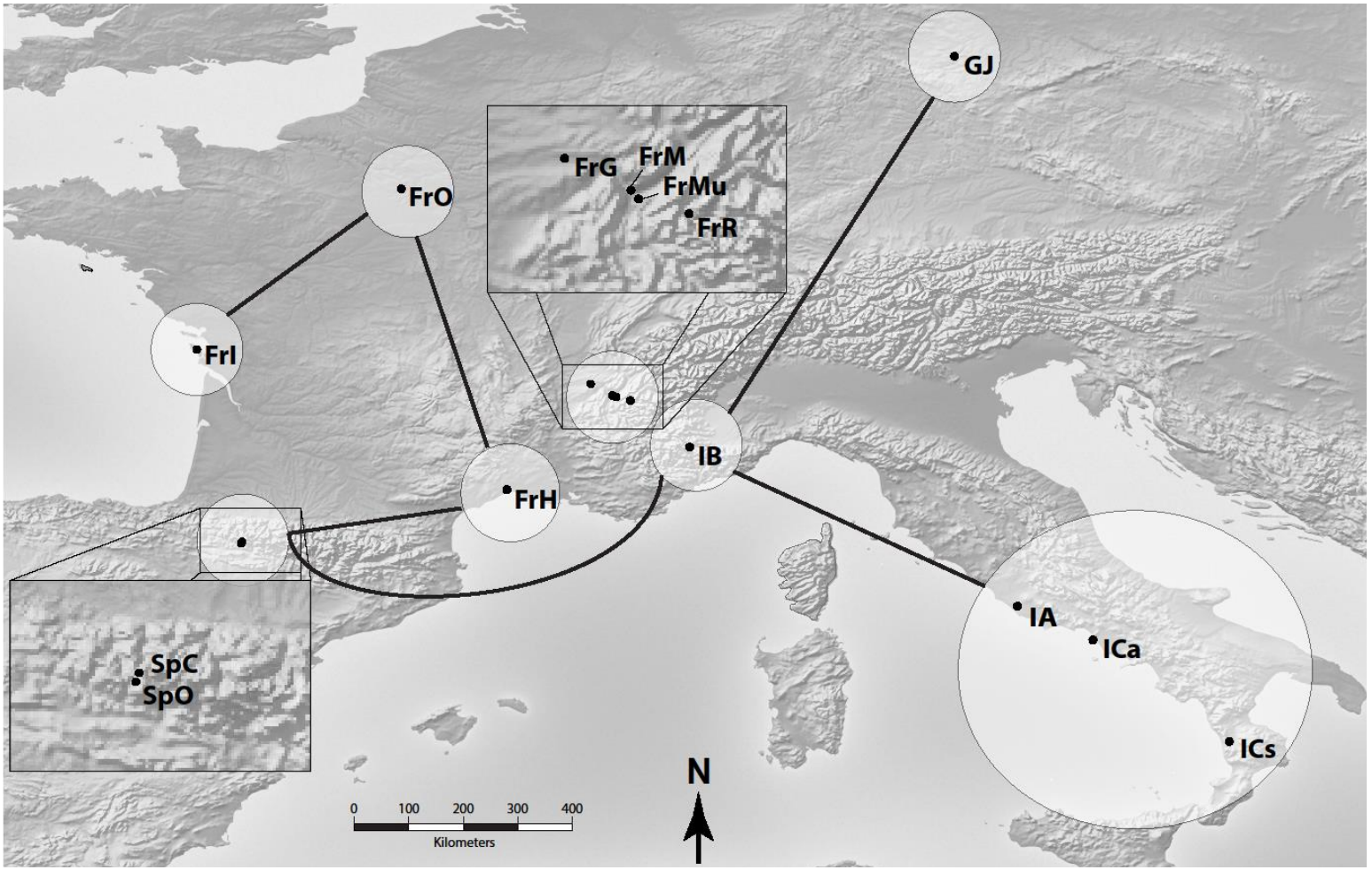


Figure 2.2 Scatter Plot of Phenotypic Differentiation (P_{ST}) of Temperature-Sensitive Floral Reflectance Plasticity and Neutral Genetic Differentiation (F_{ST}) +/- 95% CI between 14 *Plantago lanceolata* Populations. (A) where 95% CI for P_{ST} and for F_{ST} did Not Include the $P_{ST} = F_{ST}$ line, Indicating $P_{ST} > F_{ST}$; and (B) where 95% CI for P_{ST} or for F_{ST} Included the $P_{ST} = F_{ST}$ line Indicating Statistical Support for a Difference was Lacking. The Diagonal Line Indicates $P_{ST} = F_{ST}$.

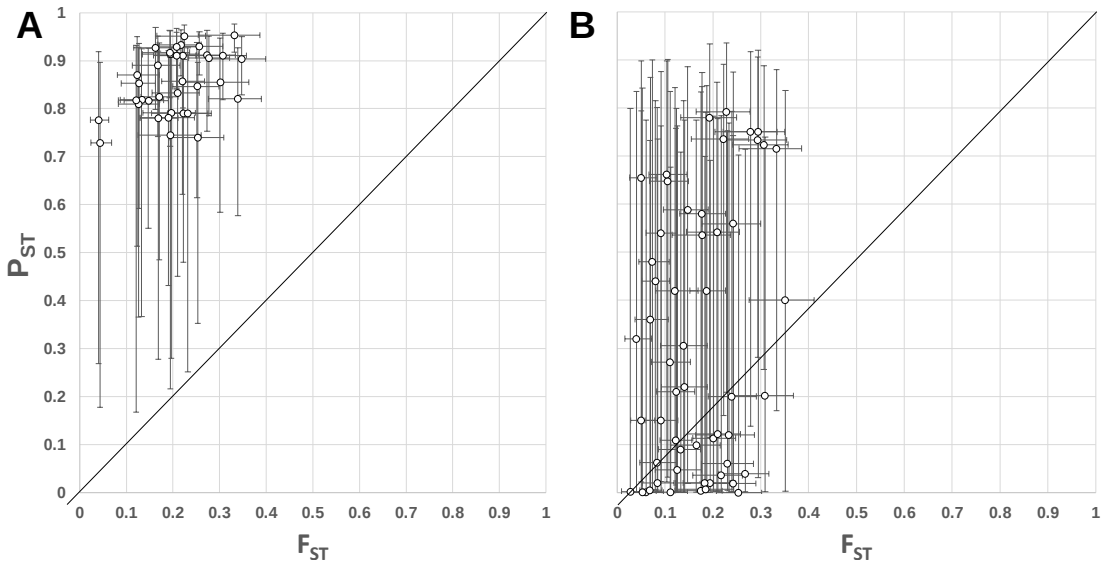
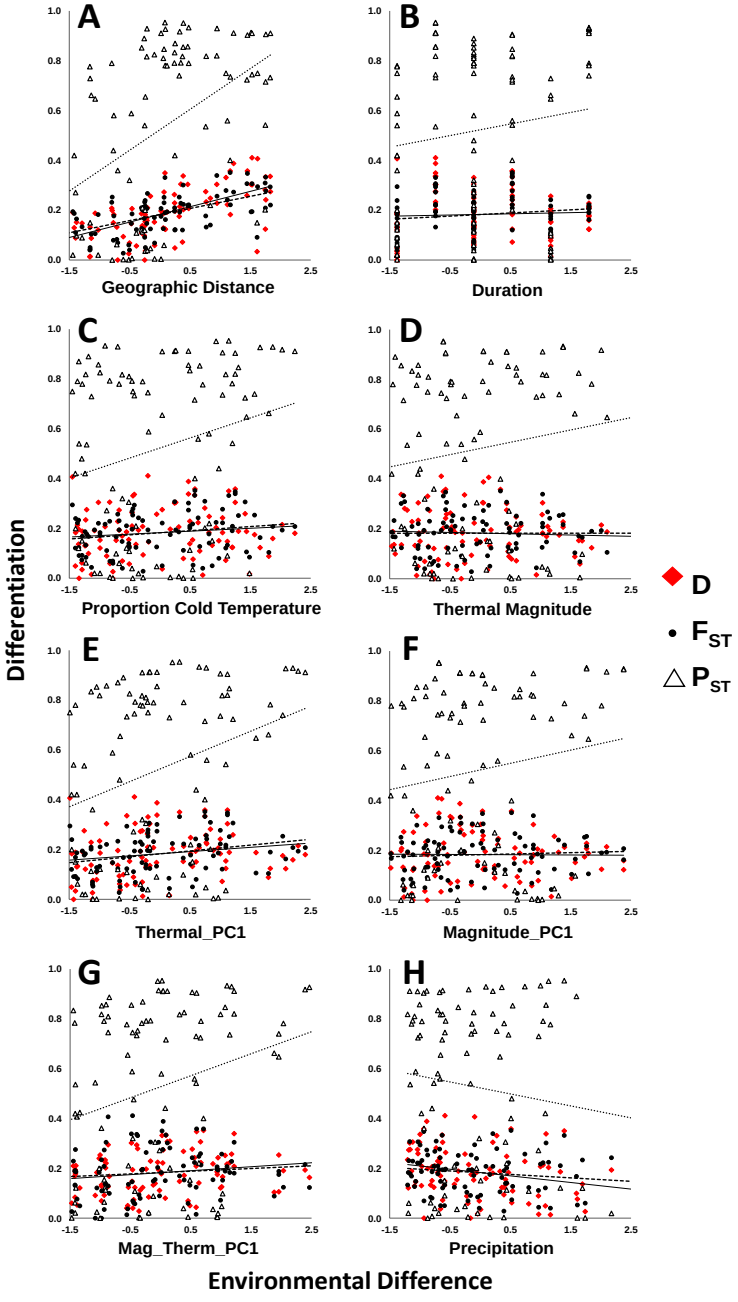


Figure 2.3 Linear Regressions of Phenotypic Differentiation (P_{ST}, triangle, dotted line) of Temperature-Sensitive Floral Reflectance Plasticity and Neutral Genetic Differentiation (F_{ST}, circle, dashed line; Jost's D, diamond, solid line) on an Axis (x) of Increasing Standardized Environmental Difference Between 14 *Plantago lanceolata* Populations. Along the x-axis (A) geographic distance, or environmental properties of the reproductive season diverge between populations from left to right. Environmental variables are (B) season duration, (C) proportion of the season under 15°C, (D) season thermal magnitude, principal components axes (E) Thermal_PC1, (F) Magnitude_PC1, (G) Mag_Therm_PC1 and (H) total season precipitation.



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Supplementary Information

Table S2.1 Scoring Error for AFLP Markers was Determined using Individual Genotypes Repeated Within and Between FAM and TAMRA (TAM) Dyes as the Percentage of Markers in Disagreement.

	TAM	FAM	BETWEEN
CCAA			
Individuals	5	4	14
Markers in disagreement	13	9	59
Total markers	226	196	677
CCAC			
Individuals	3	2	0
Markers in disagreement	5	0	0
Total markers	113	90	0
CCAG			
Individuals	7	2	1
Markers in disagreement	10	3	2
Total markers	255	74	37
CCAT			
Individuals	7	5	1
Markers in disagreement	17	6	4
Total markers	189	112	28
CCTA			
Individuals	0	5	0
Markers in disagreement	0	14	0
Total markers	0	295	0
CCTC			
Individuals	2	5	0
Markers in disagreement	4	12	0
Total markers	92	209	0
CCTG			
Individuals	0	8	0
Markers in disagreement	0	16	0
Total markers	0	272	0
CCTT			
Individuals	3	2	0
Markers in disagreement	5	8	0
Total markers	120	46	0
Percent Error (±2 SE)	**5.51±2.29%**	**5.06±1.79%**	**8.86±1.67%**

Table S2.2 Regional Pairwise Genetic Differentiation (Populations in Region) as F_{ST} (2 Standard Deviations). Populations of *Plantago lanceolata* from southern Europe were grouped into geographic regions based on physical location and inter-regional genetic differentiation was calculated as illustrated in Figure 2.3. Values were calculated in Hickory using 313 AFLP markers from 315 genotypes. Population symbols identified in Table 1.

Region (Population(s))	**South France** (FrH)	**West France** (FrI)	**S.E. France Alps** (FrG, FrM, FrMu, FrR)	**North France** (FrO)	**Germany** (GJ)	**North Italy** (IB)	**South Italy** (IA, ICa, ICs)	**Spain** (SpC, SpO)
South France (FrH)	-	-	-	-	-	-	-	-
West France (FrI)	0.149 (0.047)	-	-	-	-	-	-	-
S.E. France Alps (FrG, FrM, FrMu, FrR)	0.215 (0.042)	0.245 (0.043)	-	-	-	-	-	-
North France (FrO)	0.052 (0.029)	0.082 (0.039)	0.186 (0.052)	-	-	-	-	-
Germany (GJ)	0.144 (0.047)	0.278 (0.064)	0.273 (0.049)	0.193 (0.058)	-	-	-	-
North Italy (IB)	0.107 (0.044)	0.178 (0.044)	0.196 (0.05)	0.122 (0.05)	0.021 (0.012)	-	-	-
South Italy (IA, ICa, ICs)	0.107 (0.036)	0.212 (0.037)	0.228 (0.044)	0.164 (0.046)	0.225 (0.039)	0.008 (0.006)	-	-
Spain (SpC, SpO)	0.075 (0.023)	0.195 (0.035)	0.239 (0.032)	0.117 (0.035)	0.129 (0.034)	0.013 (0.009)	0.187 (0.031)	-

Table S2.3 Mantel Correlations between Geographic Distance, Neutral Genetic Differentiation (F_{ST} and Jost's D) and Environmental Properties of the Reproductive Season. Mantel correlation coefficients and p-values are obtained from permutation tests. Bold type indicates $p < 0.05$ after controlling for multiple comparisons. Italic type indicates $0.05 < p < 0.10$.

			r	p
Distance	~	Duration	0.04	0.731
F_{ST}	~	Duration	0.15	0.190
Jost's D	~	Duration	0.05	0.659
Distance	~	DegMoB15°C	0.12	0.303
F_{ST}	~	DegMoB15°C	0.20	*0.079*
Jost's D	~	DegMoB15°C	0.12	0.277
Distance	~	Magnitude	-0.07	0.618
F_{ST}	~	Magnitude	0.00	0.997
Jost's D	~	Magnitude	-0.05	0.692
Distance	~	Thermal_PC1	0.17	0.208
F_{ST}	~	Thermal_PC1	0.29	**0.030**
Jost's D	~	Thermal_PC1	0.17	0.151
Distance	~	Magnitude_PC1	0.00	0.986
F_{ST}	~	Magnitude_PC1	0.07	0.643
Jost's D	~	Magnitude_PC1	0.17	0.151
Distance	~	Mag_Therm_PC1	0.11	0.449
F_{ST}	~	Mag_Therm_PC1	0.20	0.178
Jost's D	~	Mag_Therm_PC1	0.11	0.394
Distance	~	Precipitation	-0.28	0.127
F_{ST}	~	Precipitation	-0.17	0.414
Jost's D	~	Precipitation	-0.27	*0.083*

Table S2.4 Multiple Regression of Distance Matrices Test Results of the Phenotypic Differentiation (P_{ST}) in Temperature-Sensitive Floral Reflectance Plasticity Matrix on Matrices of Geographic Distance, Genetic Differentiation (F_{ST} and Jost's D), and Environmental Differences between 14 *Plantago lanceolata* Populations while Accounting for Geographic Distance. Environmental variables examined were reproductive season duration (Duration), the proportion of the reproductive season under 15°C (DegMoB15°C), the magnitude of thermal variation of the reproductive season (Magnitude), three principal components axes integrating these variables (Thermal_PC1, Magnitude_PC1, Mag_Therm_PC1, see text for details), and total reproductive season precipitation (Precipitation). Regression coefficients and p-values are obtained from permutation tests. Bold type indicates $p < 0.05$ after controlling for multiple comparisons. Italic type indicates $0.05 < p < 0.10$.

	coefficient	p
A. $P_{ST} \sim F_{ST}$ + Distance		**0.010**
$r^2 = 0.18$		
Intercept	2.94E-11	*0.072*
F_{ST}	1.29E-01	0.403
Distance	3.28E-01	**0.032**
B. $P_{ST} \sim$ Jost's D + Distance		**0.006**
$r^2 = 0.18$		
Intercept	3.17E-11	0.275
Jost's D	1.77E-01	0.189
Distance	2.94E-01	*0.055*
I. $P_{ST} \sim$ Distance + Duration		**0.013**
$r^2 = 0.18$		
Intercept	3.24E-11	0.240
Distance	4.02E-01	**0.006**
Duration	1.18E-01	0.215
J. $P_{ST} \sim F_{ST}$ + Distance + Duration		**0.019**
$r^2 = 0.19$		
Intercept	3.27E-11	0.240
F_{ST}	1.09E-01	0.490
Distance	3.37E-01	**0.028**
Length	1.03E-01	0.288
K. $P_{ST} \sim$ Jost's D + Distance + Duration		**0.013**
$r^2 = 0.20$		
Intercept	3.54E-11	0.133
Jost's D	1.72E-01	0.198
Distance	2.92E-01	*0.057*
Length	1.14E-01	0.230
C. $P_{ST} \sim$ Distance + DegMoB15°C		**0.008**
$r^2 = 0.20$		
Intercept	2.44E-11	0.983
Distance	3.84E-01	**0.007**
DegMoB15°C	1.98E-01	**0.048**
D. $P_{ST} \sim F_{ST}$ + Distance + DegMoB15°C		**0.012**
$r^2 = 0.21$		

Intercept	2.53E-11	0.961
F_{ST}	9.10E-02	0.557
Distance	3.30E-01	**0.029**
DegMoB15°C	1.86E-01	*0.060*
E. P_{ST} ~ Jost's D + Distance + DegMoB15°C		**0.009**
$r^2 = 0.22$		
Intercept	2.75E-11	0.774
Jost's D	1.63E-01	0.217
Distance	2.80E-01	*0.063*
DegMoB15°C	1.91E-01	*0.052*
F. P_{ST} ~ Distance + Magnitude		**0.010**
$r^2 = 0.19$		
Intercept	3.02E-11	0.412
Distance	4.19E-01	**0.005**
Magnitude	1.66E-01	0.109
G. P_{ST} ~ F_{ST} + Distance + Magnitude		**0.014**
$r^2 = 0.20$		
Intercept	3.09E-11	0.332
F_{ST}	1.19E-01	0.442
Distance	3.47E-01	**0.022**
Magnitude	1.61E-01	0.120
H. P_{ST} ~ Jost's D + Distance + Magnitude		**0.010**
$r^2 = 0.21$		
Intercept	3.34E-11	0.145
Jost's D	1.78E-01	0.182
Distance	3.05E-01	**0.043**
Magnitude	1.67E-01	0.106
L. P_{ST} ~ Distance + Thermal_PC1		**0.007**
$r^2 = 0.17$		
Intercept	2.65E-11	0.994
Distance	3.71E-01	**0.008**
Thermal_PC1	2.19E-01	**0.044**
M. P_{ST} ~ F_{ST} + Distance + Thermal_PC1		**0.010**
$r^2 = 0.22$		
Intercept	2.71E-11	0.949
F_{ST}	6.93E-02	0.659
Distance	3.31E-01	**0.029**
Thermal_PC1	2.06E-01	*0.062*
N. P_{ST} ~ Jost's D + Distance + Thermal_PC1		**0.007**
$r^2 = 0.23$		
Intercept	2.93E-11	0.521
Jost's D	1.53E-01	0.242
Distance	2.73E-01	*0.070*
Thermal_PC1	2.09E-01	*0.051*
O. P_{ST} ~ Distance + Magnitude_PC1		**0.012**
$r^2 = 0.19$		
Intercept	3.84E-11	0.137
Distance	4.07E-01	**0.005**
Magnitude_PC1	1.50E-01	0.143

P. P_{ST} ~ F_{ST} + Distance + Magnitude_PC1		**0.018**
r^2 = 0.20		
Intercept	3.86E-11	0.137
F_{ST}	1.15E-01	0.460
Distance	3.38E-01	**0.027**
Magnitude_PC1	1.43E-01	0.168
Q. P_{ST} ~ Jost's D + Distance + Magnitude_PC1		**0.013**
r^2 = 0.21		
Intercept	4.17E-11	*0.095*
Jost's D	1.79E-01	0.180
Distance	2.93E-01	*0.055*
Magnitude_PC1	1.52E-01	0.138
R. P_{ST} ~ Distance + Mag_Therm_PC1		0.006
r^2 = 0..21		
Intercept	2.40E-11	0.987
Distance	3.83E+01	**0.006**
Mag_Therm_PC1	2.17E-01	*0.053*
S. P_{ST} ~ Distance + F_{ST} + Mag_Therm_PC1		**0.010**
r^2 = 0..22		
Intercept	2.48E-11	0.968
F_{ST}	8.80E-02	0.569
Distance	3.31E-01	**0.028**
Mag_Therm_PC1	2.05E-01	*0.069*
T. P_{ST} ~ Distance + Jost's D + Mag_Therm_PC1		**0.007**
r^2 = 0..23		
Intercept	2.70E-11	0.791
Jost's D	1.63E-01	0.215
Distance	2.80E-01	*0.064*
Mag_Therm_PC1	2.11E-01	*0.059*
U. P_{ST} ~ Distance + Precipitation		0.013
r^2 = 0.17		
Intercept	2.86E-11	0.838
Distance	4.05E-01	**0.007**
Precipitation	-5.90E-03	0.972
V. P_{ST} ~ F_{ST} + Distance + Precipitation		**0.020**
r^2 = 0.18		
Intercept	2.94E-11	0.701
F_{ST}	1.30E-01	0.408
Distance	3.27E-01	**0.035**
Precipitation	-5.80E-03	0.973
W. P_{ST} ~ Jost's D + Distance + Precipitation		**0.012**
r^2 = 0.18		
Intercept	3.16E-11	0.345
Jost's D	1.78E-01	0.196
Distance	2.86E-01	*0.054*
Precipitation	1.12E-02	0.947

Figure S2.1 Mean Panmictic Heterozygosity ± 95% CI of 14 *Plantago lanceolata* Populations from Southern Europe. Values calculated in Hickory using 313 AFLP markers from 315 genotypes. Locations are approximate, symbols as in Table 2.1. Pop-out boxes zoomed 4x.

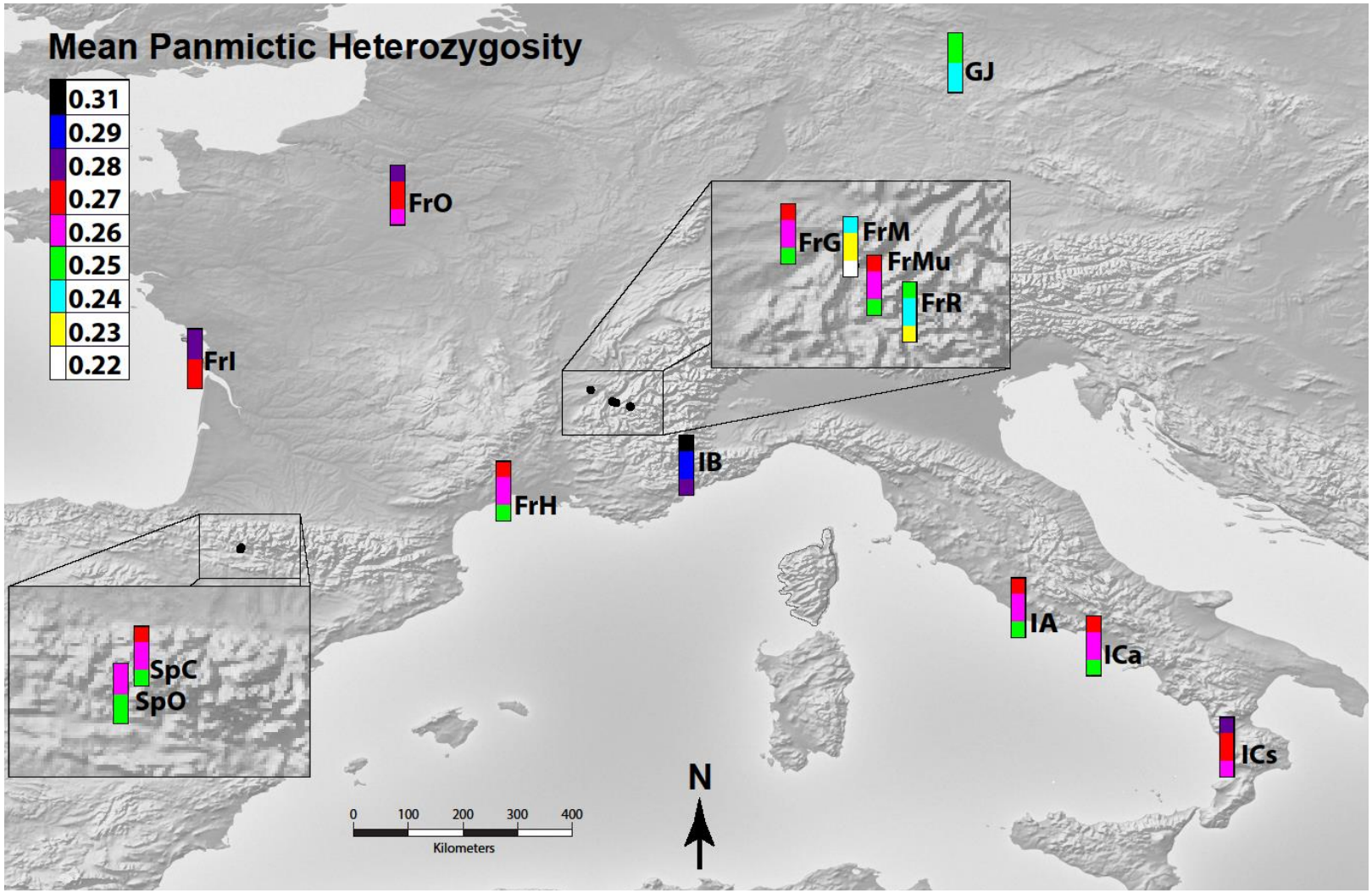


Figure S2.2 Delta K of STRUCTURE Runs From K=2-10 Suggest AFLP Data From 14 *Plantago lanceolata* Populations From Southern Europe Best Fit into 7 Groups. Runs Were Combined and Interpreted With Structure Harvester (EARL 2012), Using the Methods of Evanno *et al.* (2005).

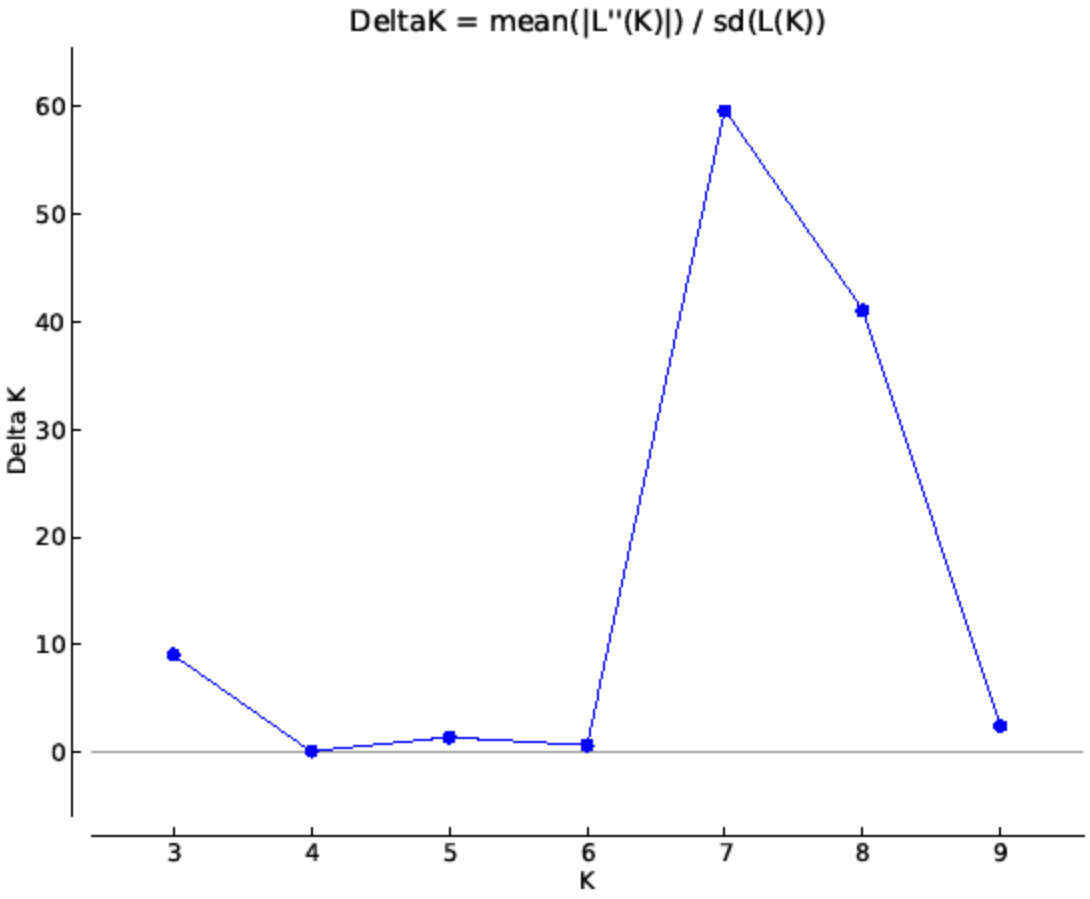


Figure S2.3 Mean (± SD) of Estimated Ln Probability of Data from STRUCTURE Runs from K=2-10 Suggest AFLP Data from 14 *Plantago lanceolata* Populations from Southern Europe Best Fit into 7 or 8 Groups. Runs were combined and interpreted with Structure Harvester (EARL 2012), using the methods of Pritchard *et al.* (2000).

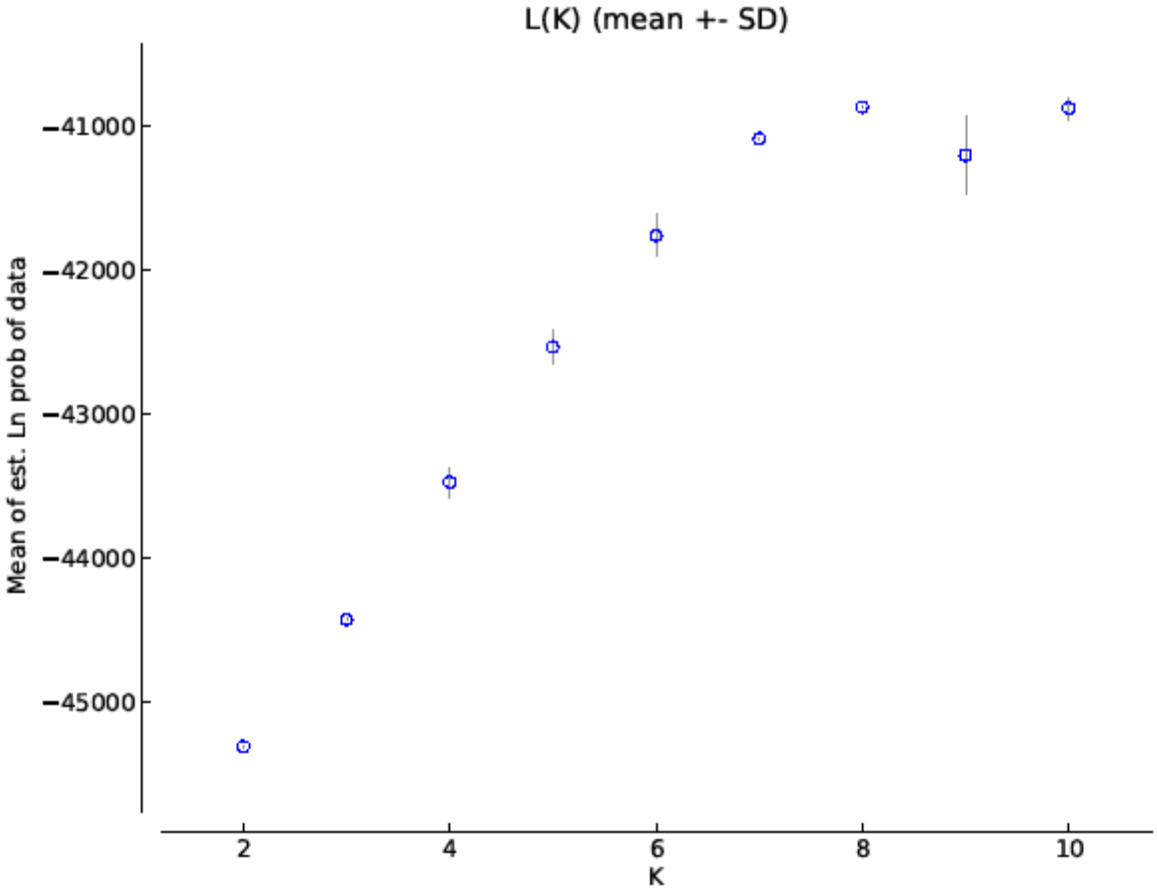
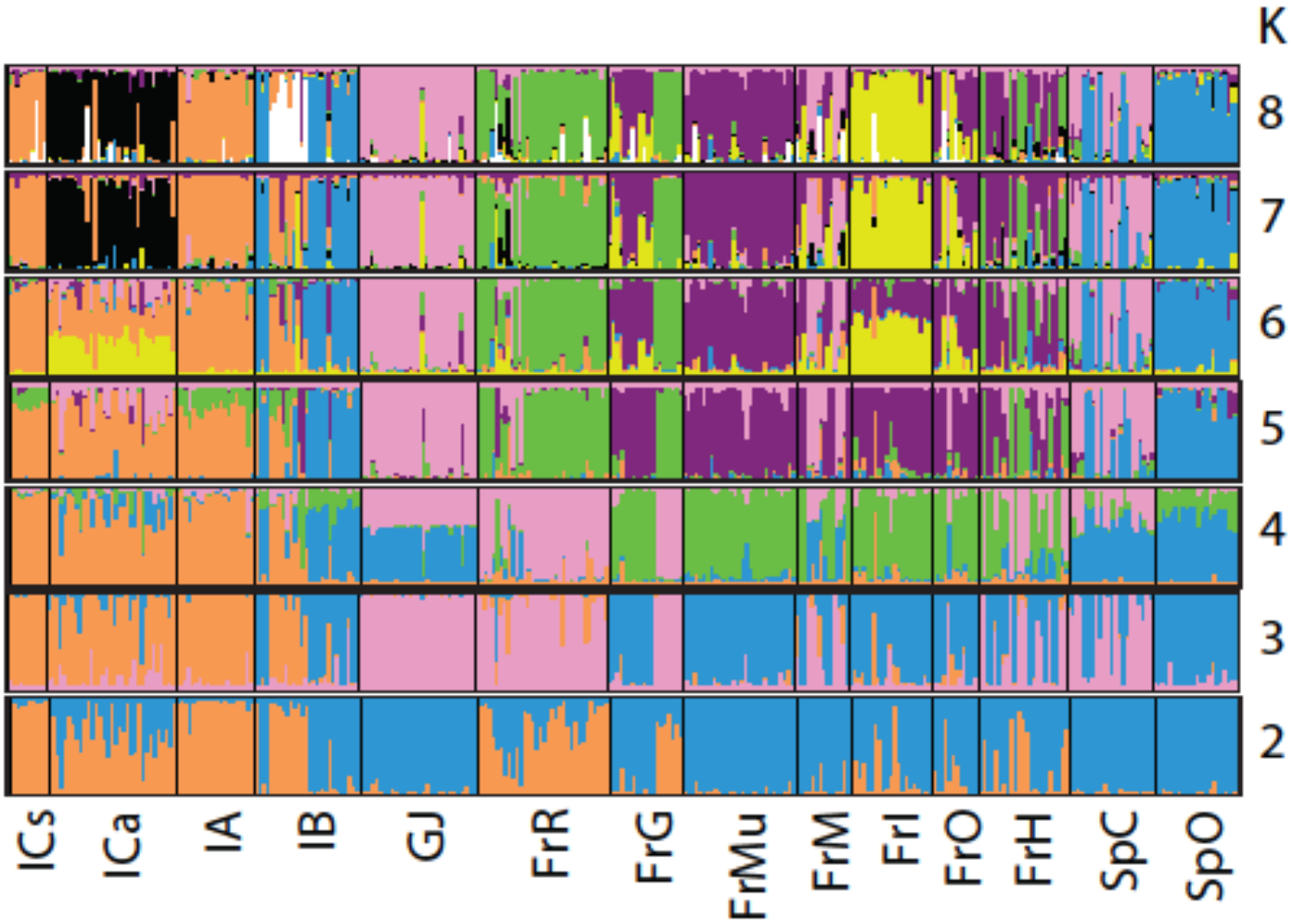


Figure S2.4 STRUCTURE Admixture Plots of 14 *Plantago lanceolata* Populations from Southern Europe. Calculated with 313 AFLP markers from 315 genotypes. CLUMPP was used to average admixture proportions over runs (Jakobsson & Rosenberg 2007) and Distruct (Rosenberg 2004) for visualization.



CHAPTER III

THE GENETICS OF THERMAL PLASTICITY IN *PLANTAGO LANCEOLATA*: QTL MAPPING

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Abstract

In many species, temperature-sensitive phenotypic plasticity (i.e., an individual's phenotypic response to temperature) displays positive correlations with latitude, a pattern presumed to reflect local adaptation. We took advantage of natural geographic variation in *Plantago lanceolata* to examine the genetic architecture underlying latitudinal differences in thermal plasticity. We produced an F_2 mapping family from parents derived from distant northern and southern European populations that exhibited high (northern parents) and low (southern parents) thermal plasticities of floral reflectance. We then grew parents and offspring in two environments (cool and warm) mimicking what plants would encounter in nature. We obtained genetic markers via genotype-by-sequencing (ddRADseq), produced a recombination map and performed QTL mapping of thermal plasticity and single-environment trait values for six traits: floral reflectance, flowering time, rosette diameter, leaf length, leaf fresh mass, and leaf area.

Our data provide critical genetic support for the hypothesis that temperature-sensitive floral reflectance plasticity in *P. lanceolata* is adaptive in high latitude environments where growing seasons are cool and short. We found one single QTL underlying the thermal plasticities

of three traits, floral reflectance, flowering time and leaf length. Our data confirms thermal plasticity in *P. lanceolata* has a genetic basis. Floral reflectance plasticity and flowering time plasticity QTLs colocalized with, and shared phenotypic effects with corresponding single environment loci. The leaf length plasticity QTL did not colocalize with any single-environment loci, and was influenced by cytoplasm. We did not find evidence that plasticity QTLs of different traits were pleiotropic, suggesting thermal responses of different traits are free to evolve independently. Additionally, genotypic differences at plasticity QTLs paralleled phenotypic patterns of plasticity along latitudinal clines. Northern genotypes (Danish and Swedish) increased the magnitude of thermal plasticity, while southern genotypes (French and Italian) decreased plasticity. This provides genetic support that observed latitudinal clines of thermal plasticity reflect adaptation.

Keywords

Phenotypic plasticity, temperature, adaptive plasticity, QTL mapping, geographic clines, linkage map, thermal plasticity.

Introduction

Phenotypic plasticity, the ability of a genotype to alter its phenotype in response to environmental change, is a fundamental characteristic of all life on Earth, and has been shown to be adaptive (Ghalambor *et al.* 2015; Nicotra *et al.* 2015). Temperature-sensitive plasticity, in particular, is vital for survival and reproduction in many species because basic metabolic activities function only within a limited range of temperatures. For ectotherms, which rely on external heat sources to mediate internal body temperature, temperature-sensitive plasticity allows organisms to acclimate to a new temperature through adjustments in: 1) behavior e.g. movements through microhabitats and solar tracking (Clench 1966; Ehleringer & Forseth 1980;

Huey 1991; Kudo 1995; Webster & Weathers 1990); 2) phenology of sensitive life stages e.g. bud, flower, and fruit emergence, laying/birthing date in animals (Crick *et al.* 1997; Fitter & Fitter 2002; Visser & Holleman 2001); and 3) cellular physiology, e.g. of cellular membranes and gene expression (Angilletta Jr *et al.* 2002; Hazel 1995; Huey & Bennett 1990; Huey & Stevenson 1979; Lacey & Herr 2005; Marmur & Doty 1962; Somero 1995).

In many species, thermal plasticity displays positive correlations with latitude, e.g. in developmental rate (Laugen *et al.* 2003), body size (Liefting *et al.* 2009), thermal tolerance (Addo-Bediako *et al.* 2000; Ghalambor *et al.* 2006), leaf shape (Royer *et al.* 2009), flower/seed number (Molina-Montenegro & Naya 2012), and flower reflectance (Lacey *et al.* 2010). These latitudinal patterns are presumed to reflect local adaptation, in the sense that temperature-sensitivity is believed to be more adaptive in thermally variable environments where growing seasons are cooler and shorter (Huey & Stevenson 1979; Lacey *et al.* 2010; Wieser 1973). While this hypothesis is reasonable, it has not been thoroughly tested. Demonstrating local adaptation requires showing that thermal plasticity has a genetic basis and varies genetically along a latitudinal gradient. Ultimately, we would like to identify the genes underlying the plasticity. Doing so can help us understand the molecular mechanisms by which organisms respond to environmental changes. Such information can give us clues as to how plasticity has evolved in the past and potentially provide us with useful tools to modify crop species and improve yield in future environments. Also, we must show that the clinal variation arises from natural selection, and not by chance alone. Satisfying these requirements is non-trivial.

Despite the abundance of ways by which organisms respond to changes in their thermal environment, we still know little about the genetic architecture underlying geographic variation in most ecologically and agriculturally relevant traits, e.g., the number of genes, their chromosomal locations, or their phenotypic effects (Alonso-Blanco & Méndez-Vigo 2014; Des Marais *et al.*

2013; Dittmar *et al.* 2016; Gerken *et al.* 2015; Remington 2015). The most comprehensively studied and best understood trait known to respond to temperature in plants is the transition from vegetative growth to flowering in *Arabidopsis thaliana.* However, quantitative trait locus (QTL) mapping studies exploring the genetic variation of flowering time under different thermal environments are only just beginning to emerge (Dittmar *et al.* 2014; Ilk *et al.* 2015; Springate & Kover 2014; Vasseur *et al.* 2014), and we have found only one study examining the genetic architecture of thermal plasticity of flowering time (Méndez-Vigo *et al.* 2016), Likewise, our knowledge of the genes underlying adaptive thermal plasticity of other traits remains scarce. Ultimately, we would like to know whether or not: 1) few or many genetic loci control adaptive traits (Fisher 1919; Fisher 1930; Orr 1998, 2005), 2) genetic loci typically exhibit small or large phenotypic effects (Remington 2015; Rockman 2012), 3) pleiotropic genes affect adaptive thermal responses (Anderson *et al.* 2011; Des Marais *et al.* 2013; Méndez-Vigo *et al.* 2016), and 4) epistatic interactions influence thermal plasticity (Gaertner *et al.* 2012; Leinonen *et al.* 2013; Taylor & Ehrenreich 2015; Zeng 1993).

We took advantage of natural geographic variation in *Plantago lanceolata* to examine the genetic architecture underlying latitudinal differences in thermal plasticity of reproductive and vegetative traits. Our study builds upon a body of previous work showing that thermal plasticity in floral reflectance: 1) is genetically variable within and among populations (Lacey & Herr 2005), 2) improves seed production at cool, but not warm, temperatures relative to the absence of plasticity (Lacey *et al.* 2012), and 3) is positively correlated with latitude and altitude in its native Europe (Lacey *et al.* 2010). Also, data from a recent population-genetic analysis provide evidence that the clinal variation in thermal plasticity arises from natural selection, as well as genetic drift (Marshall *et al.* In Prep.).

For our study, we produced an F_2 mapping family from parental genotypes derived from distant northern and southern European populations that exhibited high (northern parents) and low (southern parents) thermal plasticities of floral reflectance from over 300 genotypes sampling 29 European populations previously studied by Lacey et al. (2010). We then grew parents and F_2s in two controlled thermal environments (cool and warm) that mimicked what plants would encounter in their natural environment. We developed a de novo genetic recombination map from genetic markers attained via double-digest restriction associated digest sequencing (ddRADseq) and performed QTL mapping to examine the genetic architectures underlying thermal plasticity and single-environment trait values. Because parental genotypes differed in their thermal plasticity of several traits, in addition to floral reflectance, we also explored the genetic architecture of these traits: flowering time, rosette diameter, leaf length, leaf fresh mass, and leaf area. Our data allowed us to address the following questions:

1. *Where in the genome are plasticity QTLs located?* Identifying the genomic locations of QTLs underlying variation in thermal plasticity represents a critical first step to determining the genes controlling temperature-sensitivity and can guide the development of directed sequencing and fine mapping studies aimed at identifying allelic-gene variants (Glazier *et al.* 2002). Here, we used QTL mapping to evaluate the phenotypic effects of QTL-alleles derived from divergent natural populations on variation in temperature-sensitivity.

2. How many loci underlie thermal plasticity and they have small or large phenotypic effects? Thermal plasticity often occurs along a continuous spectrum in nature, and therefore, we expected the genetic architecture of thermal-plasticity to be quantitative and consist of several QTLs of various effect sizes. We expected that our sample size would allow us to detect large and intermediate effect QTLs associated with variation for thermal plasticity in our mapping

population, and we expected to slightly overestimate QTL effect sizes (Beavis 1994; Beavis 1998).

3. Are plasticity QTLs the same as, or different from single-environment QTLs? For traits where phenotypic variation in thermal plasticity and variation in a single environment are highly correlated, we expected that QTLs for plasticity and the single environment QTLs would colocalize, indicating shared genetic control.

4. Is there a common genetic mechanism by which different traits respond to temperature changes? If pleiotropic genetic control of thermal plasticity in different traits were to exist, then we would expect to find plasticity QTLs underlying different traits to colocalize, and we would expect to see strong genetic correlations among thermal plasticities of these traits.

5. Do overlapping QTLs display similar additive, dominant and/or interaction effects? Colocalization of QTLs from different traits, by itself, does not allow us to differentiate between pleiotropic gene effects or closely linked genes (Paterson *et al.* 1990). However, traits with shared genetic mechanisms should, in addition to displaying colocalization of QTLs, also display QTLs with effects influencing trait variation of similar magnitude and direction. Colocalized QTLs exhibiting different QTL effects imply different genes and genetic mechanisms underlying trait variation and suggest the common QTLs are driven by closely linked, but different genes.

6. Are interactions between genetic loci an important component of the genetic architecture of thermal plasticity? We expect to find epistasis if two or more genes from distinct genomic locations involved in the biochemical response to temperature have coevolved in divergent directions between northern and southern populations.

Methods

Biology of Plantago lanceolata

Plantago lanceolata L. (ribwort plantain, English plantain), a widespread perennial herb native to Eurasia, has a haploid number of 6 chromosomes (Tjebbes 1928) and 1.2-1.4 Gbp estimated from C-DNA values (Bennett *et al.* 1998; Grime *et al.* 1985, Lacey unpublished data). Because the genome has yet to be sequenced, it has few genomic resources available (Bennett *et al.* 1998; Grime *et al.* 1985; Primack & Antonovics 1982; Wong & Murray 2012). Despite these challenges, *P. lanceolata* provides many advantages for studying the genetics of thermal plasticity. First and foremost, many fitness related traits including survival, flowering traits (e.g. floral reflectance, flowering time, inflorescences per plant, capsules per inflorescence, scape length, spike length), vegetative traits (e.g. leaf number, length, width, mass, angle), and seed traits (e.g. seed mass, yield, percent germination), exhibit phenotypic plasticity to abiotic conditions in field and greenhouse environments. Moreover, phenotypic plasticity is genetically variable within and among natural populations (Antonovics & Primack 1982; Lacey & Herr 2005; Lacey *et al.* 2010; Primack & Antonovics 1981; Schmitt *et al.* 1992; Stiles *et al.* 2007; Van Tienderen 1990; Van Tienderen 1992; Van Tienderen & Hinsberg 1996; Van Tienderen & van der Toorn 1991; Wolff 1990; Wolff & Van Delden 1987). Furthermore, thermal plasticity of multiple traits in *P. lanceolata* (e.g., flowering time, spike and scape length) can affect reproductive success (Alexander & Wulff 1985; Case *et al.* 1996; Herrera 2013; Herrera & Lacey In prep.; Lacey & Herr 2005; Lacey *et al.* 2012; Lacey *et al.* 2010; Marshall *et al.* In Prep.).

Additionally, the species has many qualities that make it useful for experimentation. One can obtain true measures of phenotypic plasticity and control for age by cloning individuals into multiple cuttings, which can be phenotyped under different conditions. Also, flowering characteristics in *P. lanceolata* facilitate controlled genetic crossing. The transition from

vegetative growth to flowering is controlled by photoperiod. Plants will remain in vegetative growth under continuous exposure to short days and only flower in long day conditions (Baroni *et al.* 2000; Snyder 1948). In addition, flowers in *P. lanceolata* are protogynous and an outcrossing mating system is enforced by self-incompatibility (Ross 1973; Van Damme 1983).

There is strong evidence that temperature-sensitivity of floral reflectance and color of spikes (i.e. inflorescences of tightly-packed flowers) is adaptive in high latitude environments where thermally variable growing seasons are cool and short. Floral reflectance plasticity, which is determined by the ambient temperature experienced during flower development, influences internal spike temperature, allowing individuals to partially thermoregulate the temperature of developing gametes, embryos, and seeds (Lacey & Herr 2005). Poorly reflective spikes are consistently warmer (~1-2°C) than highly reflective spikes when placed in a common thermal environment, and warming spikes likely improves seed production in cool temperatures (Lacey & Herr 2005; Lacey *et al.* 2012). Across the European landscape, floral reflectance plasticity displays latitudinal and altitudinal clines where populations with higher mean plasticity inhabit climates characterized by shorter and cooler reproductive seasons. Populations with lower plasticity inhabit climates with longer and warmer reproductive seasons (Lacey *et al.* 2010). This geographic pattern is significantly influenced by selection, which increasingly favors thermal plasticity of floral reflectance in short and cool reproductive seasons (Marshall *et al.* In Prep.).

Crossing Design

We reciprocally outcrossed two northern genotypes from Denmark and Sweden displaying high thermal plasticity of floral reflectance with two southern genotypes from Italy and France displaying low thermal plasticity of floral reflectance to produce an F_2 mapping population (Figure 3.1; Table 3.1). Parental genotypes represented the extremes in thermal

plasticities found in a sample of 29 European *P. lanceolata* populations (Lacey *et al.* 2010, Table S3.1). They were the progeny of genotypes collected from wild populations.

In 2012, we reciprocally crossed the northern parents with southern parents (Danish x Italian and Swedish x French) to produce two hybrid F_1 families with reciprocal (northern vs. southern) cytoplasms (Figure 3.1). Crossing was conducted in growth chambers at 20ºC, 16-h day/15ºC, 8-h night, on multiple clones of each parental genotype. Plants were watered and fertilized with half-strength Hoagland's solution once a day. We conducted controlled crosses by sealing maternal spikes prior to stigma emergence in pollination bags and then introducing pollen into the bags. Seeds were harvested, counted and stored at room temperature until sowing. Likewise, in 2013 we reciprocally crossed a single Danish x Italian F_1 hybrid with northern (Danish) cytoplasm, with a single French x Swedish F_1 hybrid with southern (French) cytoplasm to produce the F_2 mapping population with reciprocal (Danish/French) cytoplasms (Figure 3.1). After crossings were completed, we maintained F_0 and F_1 parental genotypes in growth chambers at 20ºC, 8-h day/15ºC, 16-h night to promote vegetative growth until needed for phenotyping.

Phenotyping

Plant growth and phenotyping of plants from all three generations occurred in 2 cohorts subjected to the same 42-week regime. Clones of parental F_0 and F_1 genotypes were interspersed with and phenotyped along with each cohort as described below. Cohort 1 consisted of 260 F_1 seeds (65 / reciprocal family) and 312 F_2 seeds (156 / reciprocal family) sown in November 2013, and cohort 2 consisted of 449 F_2 seeds (226 with Danish cytoplasm and 223 with French cytoplasm) sown in September 2014, respectively. On day 0, we planted seeds in 118mL pots in growth chambers set at 20ºC, 8-h day/15ºC, 16-h night. On day 42, we transferred seedlings to 473mL pots. On day 112 we split all genotypes into 4 clones with a razor and applied Bontone II® rooting powder to cut sites, then planted clones in 473mL pots. We also split F_0 and F_1 parental

genotypes into 8 clones each and randomly distributed them into each cohort. On day 140, we moved two clones per genotype (four clones per parent) to different cool (three chambers: 15ºC, 8-h day/10ºC, 16-h night) and warm growth chambers (three chambers: 27ºC, 8-h day/20ºC, 16-h night). On days 165-168 we measured vegetative components representing plant size and shape. We measured rosette diameter as the maximum plant diameter to the nearest 0.1 cm. We measured leaf traits by removing the longest two leaves from each plant and averaging their values. We measured leaf length to the nearest 0.1 cm, and leaf area using a portable area meter (LI-3000C) to the nearest 0.01cm^2. We measured fresh leaf mass to the nearest 0.01g using a digital scale immediately after leaves were removed. On day 168, we initiated flowering by extending the day length in growth chambers to 16-h day/8-h night. We monitored plants for emergence of flowering spikes every other day for the following 126 days. Once flowering began, we measured reproductive components representing floral reflectance and flowering time. We measured floral reflectance as the average percent of light reflected at 850nm from two spectral scans conducted on a single pre-flowering spike prior to stigma emergence, using a spectrophotometer with an integrating sphere (for methodology, see Lacey and Herr 2005). We measured flowering time as the number of days after flowering was initiated (day 168), until the first flowering spike emerged completely from the leaf axil. We removed each plant from growth chambers once measurements were completed. On day 294 each cohort concluded.

Throughout the experiment we took measures to maintain thermal differences between temperature treatments and reduce differences among other abiotic conditions. We verified temperature at plant height in each chamber daily with thermometers and maintained light intensity at plant height between 300-325 μmol throughout the experiment. We randomly placed multiple clones of each genotype in different growth chambers for each temperature treatment. We used chambers set at cool temperature during cohort 1 as warm temperature chambers in

cohort 2, and vice versa. Throughout the experiment, plants were potted in Fafard 52 mix soil and watered daily. During each cohort, we fertilized plants daily between days 42-144 and 210-294, with 0.2 Tbsp. of Miracle-Gro® all-purpose plant food per gallon of water and trimmed leaves on all plants to ~10 cm length on days 112, 196-198 and 217-220.

Phenotypic Analyses

We compared parental phenotypes in each thermal environment with one-way analysis of variance (*aov*) and Tukey's post hoc tests (*TukeyHSD)* performed for each trait by temperature treatment combination in *R 3.2.3*, with each parental genotype represented by multiple clones (R Development Core Team 2013). We estimated broad-sense heritability for each trait at cool and warm temperature, and for trait plasticity using the formula:

$$h^2 = \frac{\sigma_{F2}^2 - \sqrt{\sigma_N^2 \ x \ \sigma_S^2}}{\sigma_{F2}^2}$$

where σ_N^2 and σ_S^2 represent variances of parents from northern and southern populations, respectively, and σ_{F2}^2 is the F_2 variance (Mahmud & Kramer 1951). We estimated parental variances (σ_N^2 and σ_S^2) in cool and warm temperature from the mean trait value of clones of northern and southern parental genotypes. We calculated parental variances for plasticity by estimating multiple plasticity values for each genotype, each of which was calculated by subtracting the mean trait value of a randomly selected clone in cool from the mean trait value of a randomly selected clone in warm without resampling. Therefore for our variance calculations of plasticity, each parental genotype contributed a number of plasticity estimates equal to the fewest number of clones measured in either environment.

For each genotype we calculated mean trait values in each thermal environment by averaging trait values of clones. Trait plasticity was calculated as the warm-temperature mean

trait value minus the cool-temperature mean trait value. We used genotypic mean trait values and trait plasticities of F_2s to calculate genotypic variance and trait correlations with *cor* and *cov* in *R* (R Development Core Team 2013). We tested F_2 trait distributions for normality with the Shapiro-Wilk test *shapiro.test* in *R/stats* and kurtosis by Pearson's Kurtosis statistic *kurtosis* in *R/moments* (Komsta & Novomestky 2015; R Development Core Team 2013).

Genotyping

We collected 100mg of young leaf tissue from 465 individual genotypes (4 F_0, 2 F_1, 459 F_2) and stored it at -80ºC until extractions were performed. DNA was extracted using the MasterPure™ plant leaf DNA purification kit. Integrity of high molecular weight DNA bands were verified visually on 1% agarose gels run in 1x TAE buffer, stained with 0.2µg/mL ethidium bromide and viewed with the Bio-Rad ChemiDoc XRS system.

Plantago lanceolata has neither a sequenced genome, nor readily available genetic markers. Therefore, we performed the double-digest restriction-site associated sequencing protocol, *ddRADseq* (Peterson *et al.* 2012) to develop reproducible genetic markers evenly spread across the genome that could be used to produce a genetic recombination map. We selected four non-methylation-sensitive enzymes with an optimal reaction temperature of 37ºC to determine which restriction enzymes would be appropriate for this project. Two were 'common cutters' with 4-nucleotide recognition sites, MseI and MspI; and two were 'rare cutters' with 6-nucleotide recognition sites, EcoRI and PstI. We performed single digestions (each restriction enzyme alone) and double digestions (each combination of common + rare cutter) on genomic DNA from each of the F_0 parents (for details see supplementary methods). We subjected digested DNA samples to a dilution series and ran them on an Agilent 2100 Bioanalyzer High Sensitivity chip. The number of sequencable fragments produced from each combination of restriction enzymes was estimated using the methods described in Peterson et al. (2012). After digestion with EcoRI and MspI, we

estimated a size selection window of 200-400bp to produce ~38,000 sequencable fragments per individual. Therefore, to capture DNA fragments of 200-400bp ligated to 120bp of adapters, we used a size selection window of 320-520bp for library preparation.

We sent genomic DNA samples of 465 individual genotypes (4 F_0, 2 F_1, 459 F_2) to the genomics core lab at Texas A&M University Corpus Christi for library preparation where SPRI size selection was used to purify high molecular weight genomic DNA. Illumina library preparation was conducted using the restriction enzymes EcoRI and MspI with a size selection window of 320-520bp. For each run 100bp PE sequencing was performed on a single Illumina lane of 196 pooled individuals. We estimated this volume to produce ~38,000 reads per individual with 40x coverage.

Linkage Mapping

We used the following workflow in STACKS v. 1.35-1.37 to process ddRADseq reads and produce the genetic markers (Catchen *et al.* 2013; Catchen *et al.* 2011). We filtered raw reads from each sequencing run to remove erroneous and low-quality reads, and demultiplex (see Supplementary Laboratory Methods for details). Then we sorted and scanned reads from each individual with a minimum of 5x coverage and maximum of 2 alleles per locus against a catalog of loci from F_0 and F_1 parental genotypes and exported matching reads as haplotypes for each genetic locus (marker).

We conducted recombination mapping using the 118 F_2s (25.7%) with the highest sequence coverage. In Microsoft Excel, we removed genetic markers if they did not contain allelic differences between F_1 genotypes, could not be traced to F_0 genotypes, or were scored in fewer than 70% of the 118 F_2s. Based upon the alleles identified and their segregation patterns, each genetic marker was categorized as either fully informative (segregating 1:1:1:1; Type A), or partially informative (segregating 1:2:1; Type B, or 1:1; Type D), as described in (Wu *et al.*

2002). Then, we removed markers with extremely skewed segregation ratios, (i.e., $p<0.0001$ from chi-squared tests of observed vs. expected segregation ratios of each marker). Filtering produced a set of 555 genotyped markers utilized create a genetic recombination map.

We used the Kosambi mapping function in the *R/OneMap* package to calculate marker order and genetic distance (Kosambi 1943; Margarido *et al.* 2007; R Development Core Team 2013). Denovo linkage mapping proceeded in three phases based upon segregation ratio p-values. First, we grouped markers with segregation p-values ≥ 0.05 using recombination frequencies ≤ 0.40 and LOD scores ≥ 4.0. Within a linkage group (LG), we estimated preliminary marker order using the *order.seq* function. We evaluated the resulting order using the recombination fraction matrix. Markers that did not show recombination frequencies monotonically increasing with distance from the diagonal were relocated using the *try.seq* and *make.seq* functions, or removed. Once all markers within the LG displayed a monotonic recombination frequency pattern, we forced each other marker initially grouped with those markers onto the LG, one at a time, to determine if they fit soundly at any position along the linage group. If forcing a marker onto the LG resulted in map expansion or violation of monotony we relocated or removed it. Second, we added markers with segregation ratio p-values ≥ 0.01, and third, we added markers with p-values ≥ 0.0001 to LGs using the same criteria (map expansion and violation of monotony). Finally, we forced all remaining markers that did not fit soundly on any of the LGs together onto a single, separate LG and evaluated position using the same criteria. Once all markers were tested, we evaluated the order of each LG using the *ripple.seq* function with a sliding window size of 4, LOD threshold of 2.0, and tolerance value of 0.1. We examined alternative orders that produced lower LOD scores for map expansion and violation of monotony along the LG. In cases when reordering did not produce a better overall linkage map, we removed the least informative markers.

Marker Distribution and Genome Coverage

We compared marker density with expected marker density under the Poisson distribution to evaluate marker distribution among LGs on the final linkage map. We calculated average marker spacing *s* by dividing the summed length of all LGs by the number of marker intervals in the final linkage map. We estimated the length of each LG *i* as $G_i = M_i + 2s$, where M_i is the map distance between terminal markers of LG *i*. The expected distance between the chromosome end and the terminal marker is *s* under a uniform probability distribution. The number of markers m_i in LG *i* would be a sample from a Poisson distribution with parameter $\lambda_i = mG_i/\Sigma_i G_i$, where *m* is the total number of markers, if the marker density underlying all chromosomes were the same (Remington *et al.* 1999). We evaluated the probabilities $P(X \leq m_i)$ and $P(X \geq m_i)$ under the cumulative Poisson distribution (Remington *et al.* 1999). We estimated the proportion of the genome *c*, within 10 cM, and within 20 cM of a marker, using the formula:

$$c = 1 - e^{2dn/L}$$

where *L* is the estimated genome length, *n* is the number of markers, and *d* is the specified distance, assuming a random marker distribution (Lange & Boehnke 1982).

QTL Mapping

We performed genome-wide interval mapping scans with the *scanone* function in *R/qtl* package to identify genomic regions underlying phenotypic variation in cool and warm thermal environments and thermal plasticity in R 3.2.3 (Broman *et al.* 2003; R Development Core Team 2013). We carried out analyses separately on each trait from each environment, and for trait plasticity. We analyzed reciprocal progeny together and included cytoplasmic origin as an additive covariate. We used 1,000 permutations to determine genome-wide LOD thresholds of p = 0.05 for each trait (Churchill & Doerge 1994).

We used the *makeqtl* and *fitqtl* functions to estimate the genetic architecture of each trait in both thermal environments, and for thermal plasticity. We made all putative QTL peaks with LOD ≥ 3.0 identified by *scanone* into a QTL with *makeqtl*. We used two methods to test the significance of each putative QTL, cytoplasm type (as an additive covariate), and two-way interactions between QTLs and between QTL and cytoplasm type. First, we placed all putative QTLs and the cytoplasm covariate into an additive model containing all main QTL effects and all two-way interactions. The general form of the model was:

$$trait = Q_i + Q_{ii} + Cytoplasm + Q_i * Q_{ii} + Q_i * Cytoplasm + Q_{ii} * Cytoplasm$$

where Q_i= QTL_1 and Q_{ii}= QTL_2. Then we and executed *fitqtl* on the model. We performed an iterative stepwise reduction by removing terms, one at a time, with the highest p-value greater than 0.05. This process was repeated until all terms in the model reached p-values ≤ 0.05. Second we evaluated *fitqtl* models for each trait by iterative stepwise addition. Here we began with only the putative main effect QTLs and cytoplasm terms in the model. We reduced the model until all terms reached p-values ≤ 0.05. Then we added two-way interactions, one at a time, and retained significant terms. To avoid overlooking important interactions when an interaction was identified and added to the model, we also tested each two-way interaction in the model with previously added interactions excluded. Both methods for evaluating the genetic architecture with *fitqtl* models produced the same 'best' genetic architecture model for each trait/environment. The best genetic architecture model was achieved when all model parameters achieved p-values ≤ 0.05. We also retained two secondary models (i.e. for leaf length in cool temperature and germination) that contain parameters with suggestive significance levels ($p < 0.07$) that may be biologically meaningful.

Each QTL that contributed to the genetic architecture was labeled as **[trait].[environment].[LG]** and abbreviated as follows, **trait**: floral reflectance (REF), flowering time (FT), rosette diameter (ROS), leaf length (LL), leaf fresh mass (Mass), leaf area (LA), and germination time (Germination); **environment**: Cool, Warm, or Plasticity; and **LG**: numbered 1-6 from longest to shortest, corresponding to the genetic map (Figure 3.2).

We partitioned each QTL that contributed to the genetic architecture into QTL effects as additive effects, dominance effects, and the difference between the two heterozygous classes using a custom *glm* script in *R* that partitions the effects of one QTL at a time from outcross F_2 data (Remington *et al.* 2013). Additionally, using this script we examined whether cytoplasm contributed a significant additive effect at each QTL locus, and if significant cytoplasm by additive, cytoplasm by dominance, or cytoplasm by difference between heterozygous class interactions were present. We were interested in identifying the magnitude and direction of QTL effects contributing to the genetic architecture of each trait to determine if similar effects were observed between QTLs underlying: 1. thermal plasticity and either thermal environment for each trait, 2. plasticity of different traits and 3. highly correlated traits. We estimated and plotted genotypic means and standard errors of significant QTL by cytoplasm and QTL by QTL interactions with the *effectplot* function in *R/qtl* (Broman *et al.* 2003; R Development Core Team 2013).

Results

Germination

Among all seeds from F_1 and F_2 generations 83% germinated. Percent germination among reciprocal F_1 families ranged from 78-94% and did not show consistent latitudinal differences between families, in agreement with germination data from additional F_1 hybrids derived from European parents (Lacey unpublished data). In the first and second cohort,

respectively, 78% and 88% of F_1s with northern cytoplasm germinated, and 85% and 93% of F_1s with southern cytoplasm germinated. Among the F_2 mapping population 81% of seeds germinated. Percent germination was higher among F_2s with Danish cytoplasm (92%) than F_2s with French cytoplasm (70%).

Phenotypic Patterns of Thermal Plasticity

A. Parents

Parental genotypes differed in thermal plasticity, and patterns differed among traits. Also, with one exception (flowering time) the trait-specific differences in plasticity are explained largely by genotypic variation in cool, but not warm temperature. Thermal plasticity of floral reflectance was greater in northern (i.e. Denmark and Sweden) than in southern parents (i.e. Italy and southern France). All parents produced highly reflective flowers in the warm 'southern' environment, but northern parents significantly reduced reflectance more than did southern parents in the cool 'northern' environment (Tables 3.1, 3.2A-B, Figure 3.3).

This latitudinal difference was also seen when looking at flowering time plasticity, but there were also genotype-specific differences within latitude (Table 3.1). At cool temperature northern parents flowered significantly later than did southern parents (Table 3.1, Table 3.2C). However, in warm temperature the Swedish parent flowered significantly later than did the others, which had similar flowering times (Table 3.2D). Consequently, plasticity was lowest in the Swedish parent, highest in the Danish parent and intermediate in southern parents (Table 3.1).

Parents also differed in vegetative traits. Plasticity in both rosette diameter and leaf length showed a latitudinal pattern similar to that for floral reflectance. In warm temperature, all parents grew similarly sized rosettes and leaves of similar length, but at cool temperature, northern parents reduced rosette size and leaf length significantly more than did southern parents (Tables 3.1, 3.2E-H). The southern parents displayed negligible temperature sensitivity.

Parental genotypes showed a latitudinal difference in plasticity for leaf fresh mass and leaf area, in that northern and French parents produced similar leaf fresh mass and area at warm temperature, but only the northern parents reduced mass and area at cool temperature (Table 3.2I-L). However, there were again genotype-specific differences within latitude. The leaf fresh mass and area for the Italian parent was much greater than for other parents at both temperatures, and was approximately 50% greater at cool temperature than at warm temperature. Thus, although the magnitude of thermal plasticity in the Italian parent resembled that for northern parents, the environmental effect was in the opposite direction.

B. F_2 Genotypes

Heritability was found for the thermal response of each trait (Table S3.2). For all traits we examined, mean plasticity of F_2s was near the mid-parent value and F_2 distributions were wider than the phenotypic range of the parents (Figure 3.4). Most F_2s (>99%) reduced reflectance and delayed flowering in cool relative to warm temperature, similar to parental genotypes (Table 3.1, Figure 3.4A-F). The majority of F_2s had smaller rosette diameters (76%) and produced shorter leaves (82%) in cool relative to warm temperature like northern parents. The remainder either responded to temperature in the opposite direction producing larger rosettes (15%) and longer leaves (10%) in cool temperature, or displayed negligible thermal responses for these traits (i.e. plasticities <1 cm in rosette diameter, <0.5 cm in leaf length; Figure 3.4G-L). Most F_2s responded to temperature by also reducing leaf fresh mass (61%) and leaf area (55%) in cold relative to warm temperature, as did northern parents. Of those that did not, more produced leaves with greater fresh mass (34%) and area (35%) at cold temperature, similar to the Italian parent. A very few F_2s displayed negligible thermal sensitivity (i.e. plasticities <0.01 g in mass, <0.5 cm^2 in area) similar to the French parent (Figure 3.4M-R).

C. F_2 Genotypic Correlations

In the F_2 population, significant genotypic correlations were found between thermal plasticity of each trait and trait values in both cool and warm temperature (Table 3.3). However, the values of the correlations were strong for only a few cases. Thermal plasticity of floral reflectance was unique in that it was very strongly negatively correlated with trait values in cool temperature ($r = - 0.99$, Table 3.3) and only weakly correlated with trait values in warm temperature. Thus, thermal plasticity in floral reflectance was primarily driven by decreased reflectance in the cool environment. The correlation between plasticity of flowering time and its trait value in cool temperature was also higher than with the trait value at warm temperature, though the correlation (- 0.61) was lower than for reflectance. Vegetative traits showed the opposite pattern. Correlations ($r > 0.50$) were higher between plasticity and trait values in warm temperature (Table 3.3). Thus, trait variation in the thermal environments contributed to variation in thermal plasticity differently among flowering and vegetative traits. Correlations between reproductive and vegetative plasticities were very weak (absolute values of coefficients < 0.11).

All vegetative traits were highly correlated with each other in each thermal environment, and all correlation coefficients among thermal plasticities of vegetative traits were greater than 0.51, indicating that leaf traits responded to temperature similarly (Table 3.3). Thus, F_2 individuals resembled the parents in that F_2s with longer leaves had greater leaf mass, leaf area, and larger rosettes (Table 3.3).

Genotypic correlations among germination time with reproductive and vegetative traits were also detected. Although weak, correlations indicated that late germinating individuals were likely to flower earlier at both temperatures and display higher reflectance in warm temperature (Table 3.3). Late germinating individuals also grew larger and were likely to display thermal sensitivity in rosette diameter and leaf length (Table 3.3).

Genetic Mapping

Illumina sequencing of the double digest restriction-site associated DNA sequencing (ddRADseq) libraries produced 69K - 2.7M reads in F_0 parents with mean coverage of 13 - 31x, 24K – 1M reads at 13-29x in F_1 parents, and a mean of 597K reads at 16x coverage in F_2s (see Table S3.3). Using the bioinformatics processing steps in Stacks (see Supplementary Laboratory Methods for details) produced 11,295 haplotypes from forward reads and 10,387 haplotypes from reverse reads. Filtering out markers that 1) could not be traced to F_0 parents, 2) did not display allelic differences between F_1 parents, 3) were scored in less than 70% of the 118 F_2s with highest coverage, and 4) had segregation ratio p-values ≤ 0.0001, reduced the number of markers to 555. Of the 555 markers used for genetic mapping, 232 displayed segregation ratio $p>0.05$, 122 $0.05>p>0.01$, and 201 markers with segregation p $0.01>p>0.0001$. Additionally, the 555 markers represented 3 segregation patterns, 3 displayed segregation in 1:1:1:1 ratios (type A), 426 segregated in 1:2:1 ratios (type B), and 126 segregated in 1:1 ratios (type D) (Margarido et al. 2007). During recombination mapping, genetic markers were excluded that could not be mapped to a single unique position as indicated by map expansion and/or a monotonic increase in recombination frequency with distance (see Methods).

The final genetic linkage map consists of 47 markers along 6 linkage groups with a combined length of 415.1 cM Kosambi (Figure 3.2). One of the 47 mapped genetic markers was type A with a 1:1:1:1 segregation pattern, 36 were type B with 1:2:1 segregation patterns, and 10 were type D with 1:1 segregation patterns (Margarido *et al.* 2007; R Development Core Team 2013, Figure 3.2B). Markers with skewed segregation ratios tended to cluster together, and one of the six linkage groups (LG 3) consisted entirely of highly skewed markers (Figure 3.2B).

Our final linkage map has average marker spacing of 10.1 cM, which is ideal spacing to maximize the resolving power of a marker-QTL linkage experiment (Darvasi *et al.* 1993).

Assuming markers are evenly spaced and each linkage group corresponds to a single chromosome, the average distance between chromosome ends and terminal markers equal the average marker spacing, 10.1 cM. These assumptions provide an estimated map length of 536.3cM. Our statistical tests of marker distribution among linkage groups compared the number of markers on each linkage group m_i to the expected number of markers based upon linkage group length as $\lambda_i = 47G_i/ 536.3$. We did not detect significant differences in marker density among linkage groups, Poisson probabilities for deviations of m_i from λ_i in either direction were greater than 0.329 (Table S3.4). Using the formula $c = 1 - e^{-2dn/L}$ (see Materials and Methods) and estimating L as 536.3 cM, an estimated 82.7% of the genome is within 10 cM of a genetic marker, and 97.0% is within 20 cM (Lange and Boehnke 1982).

QTL Mapping

Plasticity QTLs

Each trait produced genome-wide scans that were unique between temperature treatments, and between each temperature treatment and plasticity (Figure 3.5). We found one trait-specific QTL underlying thermal plasticity in each of three traits: reflectance (REF.Plasticity.6), flowering time (FT.Plasticity.2) and leaf length (LL.Plasticity.3), which explained 17.6%, 5.1%, and 2.8% of the F_2 variation in thermal plasticity of these traits, respectively (Table 3.4A, D, J). The QTLs underlying reflectance plasticity and flowering time plasticity had corresponding single-environment QTLs with similar effects in cool, but not warm temperature (Figure 3.6). Physical locations of thermal plasticity QTLs and corresponding single-environment QTLs overlapped perfectly for floral reflectance (REF.Plasticity.6 and REF.Cool.6), and were 4 cM apart and within the same primary QTL peak for flowering time (FT.Plasticity.2 and FT.Cool.2). Furthermore, variation in thermal plasticity of F_2s displayed a higher correlation with trait variation in the thermal environment where the corresponding single-environment QTL

was found (i.e. cool temperature) than with trait variation in the warm thermal environment (Table 3.3).

All three plasticity QTLs displayed significant additive effects (Figure 3.7). At these QTL the additive effect (2a) of substituting both northern alleles for southern alleles increased the magnitude of plasticity in the same direction as was observed in the northern relative to the southern parents. At the location of REF.Plasticity.6 (and REF.Cool.6) northern alleles increased the magnitude of thermal plasticity in floral reflectance (i.e. percent of light reflected at 850 nm) by 18.5% relative to southern alleles, which represented 78% of the difference between mean trait values of northern vs. southern parents (Figure 3.7). Likewise, these northern alleles reduced reflectance in cool temperature. At the QTL location of FT.Plasticity.2 the effect of substituting both northern alleles increased the magnitude of flowering time plasticity (i.e., delayed flowering onset) by 16 days, i.e., 2a= 203% of the difference between parents (Figure 3.7). Nearby, at QTL FT.Cool.2, northern alleles delayed flowering in cool temperature by nearly the same amount of time. The effect of substituting both northern alleles at the location of LL.Plasticity.3 increased the thermal response of leaf length by 2.5 cm, 64% of the mean difference between northern vs. southern parents (Figure 3.7).

In addition to additive effects, two other types of QTL effects were found at plasticity QTLs. The QTLs underlying thermal plasticity of floral reflectance and flowering time plasticity displayed a significant difference between heterozygote classes, indicating that allelic effects at these loci were genotype-specific (Figure 3.7). Heterozygotes with Swedish/Italian genotypes at the locus where the floral reflectance plasticity QTL and cool reflectance QTL colocalized produced darker, less reflective flowers in cool temperature and exhibited greater thermal plasticity for floral reflectance than did Danish/French heterozygotes. At both the flowering time plasticity QTL and cool flowering time QTL Danish/French heterozygotes flowered later in cool

temperature and exhibited greater thermal plasticity for flowering time than Swedish/Italian heterozygotes. We did not identify significant effects of cytoplasm type at QTLs underlying floral reflectance plasticity or flowering time plasticity. The leaf length plasticity QTL did display a significant effect of cytoplasm type. The northern Danish cytoplasm increased leaf length plasticity compared to the southern French cytoplasm (Figure 3.7). We did not find evidence of dominance for any plasticity QTLs.

Environment-Specific Genetic Architecture

By growing clones of identical genotypes in two discrete thermal environments to examine the genetic architecture of thermal plasticity, this study offered us the opportunity to compare genetic architectures of traits measured on the same genotypes across environments. In each trait we examined, the genetic architecture underlying trait variation was environment-specific (Figure 3.7). Among all traits we found a total of 18 QTLs in the cool environment and 20 QTLs in the warm environment. Of these, we identified only one pair of QTLs (LL.Cool.4/LL.Warm.4a) that displayed identical effects in the same direction *and* colocalized in both thermal environments (Figures 3.6, 3.7). Eight other pairs of QTLs across environments that either colocalized to the same physical location (2 pairs), or fell within the same QTL peak (6 pairs), shared some QTL effects but were not identical (Figures 3.5-3.7).

We did not identify significant effects of cytoplasm type at QTLs underlying floral reflectance or flowering time from either thermal environment, or their thermal plasticities. Among vegetative traits we found 4/14 QTLs in cool temperature and 18/18 QTLs in warm temperature possessed significant effects of cytoplasm type. Furthermore, the effect of cytoplasm was consistent at all 22 of these QTLs, the Danish cytoplasm increased rosette diameter and leaf size relative to the French cytoplasm (Figure 3.7). In addition, all four QTLs underlying

germination time exhibited significant effects of cytoplasm type where the Danish cytoplasm accelerated germination relative to the French cytoplasm (Figure 3.7).

We found six significant nuclear QTL by cytoplasm interactions. Of these, four were identified at the genome-wide level with *fitqtl models* (Figure 3.8A), two with single QTL *a-d-i models* (Figure 3.8B), and only one (FT.Cool.4) was significant in both analytical methods. In each of the five QTLs where interactions were significant in only one method, single QTL *a-d-i models* found a significant effect of cytoplasm type (Figure 3.7). The two QTLs where the nuclear by cytoplasm interaction was identified in *a-d-i models* only (LL.Warm.3 and ROS.Warm.3) exhibited complex effects. At these QTLs, significant interactions were found between cytoplasm type and both the additive effect, and the difference between heterozygote effect (Figure 3.7). The QTL where the nuclear by cytoplasm effect was identified with both models, FT.Cool.4 was unique. Here the entire QTL effect was driven by an interaction between cytoplasm type and the difference between heterozygote classes (Figure 3.8A). At this locus F_2s with Danish/French alleles flowered 10.7 days later than F_2s with Swedish/Italian alleles, but only in the cytoplasmic background from the French maternal line (Figure 3.8A).

In two traits, leaf area in cool temperature and leaf fresh mass in warm temperature, we found a significant interaction between nuclear QTL (Figure 3.9). These nuclear interactions were complex and did not produce meaningful biological conclusions about the underlying effects of specific alleles.

Discussion

This study was incredibly useful for illuminating the genetic architecture underlying the thermal plasticities of multiple traits in a perennial herb with few available genetic resources. The use of multiple clonal replicates of parent and offspring genotypes allowed us to reduce

environmental noise while phenotyping, improving our measures of genotypic responses to temperature. We used a genotyping by sequencing approach to produce genetic markers and assemble a linkage map with 47 markers evenly spaced along 6 linkage groups with an average marker spacing of 10.1 cM. This was ideal spacing to maximize the resolving power of our marker-QTL linkage experiment (Darvasi *et al.* 1993).

Plasticity QTLs

Our identification and characterization of thermal plasticity QTLs demonstrate that thermal plasticity in *P. lanceolata* has a genetic basis. This finding is consistent with temperature-sensitivity of flowering time in *A. thaliana* (Méndez-Vigo *et al.* 2016), and phenotypic plasticity of plants in general (Bloomer *et al.* 2014; Hausmann *et al.* 2005; Kliebenstein *et al.* 2002; Lacaze *et al.* 2009; Ungerer *et al.* 2003). The phenotypic patterns and genetic architectures we found underlying thermal plasticities and single environment trait values were trait-specific. We found one single QTL in each of the genetic architectures underlying the thermal plasticities of three traits, floral reflectance, flowering time and leaf length, although no QTLs for thermal plasticities of rosette diameter, leaf mass or leaf area.

We found genotypic differences at plasticity QTLs paralleled phenotypic patterns of thermal plasticity along latitudinal clines that are consistent with local adaptation. Genotypes from northern (Danish and Swedish) populations increased the magnitude of thermal plasticity, while genotypes from southern (French and Italian) populations decreased plasticity. Latitudinal clines of higher thermal plasticity in higher latitude environments appear common, and have been reported for thermal plasticity of a multitude of traits in diverse taxa, e.g. developmental rate in frogs (Laugen *et al.* 2003), body size in flies (Liefting *et al.* 2009), thermal tolerance in insects and lizards (Addo-Bediako *et al.* 2000; Ghalambor *et al.* 2006), leaf shape in trees (Royer *et al.* 2009), flower/seed number in dandelions (Molina-Montenegro & Naya 2012). However, our

study is, to the best of our knowledge, the first to report geographic patterns of genetic information that parallel phenotypic patterns of thermal plasticity.

Genetic correlations among thermal plasticities of floral reflectance, flowering time, and leaf length were weak and plasticity QTLs did not colocalize. Thus, thermal responses of these traits are at least partially, genetically unique. This implies thermal responses of these traits may be free to evolve independently along the European latitudinal gradient. Similar results have been found in *A. thaliana* where QTLs underlying thermal plasticities of rosette diameter and fitness (# of fruits) did not overlap (Springate & Kover 2014), and in *Caenorhabditis elegans* where thermal plasticity QTLs underlying age at maturity, fertility, growth rate, and egg size were all unique except for a single QTL that colocalized for plasticities of age at maturity and growth rate (Gutteling *et al.* 2006). Perhaps we might expect seemingly unrelated traits to show little genetic commonality to temperature sensitivity because temperature has such a strong influence on physiology and thermal fluctuations are so common.

Although most QTL mapping studies that examine plasticity in plants have focused only on the plastic response of a single trait (Bloomer *et al.* 2014; Hausmann *et al.* 2005; Kliebenstein *et al.* 2002; Ungerer *et al.* 2003), some studies include plastic responses of multiple traits (Lacaze *et al.* 2009; Méndez-Vigo *et al.* 2016). In contrast to our finding that genetic architectures underlying thermal plasticities of different traits were unique, plastic responses of different traits to other environmental variables appear to share a significant amount of genetic control. For example, in *A. thaliana* QTLs underlying photoperiod plasticity of flowering time and leaf number colocalized at 2 of 3 loci, and 3 of 4 QTLs underlying vernalization plasticity of flowering time and leaf number colocalized (Méndez-Vigo *et al.* 2016). Likewise, in barley, a QTL analysis of plasticity across 22 U.S. environments found about half of the plasticity QTLs underlying grain yield and thousand kernel weight colocalized, although it is not clear which

environmental variables caused the plastic response (Lacaze *et al.* 2009). Similarly, multiple QTLs underlying plasticity across 7 U.S. environments of grain yield and grain protein content colocalized (Lacaze *et al.* 2009).

Additive effects of plasticity QTLs explained much of the difference between mean trait values of northern and southern parents (2a = 78%, 203% and 64%, respectively), but a comparatively small proportion of the phenotypic variation among F_2s (18%, 5% and 3%, respectively). Additionally, F_2s displayed greater phenotypic variation of thermal plasticity than parental genotypes. These results suggest the variation in thermal plasticity of these traits is controlled by the QTL we detected and additional smaller effect loci we did not detect (Castle 1921; Lande 1981; Wright 1968). Our sample size may have limited our ability to detect QTLs with small phenotypic effects (Beavis 1994; Beavis 1998).

Floral Reflectance Plasticity

We found strong evidence that at least one large effect temperature-sensitive QTL drives floral reflectance plasticity by reducing reflectance in cool temperature. Our data show a single QTL on linkage group 6 underlies both plasticity and reflectance at cool temperature. This QTL displayed similar phenotypic effects. Thermal plasticity of floral reflectance and reflectance at cool temperature exhibited an extremely strong correlation (r= -0.99) indicating that the cool temperature trait values primarily drove the plastic response.

These results provide critical genetic support for the hypothesis that temperature-sensitive floral reflectance plasticity in *P. lanceolata* is adaptive in high latitude thermally variable environments where growing seasons are cooler and shorter than at low latitude. Northern genotypes increased the magnitude of plasticity relative to southern genotypes to nearly the same degree as they reduced reflectance in cool temperature. We add to evidence from earlier studies that is also consistent with the adaptive thermal plasticity hypothesis:

1) Floral reflectance plasticity displays significant positive correlations with latitude and altitude among European populations in the native range of *P. lanceolata* (Lacey *et al.* 2010). Moreover, the proportion of cool temperature during the reproductive season and season duration, but not the magnitude of thermal variation, best explain the geographic distribution of floral reflectance plasticity variation among European populations.
2) Patterns of neutral genetic differentiation and phenotypic differentiation strongly suggest divergence of floral reflectance plasticity in European populations has been influenced by natural selection. The proportion of the reproductive season at cool temperatures and the reproductive season duration, but not the magnitude of thermal variation, appear to have driven phenotypic divergence of floral reflectance plasticity, with higher plasticity favored in cooler and shorter reproductive seasons (Marshall *et al.* In Prep.).
3) Floral reflectance plasticity provides individual plants with the ability to partially thermoregulate flowering spikes that house delicate reproductive tissues and developing offspring because darker, poorly reflective flowers are warmer than highly reflective flowers (Lacey & Herr 2005).
4) Floral reflectance plasticity is likely to improve fitness because offspring developed in warmer temperatures display higher fitness, e.g. offspring exhibit greater germination, probability of flowering, seed set when developed in warm temperature compared with those developed in cool temperature (Lacey & Herr 2000).
5) Cool environments that limit physiological performance favor individuals who produce flowers with reduced reflectance (Lacey *et al.* 2012), e.g. cellular respiration in *P. lanceolata* is primarily temperature limited below 15ºC (Covey-Crump *et al.* 2002). In warmer environments, individuals benefit from producing highly reflective flowers which helps cool reproductive tissues (Lacey *et al.* 2012).

This study adds two important genetic components to this body of research. We found genetic evidence that there are genes underlying floral reflectance plasticity. Additionally, we found that phenotypic variation in floral reflectance plasticity between northern and southern genotypes is explained by genetic differences in the large effect QTL underlying reflectance plasticity.

Also, we found genotype-specific effects (i.e. significant differences between F_2 heterozygotes) at the floral reflectance plasticity QTL. There was evidence of within-latitude allelic variation. The data may reflect local adaptation between populations within the northern and/or southern region(s). For example, multiple population-specific alleles at the floral reflectance plasticity gene could represent an allelic series. Allelic variation at this locus may explain latitudinal variation. For example, a similar thermal response occurs in *Petunia* flowers (i.e. anthocyanin accumulation in cool vs. warm developed flowers) and continuous variation of flower color results from an allelic series at the anthocyanin 1 regulatory gene that promotes anthocyanin biosynthesis (Gerats *et al.* 1984).

Flowering Time Plasticity

Our data suggest a single QTL acts to increase flowering time plasticity by delaying flowering in cool temperature. The genetic architectures underlying flowering time plasticity and flowering time in each thermal environment were unique. The LOD profiles of linkage group 2 were similar for flowering time plasticity and flowering time in the cool environment and the plasticity QTL we detected for flowering time was very close (4 cM) to the location of the cool environment QTL on linkage group 2 (Figure 3.6). In both cool temperature, and for plasticity this QTL exhibited similar additive effects. Substituting northern for southern genotypes increased the magnitude of flowering time plasticity and delayed flowering in cool temperature, 2a = ~16 days. This represents a very large delay in flowering time, especially when compared

with findings from other plant studies! For example, a recent study examining latitudinal variation of flowering time in *A. thaliana* found substitution of the Swedish for the Italian genotype at the largest effect QTL (at *flowering locus C* (FLC)) delayed flowering in the cool and warm environments by 3.8 and 2.7 days, respectively (Dittmar *et al.* 2014). Furthermore, substitution of the Swedish for the Italian genotype at all 3 significant QTLs in the cool environment delayed flowering by a total of 8.4 days, and the same substitution at all 9 significant QTLs in the warm environment delayed flowering by 11.82 days in total (Dittmar *et al.* 2014). In a study of *Boechera stricta* hybrids derived from Montana and Colorado parents, homozygotes for the Montana allele accelerated flowering in Montana by 2.2 days (Anderson *et al.* 2011).

Flowering time in warm temperature was significantly correlated with cool temperature flowering time and flowering time plasticity. However, the genetic architecture underlying flowering time in warm temperature did not contain a QTL on linkage group 2. In the only other QTL study that we have found addressing thermal plasticity of flowering time, a similar pattern was found for two of three QTLs in *A. thaliana* (Méndez-Vigo *et al.* 2016). One plasticity QTL at FLC colocalized with QTLs in both thermal environments, and two other plasticity QTLs colocalized with a QTL in only one of the two thermal environments, near *FRIGIDA* (*FRI*) at 21ºC and near *ELF3* at 28ºC (Méndez-Vigo *et al.* 2016).

We did not find temperature-sensitivity of flowering time to differ systematically with latitude. Instead, we found the Danish parent displayed the strongest response to temperature, the Italian and French parents displayed intermediate flowering time plasticities, and the Swedish parent exhibited very little plasticity. Differences between heterozygotes matched the pattern of differences between northern parents. Danish/French heterozygotes flowered later in cool temperature and exhibited greater thermal plasticity for flowering time than Swedish/Italian heterozygotes. Therefore it is likely differences in flowering time plasticity between

heterozygotes were driven by the low temperature sensitivity of the Swedish genotype that exhibited constitutively late flowering. Likewise, a recent QTL mapping study of *A. thaliana* concluded flowering time variation may be more important in Italy than Sweden (Dittmar *et al.* 2014). Although we might expect a latitudinal cline in flowering time to have resulted from the systematic variation of environmental cues (Botto & Smith 2002; Karlsson *et al.* 1993)., our finding is consistent with flowering time variation in *A. thaliana* that, overall, lacks evidence of a latitudinal cline (Nordborg & Bergelson 1999; Stinchcombe *et al.* 2004). Instead, only in a subset of genotypes with functional copies of the temperature sensitive gene *FRI* does a significant latitudinal cline emerge, where individuals from more southern locations flower earlier than do individuals of northern origin (Stinchcombe *et al.* 2004).

Leaf Length Plasticity

We found a QTL underlying thermal plasticity in leaf length that differed in two basic ways from the plasticity QTLs underlying reflectance and flowering time. 1) The leaf length plasticity QTL did not colocalize with leaf length QTL peaks from either thermal environment. 2) In addition to the additive effect, this plasticity QTL displayed a significant effect of cytoplasm type. Both the additive and cytoplasmic effects at this plasticity QTL are consistent with the geographic pattern predicted by the hypothesis that thermal plasticity is adaptive in northern environments. Northern genotypes at this QTL increased plasticity relative to southern genotypes, and the northern (Danish) cytoplasm increased plasticity relative to the southern (French) cytoplasm independent of the nuclear genotype at this locus. This finding suggests that cytoplasmic organelles play a role in influencing leaf elongation in response to temperature. However, it remains unclear whether the observed thermal response to leaf elongation results from cytoplasmic genes (e.g. mitochondria and chloroplasts), or altered nuclear gene expression because cytoplasmic organelles play a role in the regulation of nuclear gene expression through

overlapping signaling pathways (Rhoads 2011). For example, expression of the mitochondrial enzyme *alternative oxidase, AOX*, a nuclear gene, is regulated by the mitochondria (Vanlerberghe 2013, Vanlerberghe 1996). Furthermore, temperature can influence this relationship. In multiple forb and grass species including *P. lanceolata*, leaves grown in cool temperature produce greater leaf *AOX* content than leaves grown in warm temperature (Umbach et al. 2009, Campbell et al 2007).

In addition to the differences we found in the magnitude of thermal plasticity of leaves, the directionality of the thermal response was also not uniform. While most plants grew smaller rosette diameters and leaves at cool temperature, many plants increased rosette and leaf length (Figure 3.4G, J, M, P). When considering the effect of temperature on leaf shape, data show that northern parents produced shorter leaves in cool relative to warm temperature, which led to a smaller rosette diameter, leaf area, and fresh mass. Leaf shape in the French parent was largely temperature-insensitive. The Italian parent produced leaves of the same length but which had a larger area and mass in cool temperature. This suggests that they also produced wider leaves at cool temperature. These findings complement many prior studies of *P. lanceolata* reporting variation for plasticity that appears to be maintained by different selective pressures in contrasting habitats (Herrera 2013; Lacey *et al.* 2012; Lacey *et al.* 2010; Van Tienderen 1990; Wolff 1988; Wolff & Van Delden 1987).

Cytonuclear Interactions Affecting Environment-Specific Phenotypes

Cytoplasmic variation can have large effects on phenotypic variation (Joseph *et al.* 2013). We also found significant phenotypic effects of cytoplasm type on the majority of the single-environment QTLs underlying vegetative trait variation. Furthermore, our data showed the direction of these cytoplasmic effects were consistent across environments, e.g. in both cool and warm temperature the Danish cytoplasm increased leaf length, rosette diameter, leaf mass and

leaf area, and decreased germination time relative to the French cytoplasm (Figure 3.7). There is evidence that cytoplasmic genomes can improve local fitness (Campbell *et al.* 2008; Galloway & Fenster 2001; Kimball *et al.* 2008; Leinonen *et al.* 2011; Sambatti *et al.* 2008).

In addition to simple cytoplasmic effects affecting trait variation, we also found some evidence of cytonuclear interactions. For example, the entire phenotypic effect of one QTL we found underlying cool temperature variation of flowering time (FT.Cool.4, Figure 3.8A) was determined by strong epistasis between cytoplasm type and the nuclear genotype. While the Danish cytoplasm displayed little variation between nuclear genotypes, a large genotype-specific difference occurred in the French cytoplasmic background. In the French cytoplasm, heterozygotes with Danish/French nuclear alleles delayed flowering by ~11 days relative to Italian/Swedish heterozygotes (Table S3.5E). This result is consistent with coevolution of the cytoplasmic and nuclear genomes acting to influence variation in flowering time, an important fitness related trait (Rand *et al.* 2004). Recent studies have found evidence of cytonuclear interactions with large effects on phenotypic variation e.g. plant and ear height in maize (Tang *et al.* 2013), fitness in *A.* lyrata (Leinonen *et al.* 2013), and cytonuclear incompatibilities appear in divergent eukaryote taxa from yeast (Chou *et al.* 2010), to plants (Fishman & Willis 2006; Sambatti *et al.* 2008) and animals (Gagnaire *et al.* 2012; Niehuis *et al.* 2008). Moreover, a previous study found both i) cytonuclear interactions that were consistent with coadaptation of nuclear and cytoplasmic genomes (i.e. local alleles increased fitness only when combined with local cytoplasm), and ii) other interactions that instead reduced fitness when local nuclear and cytoplasmic genes were combined (Leinonen *et al.* 2013). Cytoplasmic genomes may serve as new sources of variation to accelerate evolutionary changes because they can modify the magnitude of QTLs controlling trait variation, and thus, gene networks (Roux *et al.* 2016; Soltani *et al.* 2016). Yet, despite their importance, the genetic mechanisms underlying cytonuclear

interactions remain unknown. Further inquiry and new interdisciplinary studies are needed to determine the role of cytoplasmic genomes in adaptation (Bock *et al.* 2014; Budar & Roux 2011; Roux *et al.* 2016; Soltani *et al.* 2016).

Table 3.1 Phenotypic Means of F_0 and F_1 Genotypes used to Produce F_2 Mapping Population, and Means of F_2 Family Shown Together (All) and Separated by Cytoplasm, with Number of Genotypes Measured for Each Trait (N). The phenotypic mean of each genotype was estimated by averaging replicated clones. Plasticity values were calculated as the difference between mean phenotype in warm minus cool. Absolute percent plasticity relative to warm temperature is presented in italics.

Genotype		**Sweden**	**Denmark**	**France**	**Italy**	**Denmark x Italy**	**France x Sweden**						
Generation		F_0	F_0	F_0	F_0	F_1	F_1	F_2					
# clones in Cool		4	8[a]	6	6	8	6	All F_2s		Danish Cytoplasm F_2s		French Cytoplasm F_2s	
# clones in Warm		2	2	5	5	7	5						
Trait	Environment	Mean	Mean	Mean	Mean	Mean	Mean	Mean	N	Mean	N	Mean	N
Flowering Time (days)	Cool	90	69	34	30	45	76	73	446	72	241	73	205
	Warm	89	21	16	15	16	19	24	449	24	243	25	206
	Plasticity	-1.5 *2%*	-48.3 *230%*	-18.6 *116%*	-15.3 *102%*	-29 *181%*	-57 *300%*	-48 *200%*	439	-48 *200%*	236	-48 *192%*	203
Floral Reflectance (%)	Cool	59.41	55.65	85.98	78.45	70.86	62.29	68.08	443	67.90	239	68.28	204
	Warm	91.01	91.25	91.77	92.41	92.69	90.15	90.95	446	90.91	242	90.99	204
	Plasticity	31.60 *35%*	35.60 *39%*	5.79 *6%*	13.96 *15%*	21.83 *24%*	27.85 *31%*	22.94 *25%*	436	23.03 *25%*	236	22.83 *25%*	200
Rosette Diameter (cm)	Cool	10.1	9.6	18.3	21.0	20.0	12.7	17.2	441	17.3	240	17.2	201
	Warm	15.8	16.4	19.0	21.5	17.9	16.9	21.6	443	22.3	240	20.7	203
	Plasticity	5.7 *36%*	6.8 *41%*	0.7 *4%*	0.5 *2%*	-2.1 *12%*	4.2 *25%*	4.3 *20%*	433	4.9 *22%*	236	3.5 *17%*	197
Leaf Length (cm)	Cool	5.8	6.2	10.0	12.7	11.8	6.9	10.5	441	10.7	240	10.3	201
	Warm	10.7	11.2	11.8	12.9	12.3	9.9	13.5	443	14.1	240	12.9	203
	Plasticity	4.9 *46%*	5.0 *45%*	1.8 *15%*	0.1 *1%*	0.5 *4%*	3.1 *31%*	3.0 *22%*	433	3.4 *24%*	236	2.5 *19%*	197
Fresh Mass (g)	Cool	0.09	0.17	0.28	0.67	0.61	0.14	0.44	441	0.46	240	0.42	201
	Warm	0.21	0.28	0.25	0.47	0.48	0.25	0.50	443	0.55	240	0.45	203
	Plasticity	0.12 *57%*	0.10 *36%*	-0.03 *12%*	-0.21 *45%*	-0.13 *27%*	0.11 *44%*	0.06 *12%*	433	0.09 *16%*	236	0.02 *4%*	197
Leaf Area (cm^3)	Cool	3.31	5.28	6.51	15.99	15.67	4.33	11.98	441	12.45	240	11.40	201
	Warm	7.73	9.54	6.21	10.45	13.01	6.63	13.12	442	14.21	239	11.85	203
	Plasticity	4.42 *57%*	4.27 *45%*	-0.30 *5%*	-5.55 *53%*	-2.66 *20%*	2.30 *35%*	1.11 *8%*	432	1.70 *12%*	235	0.40 *3%*	197
Germination	-	-	-	-	-	7	6	11	441	11	240	12	201

[a] 5 clones were used to estimate % reflectance at 850nm in Cool

[b] Under 20ºC, 8H day/15ºC, 16H night

Table 3.2 Analysis of Variance and Tukey's Post Hoc Test Results Comparing Phenotypes of Parental Genotypes from Denmark (D), Sweden (S), France (F), and Italy (I) Grown in Cool and Warm Temperature Environments. In each thermal environment, each genotype was represented by multiple clones. Phenotypes measured are flowering time (FT), floral reflectance (REF), rosette diameter (ROS), leaf length (LL), leaf fresh mass (Mass), and leaf area (LA). Column headers indicate degrees of freedom (d.f.), sum of squares (Sum Sq.), mean square (Mean Sq.), F statistic (F), p-value (p), mean difference between genotypes (diff.), lower bound 95% CI (lwr.), and upper bound 95% CI (upr.). QTLs are labeled as **[trait].[environment].[LG]**, **trait**: floral reflectance (REF), flowering time (FT), rosette diameter (ROS), leaf length (LL), leaf fresh mass (Mass), leaf area (LA), and germination time (Germination); **environment**: Cool, Warm, or Plasticity; and **LG**: numbered 1-6.

	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
A. REF.Cool ~ Genotype	3.00	3389.00	1129.60	27.28	1.00E-06***
Residuals	17.00	704.00	41.40		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**
	D - I	-22.80	-33.88	-11.72	1.04E-04***
	F - I	7.53	-3.03	18.09	0.217
	S - I	-19.04	-30.85	-7.23	0.001 **
	F - D	30.33	19.25	41.41	2.90E-06***
	S - D	3.76	-8.51	16.03	0.819
	S - F	-26.57	-38.38	-14.76	3.61E-05***
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
B. REF.Warm ~ Genotype	3.00	3.73	1.24	0.91	0.472
Residuals	10.00	13.72	1.37		
C. FT.Cool ~ Genotype	3.00	12253.00	4084.00	10.08	3.43E-04***
Residuals	19.00	7696.00	405.00		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**
	D - I	39.25	6.99	71.51	0.014 *
	F - I	4.17	-30.10	38.43	0.986
	S - I	60.00	22.04	97.96	0.001 **
	F - D	-35.08	-65.65	-4.52	0.021 *
	S - D	20.75	-13.91	55.41	0.359
	S - F	55.83	19.30	92.36	0.002 **
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
D. FT.Warm ~ Genotype	3.00	8885.00	2961.50	113.70	1.79E-07***
Residuals	9.00	234.00	26.10		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**
	D - I	6.25	-7.55	20.05	0.522
	F - I	0.85	-9.84	11.54	0.994
	S - I	73.75	59.95	87.55	2.00E-07***
	F - D	-5.40	-18.73	7.93	0.605
	S - D	67.50	51.57	83.43	1.60E-06***
	S - F	72.90	59.57	86.23	2.00E-07***
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
E. ROS.Cool ~ Genotype	3.00	533.90	177.97	12.73	8.58E-05***
Residuals	19.00	265.60	13.98		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**

	D - I	-11.34	-17.78	-4.90	4.73E-04***
	F - I	-2.66	-9.44	4.13	0.693
	S - I	-10.86	-17.91	-3.80	0.002 **
	F - D	8.68	3.00	14.36	0.002 **
	S - D	0.48	-5.51	6.48	0.996
	S - F	-8.20	-14.56	-1.83	0.009 **
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
F. ROS.Warm ~ Genotype	3.00	58.34	19.45	1.35	0.319
Residuals	9.00	129.74	14.41		
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
G. LL.Cool ~ Genotype	3.00	162.40	54.15	21.48	2.53E-06***
Residuals	19.00	47.90	2.52		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**
	D - I	-6.55	-9.28	-3.82	1.08E-05***
	F - I	-2.73	-5.61	0.16	0.068
	S - I	-6.91	-9.90	-3.91	1.81E-05***
	F - D	3.83	1.41	6.24	0.001 **
	S - D	-0.36	-2.90	2.19	0.979
	S - F	-4.18	-6.88	-1.48	0.002 **
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
H. LL.Warm ~ Genotype	3.00	7.74	2.58	0.32	0.808
Residuals	9.00	71.58	7.95		
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
I. Mass.Cool ~ Genotype	3.00	0.88	0.29	18.08	8.56E-06***
Residuals	19.00	0.31	0.02		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**
	D - I	-0.50	-0.72	-0.28	2.28E-05***
	F - I	-0.39	-0.62	-0.16	0.001 **
	S - I	-0.58	-0.82	-0.34	9.50E-06***
	F - D	0.11	-0.08	0.30	0.413
	S - D	-0.08	-0.29	0.12	0.659
	S - F	-0.19	-0.41	0.02	0.092
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
J. Mass.Warm ~ Genotype	3.00	0.14	0.05	1.86	0.207
Residuals	9.00	0.22	0.02		
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
K. LA.Cool ~ Genotype	3.00	416.80	138.92	17.35	1.14E-05***
Residuals	19.00	152.10	8.01		
Tukey's post hoc		**diff.**	**lwr.**	**upr.**	**p**
	D - I	-10.72	-15.59	-5.85	3.34E-05***
	F - I	-9.48	-14.62	-4.35	2.79E-04***
	S - I	-12.68	-18.02	-7.34	1.21E-05***
	F - D	1.24	-3.06	5.53	0.850
	S - D	-1.96	-6.50	2.57	0.624
	S - F	-3.20	-8.02	1.62	0.275
	d.f.	**Sum Sq.**	**Mean Sq.**	**F**	**p**
L. LA.Warm ~ Genotype	3.00	44.02	14.67	1.46	0.290
Residuals	9.00	90.51	10.06		

Table 3.3 Pearson Correlation Coefficients and P-values (Lower Left), Covariances (Upper Right), and Variances (Diagonal) of Traits Measured in F_2 Mapping Population. Flowering time (FT), reflectance (REF), rosette diameter (ROS.DIA), leaf length (LL), leaf fresh mass (LM), and leaf area (LA) were measured on clones in cool and warm environments; plasticity (Plast.) was calculated as the difference between warm minus cool. All individuals were germinated at moderate temperature.

		GERM	Flowering Time			Reflectance			Rosette Diameter			Leaf Length			Leaf Fresh Mass			Leaf Area		
			Cool	Warm	Plast.	Cool	Warm	Plast.	Cool	Warm	Plast.	Cool	Warm	Plast.	Cool	Warm	Plast.	Cool	Warm	Plast.
GERM		**24.8**	-13.50	-20.85	-6.66	-0.65	1.41	2.06	3.51	6.41	3.75	5.19	6.79	2.03	0.46	0.47	0.04	9.39	10.08	1.46
FT	Cool	-0.18; <0.001	**213.0**	78.33	-136.1	19.37	0.85	-17.71	-6.09	-9.88	-4.65	-6.58	-9.33	-3.58	-0.58	-0.58	-0.04	-11.18	-12.59	-2.87
	Warm	-0.31; <0.001	0.39; <0.001	**183.5**	107.64	9.39	0.94	-6.97	-6.77	-17.12	-10.74	-9.02	-16.26	-7.74	-0.70	-0.97	-0.29	-10.99	-14.23	-4.09
	Plast.	-0.09; 0.069	-0.61; <0.001	0.52; <0.001	**243.7**	-9.94	0.11	10.25	-0.49	-8.58	-7.04	-2.63	-7.74	-4.48	-0.13	-0.42	-0.27	-0.39	-2.89	-1.62
REF	Cool	-0.01; 0.831	0.10; 0.025	0.05; 0.272	-0.05; 0.312	**177.0**	7.60	-172.6	1.86	-3.43	-6.32	2.41	-0.33	-3.45	0.08	0.03	-0.09	-0.50	-0.92	-1.57
	Warm	0.14; 0.00	0.03; 0.530	0.04; 0.438	0.00; 0.938	0.29; <0.001	**3.9**	-3.71	0.52	0.85	0.52	0.79	0.82	0.15	0.04	0.05	0.02	0.76	0.98	0.50
	Plast.	0.03; 0.503	-0.10; 0.043	-0.04; 0.394	0.05; 0.283	-0.99; <0.001	-0.15; 0.002	**168.9**	-1.45	4.44	6.69	-1.94	1.18	3.50	-0.04	0.02	0.10	0.91	1.95	1.90
ROS. DIA	Cool	0.18; <0.001	-0.10; 0.024	-0.12; 0.009	-0.01; 0.871	0.04; 0.452	0.07; 0.170	-0.03; 0.562	**16.2**	5.71	-10.5	8.34	5.22	-3.07	0.67	0.31	-0.35	15.00	6.38	-8.35
	Warm	0.28; <0.001	-0.15; 0.002	-0.27; <0.001	-0.12; 0.012	-0.06; 0.248	0.09; 0.048	0.07; 0.125	0.30; <0.001	**22.0**	16.8	5.30	13.12	7.82	0.39	0.76	0.36	8.88	16.93	7.98
	Plast.	0.15; 0.002	-0.06; 0.191	-0.15; 0.001	-0.09; 0.061	-0.10; 0.053	0.05; 0.275	0.10; 0.035	-0.50; <0.001	0.67; <0.001	**26.6**	-2.99	7.89	10.89	-0.27	0.44	0.71	-5.90	10.51	16.32
LL	Cool	0.38; <0.001	-0.17; <0.001	-0.25; <0.001	-0.06; 0.190	0.07; 0.149	0.15; 0.002	-0.06; 0.249	0.76; <0.001	0.42; <0.001	-0.21; <0.001	**7.4**	5.19	-2.23	0.52	0.33	-0.19	11.45	6.93	-4.44
	Warm	0.39; <0.001	-0.18; <0.001	-0.35; <0.001	-0.15; 0.002	-0.01; 0.883	0.12; 0.010	0.03; 0.584	0.37; <0.001	0.81; <0.001	0.44; <0.001	0.55; <0.001	**12.1**	6.96	0.38	0.66	0.29	8.51	14.67	6.29
	Plast.	0.13; 0.005	-0.08; 0.086	-0.19; <0.001	-0.10; 0.042	-0.09; 0.073	0.03; 0.587	0.09; 0.062	-0.25; <0.001	0.55; <0.001	0.70; <0.001	-0.27; <0.001	0.66; <0.001	**9.2**	-0.13	0.34	0.47	-2.86	7.87	10.73
LM	Cool	0.40; <0.001	-0.17; <0.001	-0.23; <0.001	-0.04; 0.426	0.03; 0.592	0.09; 0.045	-0.01; 0.768	0.73; <0.001	0.37; <0.001	-0.23; <0.001	0.83; <0.001	0.48; <0.001	-0.19; <0.001	**0.1**	0.03	-0.02	1.16	0.61	-0.54
	Warm	0.39; <0.001	-0.17; <0.001	-0.30; <0.001	-0.12; 0.015	0.01; 0.862	0.12; 0.012	0.01; 0.873	0.33; <0.001	0.68; <0.001	0.36; <0.001	0.50; <0.001	0.80; <0.001	0.47; <0.001	0.52; <0.001	**0.1**	0.03	0.65	1.23	0.60

	Plast.	0.03; 0.494	-0.01; 0.800	-0.01; 0.044	-0.08; 0.109	-0.03; 0.538	0.04; 0.420	0.03; 0.479	-0.38; <0.001	0.34; <0.001	0.61; <0.001	-0.30; <0.001	0.36; <0.001	0.68; <0.001	-0.46; <0.001	0.52; <0.001	**0.1**	-0.51	0.64	1.14
LA	Cool	0.36; <0.001	-0.15; 0.001	-0.16; <0.001	-0.01; 0.920	-0.01; 0.877	0.07; 0.120	0.01; 0.778	0.71; <0.001	0.36; <0.001	-0.22; <0.001	0.81; <0.001	0.47; <0.001	-0.18; <0.001	0.97; <0.001	0.52; <0.001	-0.43; <0.001	**27.6**	14.61	-12.8
	Warm	0.38; <0.001	-0.16; <0.001	-0.20; <0.001	-0.04; 0.466	-0.01; 0.790	0.09; 0.048	0.03; 0.564	0.30; <0.001	0.67; <0.001	0.37; <0.001	0.47; <0.001	0.78; <0.001	0.48; <0.001	0.50; <0.001	0.96; <0.001	0.52; <0.001	0.51; <0.001	**29.4**	15.08
	Plast.	0.06; 0.248	-0.04; 0.428	-0.06; 0.225	-0.02; 0.673	-0.02; 0.641	0.05; 0.313	0.03; 0.563	-0.40; <0.001	0.32; <0.001	0.60; <0.001	-0.31; <0.001	0.34; <0.001	0.67; <0.001	-0.45; <0.001	0.48; <0.001	0.96; <0.001	-0.46; <0.001	0.52; <0.001	**27.8**

Table 3.4 Overall QTL Models from *fit.qtl* Analysis in R/qtl for Each Trait Examined in Cool and Warm Temperature, and Trait Plasticity. Best models were determined when all model parameters achieved $p < 0.05$. Secondary models (under J and S) include parameters with $p < 0.07$ that may be biologically significant. ***,**,*,and ^ represent significance at $p < 0.001$, 0.01, 0.05, and 0.1.

A. REFLECTANCE PLASTICITY

Best model = y ~ REF.Plasticity.6

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
REF.Plasticity.6	18.286	17.563	<0.0001	<0.0001	***

B. REFLECTANCE COOL

Best model = y ~ REF.Cool.6

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
REF.Cool.6	20.585	19.012	<0.001	<0.001	***

C. REFLECTANCE WARM

Best model = y ~ Ref.Warm.6

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Ref.Warm.6	5.053	5.084	3.55E-05	3.87E-05	***

D. FLOWERING TIME PLASTICITY

Best model = y ~ FT.Plasticity.2

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
FT.Plasticity.2	4.999	5.108	4.00E-05	4.37E-05	***

E. FLOWERING TIME COOL

Best model = y ~ FT.Cool.2 + FT.Cool.4 + FT.Cool.6 + Cytoplasm +

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	14.033	13.488	7.72E-09	1.30E-08	***
FT.Cool.2	4.515	4.128	<0.001	1.57E-04	***
FT.Cool.4	5.711	5.255	<0.001	2.65E-04	***
FT.Cool.6	2.819	2.555	0.005	0.006	**
Cytoplasm	2.278	2.059	0.033	0.037	*
FT.Cool.4:Cytoplasm	2.259	2.042	0.015	0.018	*

F. FLOWERING TIME WARM

Best model = y ~ FT.Warm.4

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
FT.Warm.4	8.581	8.425	1.35E-08	1.57E-08	***

G. ROSETTE DIAMETER PLASTICITY

Best model = Infinitesimal

H. ROSETTE DIAMETER COOL

Best model = y ~ ROS.Cool.4 + ROS.Cool.6

Model Parameters	LOD	%var	**p(Chi)**	**p(F)**	
Full Model	6.532	6.594	3.79E-05	4.40E-05	***
ROS.Cool.4	3.108	3.081	0.003	0.003	**

ROS.Cool.6	3.428	3.428	0.001	0.001	**

I. ROSETTE DIAMETER WARM

Best model = y ~ ROS.Warm.2 + ROS.Warm.3 + ROS.Warm.4 + ROS.Warm.6 +

Model Parameters	LOD	%var	**p(Chi)**	**p(F)**	
Full Model	23.815	21.930	<0.001	<0.001	***
ROS.Warm.2	3.054	2.518	0.003	0.003	**
ROS.Warm.3	4.166	3.455	<0.001	3.31E-04	***
ROS.Warm.4	7.682	6.491	<0.001	1.71E-07	***
ROS.Warm.6	5.126	4.273	<0.001	4.26E-05	***
Cytoplasm	2.043	1.676	0.002	0.003	**

J. LEAF LENGTH PLASTICITY

Best model = y ~ LL.Plasticity.3 + Cytoplasm

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	5.045	5.224	1.14E-04	1.26E-04	***
LL.Plasticity.3	2.694	2.755	0.006	0.006	**
Cytoplasm	1.892	1.927	0.003	0.003	**

K. LEAF LENGTH COOL

Best model = y ~ LL.Cool.2 + LL.Cool.4 + LL.Cool.6a + LL.Cool.6b + Cytoplasm +

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	20.621	19.373	3.02E-13	7.68E-13	***
LL.Cool.2	5.665	4.914	<0.001	3.13E-04	***
LL.Cool.4	4.130	3.554	<0.001	3.80E-04	***
LL.Cool.6a	3.418	2.930	0.001	0.002	**
LL.Cool.6b	5.050	4.366	<0.001	5.42E-05	***
Cytoplasm	2.226	1.896	0.036	0.043	*
LL.Cool.2:Cytoplasm	1.797	1.527	0.041	0.047	*

Secondary model = y ~ LL.Cool.2 + LL.Cool.3$ + LL.Cool.4 + LL.Cool.6a +

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	22.291	20.767	1.77E-13	5.51E-13	***
LL.Cool.2	5.108	4.341	0.001	9.57E-04	***
LL.Cool.3$	1.670	1.394	0.053	0.061	^
LL.Cool.4	3.173	2.669	0.002	0.003	**
LL.Cool.6a	3.227	2.715	0.002	0.003	**
LL.Cool.6b	4.850	4.117	<0.001	8.91E-05	***
Cytoplasm	2.353	1.971	0.028	0.035	*
LL.Cool.2:Cytoplasm$	2.009	1.680	0.026	0.031	*

L. LEAF LENGTH WARM

Best model = y ~ LL.Warm.1 + LL.Warm.2 + LL.Warm.3 + LL.Warm.4a +

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	31.053	27.588	<0.001	<0.001	***
LL.Warm.1	1.870	1.422	0.035	0.043	*
LL.Warm.2	4.908	3.791	<0.001	8.46E-05	***
LL.Warm.3	1.918	1.458	0.032	0.039	*
LL.Warm.4a	2.805	2.142	0.005	0.007	**
LL.Warm.4b	3.774	2.897	0.001	8.97E-04	***

LL.Warm.6	6.190	4.813	<0.001	1.35E-04	***
Cytoplasm	5.360	4.150	<0.001	1.02E-04	***
LL.Warm.6: Cytoplasm	2.723	2.079	0.006	0.008	**

M. FRESH MASS PLASTICITY

Best model = INFINITESIMAL

N. FRESH MASS COOL

Best model = y ~ Mass.Cool.3 + Mass.Cool.5 + Mass.Cool.6 + Cytoplasm

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	9.594	9.533	3.05E-06	4.09E-06	***
Mass.Cool.3	2.409	2.305	0.011	0.013	*
Mass.Cool.5	2.665	2.553	0.007	0.007	**
Mass.Cool.6	2.562	2.453	0.008	0.009	**
Cytoplasm	0.923	0.876	0.039	0.042	*

O. FRESH MASS WARM

Best model = y ~ Mass.Warm.1 + Mass.Warm.2 + Mass.Warm.3 + Mass.Warm.4 +

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	28.285	25.475	3.33E-16	2.00E-15	***
Mass.Warm.1	6.488	5.199	0.003	0.005	**
Mass.Warm.2	6.951	5.584	0.001	0.002	**
Mass.Warm.3	5.335	4.250	<0.001	3.75E-05	***
Mass.Warm.4	1.972	1.544	0.028	0.036	*
Mass.Warm.6	3.461	2.730	0.001	0.002	**
Cytoplasm	4.046	3.201	<0.001	2.85E-05	***
Mass.Warm.1:Mass.Warm.2	3.558	2.808	0.059	0.077	*

P. LEAF AREA PLASTICITY

Best model = INFINITESIMAL

Q. LEAF AREA COOL

Best model = y ~ LA.Cool.2 + LA.Cool.3 + LA.Cool.4 + LA.Cool.5 + Cytoplasm +

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	15.463	14.911	4.26E-07	9.22E-07	***
LA.Cool.2	5.515	5.044	0.013	0.018	*
LA.Cool.3	1.885	1.692	0.034	0.041	*
LA.Cool.4	5.678	5.198	0.010	0.014	*
LA.Cool.5	2.523	2.272	0.009	0.012	*
Cytoplasm	1.121	1.002	0.023	0.027	*
LA.Cool.2: LA.Cool.4	3.698	3.350	0.048	0.061	^

R. LEAF AREA WARM

Best model = y ~ LA.Warm.2 + LA.Warm.3 + LA.Warm.4 + Cytoplasm

Model Parameters	**LOD**	**%var**	**p(Chi)**	**p(F)**	
Full Model	19.287	18.204	9.21E-15	1.75E-14	***
LA.Warm.2	3.697	3.212	0.001	8.46E-04	***
LA.Warm.3	4.521	3.945	<0.001	1.45E-04	***
LA.Warm.4	2.695	2.330	0.006	0.007	**

Cytoplasm	4.437	3.870	<0.001	8.16E-06	***

S. GERMINATION

Best model = y ~ Germination.1 + Germination.2 + Germination.3 + Germination.4					
Model Parameters	LOD	%var	**p(Chi)**	**p(F)**	
Full Model	20.397	19.184	4.70E-13	1.18E-12	***
Germination.1	6.186	5.393	<0.001	1.16E-04	***
Germination.2	3.551	3.053	0.001	0.001	**
Germination.3	5.148	4.464	<0.001	4.40E-05	***
Germination.5	2.912	2.496	0.004	0.005	**
Cytoplasm	4.246	3.664	0.001	8.43E-04	***
Germination.1:Cytoplasm	1.811	1.543	0.039	0.045	*
Secondary model = y ~ Germination.1 + Germination.2 + Germination.3 +					
Model Parameters	LOD	%var	**p(Chi)**	**p(F)**	
Full Model	22.024	20.545	2.96E-13	9.08E-13	***
Germination.1	6.801	5.848	<0.001	3.86E-05	***
Germination.2	3.584	3.030	0.001	0.001	**
Germination.3	6.776	5.825	<0.001	4.06E-05	***
Germination.5	2.542	2.138	0.008	0.011	*
Cytoplasm	5.873	5.026	<0.001	5.09E-04	***
Germination.1:Cytoplasm	1.967	1.649	0.029	0.034	*
Germination.3:Cytoplasm$	1.627	1.362	0.058	0.067	^

Figure 3.1 Diagram of Reciprocal Out-crossing Design for Creation of F_2 Mapping Family. Outer circles (cytoplasmic DNA), inner rectangles (nuclear DNA). Alleles designate inheritance from F_0 parent. Nuclear alleles in F_2s can be homozygous for northern or southern derived alleles, or heterozygous.

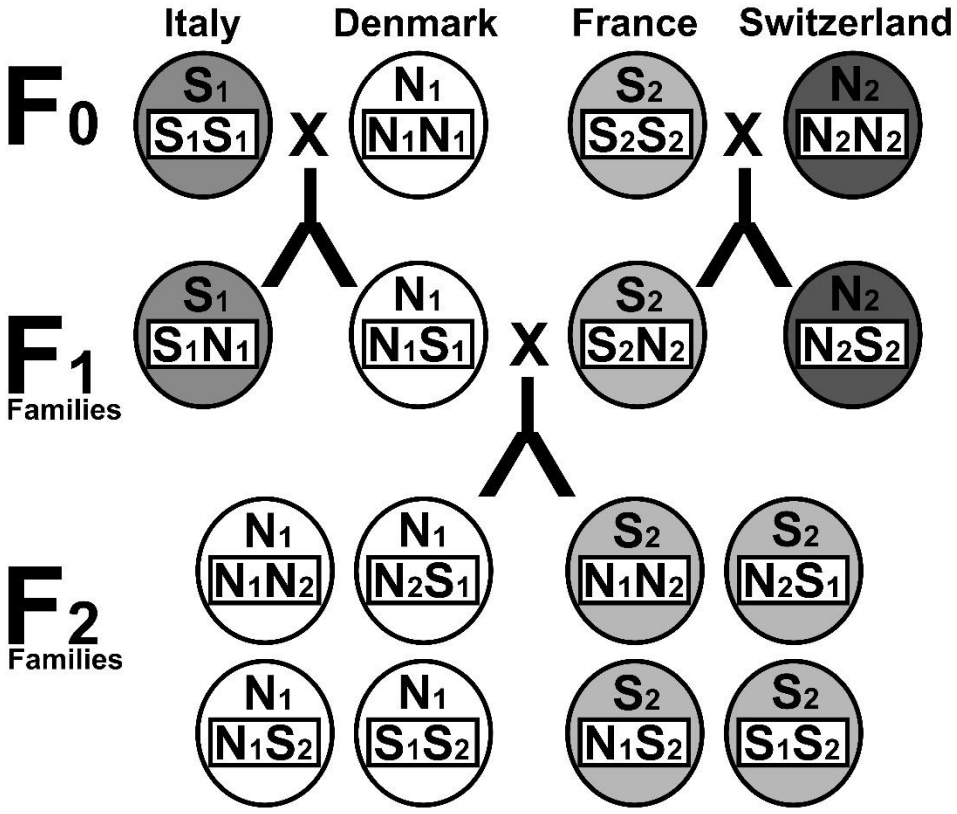


Figure 3.2 A. Heat Map of LOD (above diagonal) and Recombination Frequency (below diagonal) for Genetic Markers Ordered along 6 Linkage Groups. Color scale varies from red (small distances or LOD) to dark blue. White indicates 'could not be calculated.' B. Final linkage map showing distance in centiMorgans (Kosambi) on left of linkage group, marker name and marker type on right of linkage group. Marker type indicates segregation pattern (Margarido et al. 2007). Marker deviation from expected segregation ratio p-values < 0.05 from chi-square test are indicated as * p > 0.01 and ** p > 0.0001.

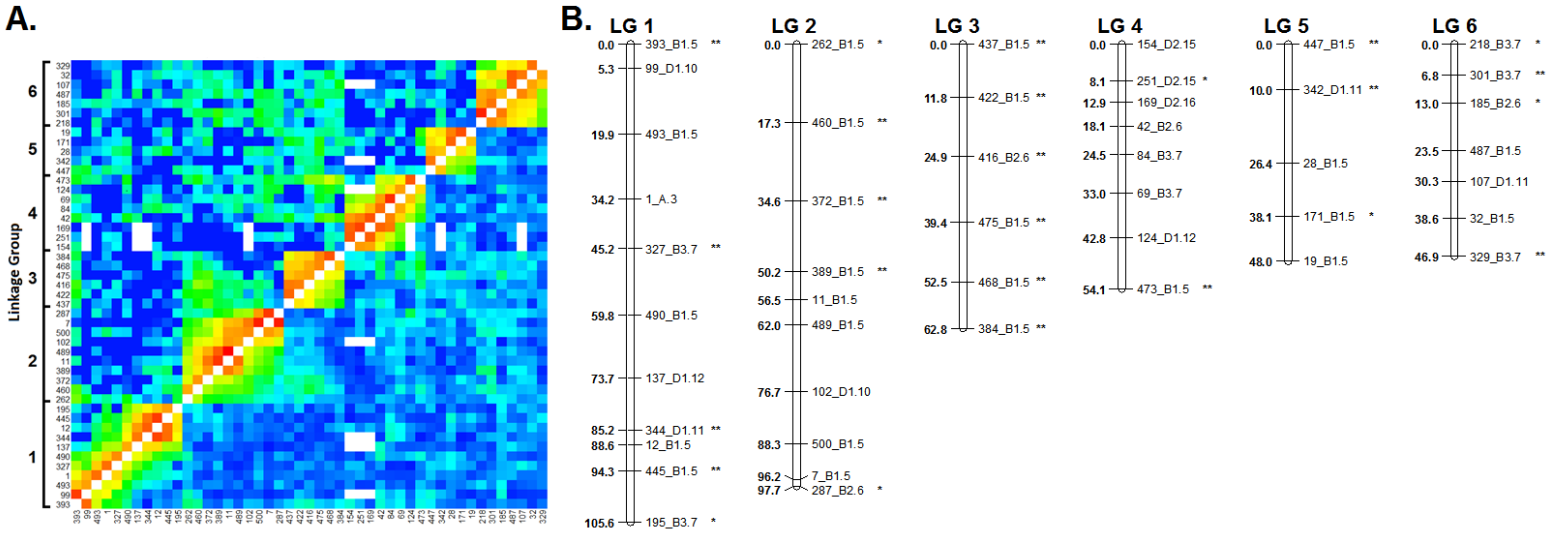


Figure 3.3 Histograms Displaying Mean Percent Reflectance of Pre-flowering Spikes at 850nm Developed in a Cool (Blue) and Warm (Red) Thermal Environment. Images display visible color variation in spikes developed at cool temperature. A. Bars show number of clones measured, black lines show mean percent reflectance for F_0 genotypes; B. Bars show number of F_1 genotypes measured, black lines show mean percent reflectance for F_1 genotypes crossed to produce F_2s; C. Bars show number of genotypes measured, black lines show percent reflectance of representative F_2 spikes imaged.

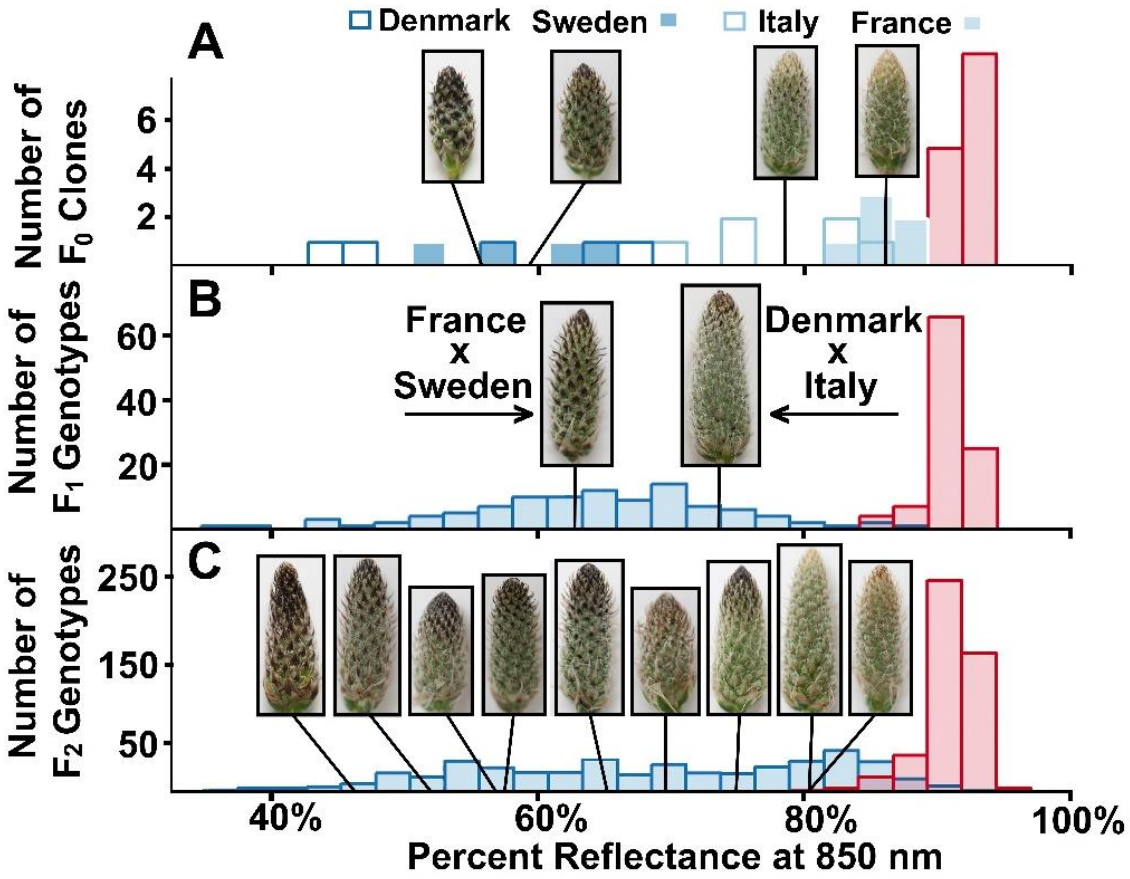


Figure 3.4 The Distribution of F_2 Means for Each Trait Measured in Cool and Warm Environment, and Plasticity Calculated as Mean Trait Value in Warm Minus Cool. 'I'/open square, 'D'/open circle, 'F'/closed square, and 'S'/closed circle represent F_0 parents from Italy, Denmark, France, and Sweden, respectively; horizontal line shows variation among clones, vertical line crosses at genotypic mean, and vertical length represents number of clones measured.

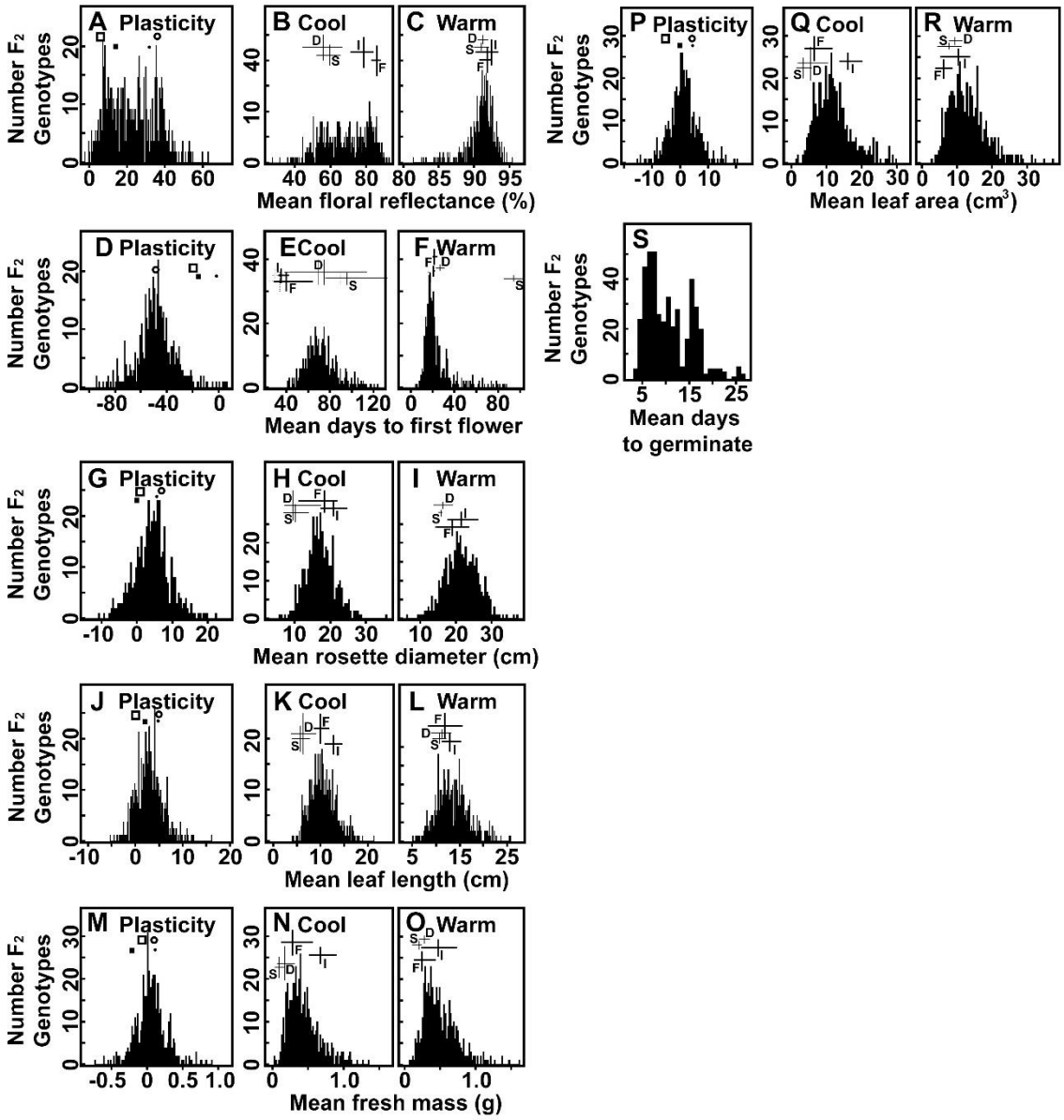


Figure 3.5 QTL Mapping Results. LOD Profiles for Flowering Time, Floral Reflectance, Rosette Diameter, Leaf Length, Leaf Area, and Leaf Fresh Mass are Shown for Trait Values in Cool (Blue) and Warm (Red) Environments, and Trait Plasticity (Black). LOD profile for germination was measured in a single environment (green). Horizontal lines represent genome wide P = 0.05 significance thresholds.

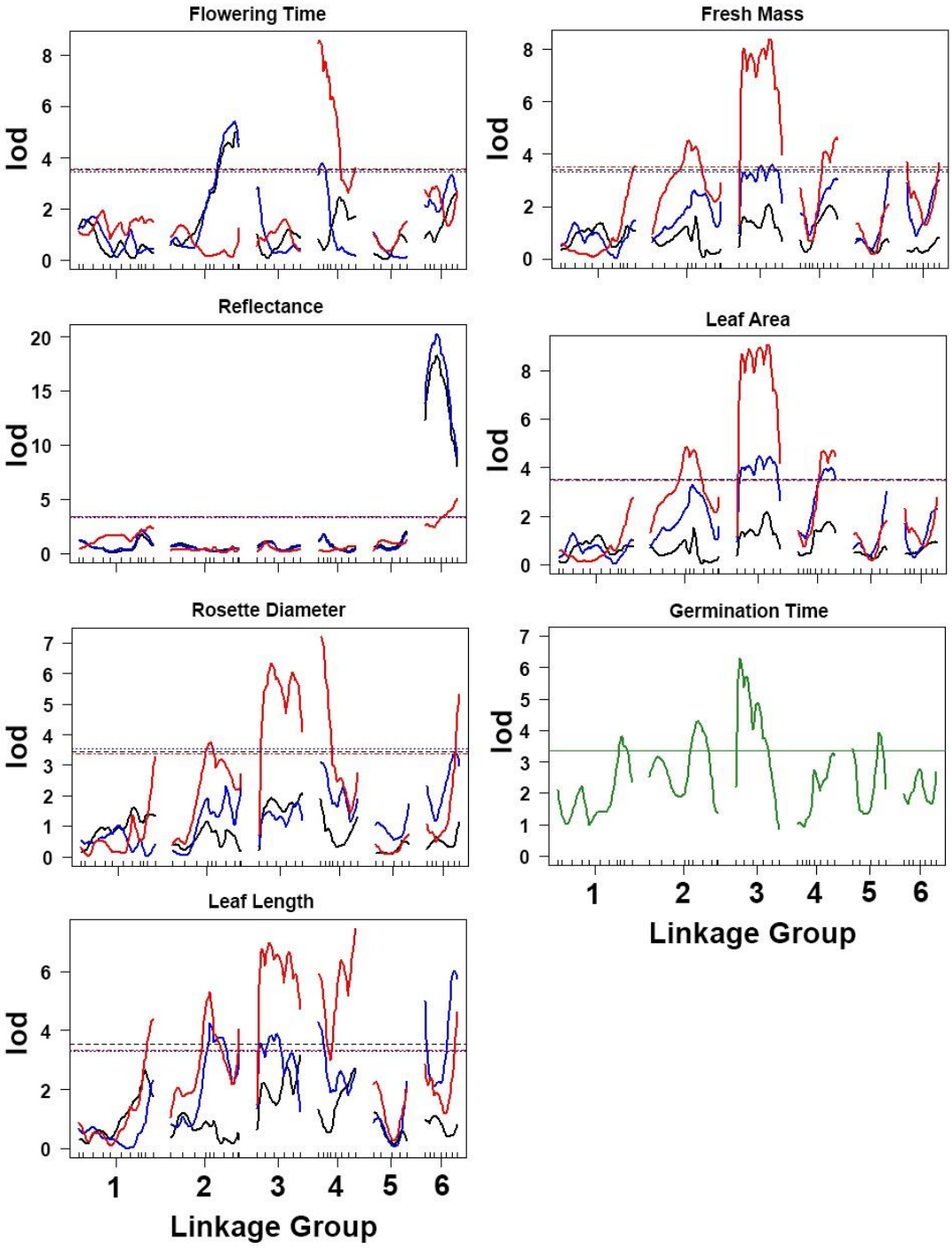
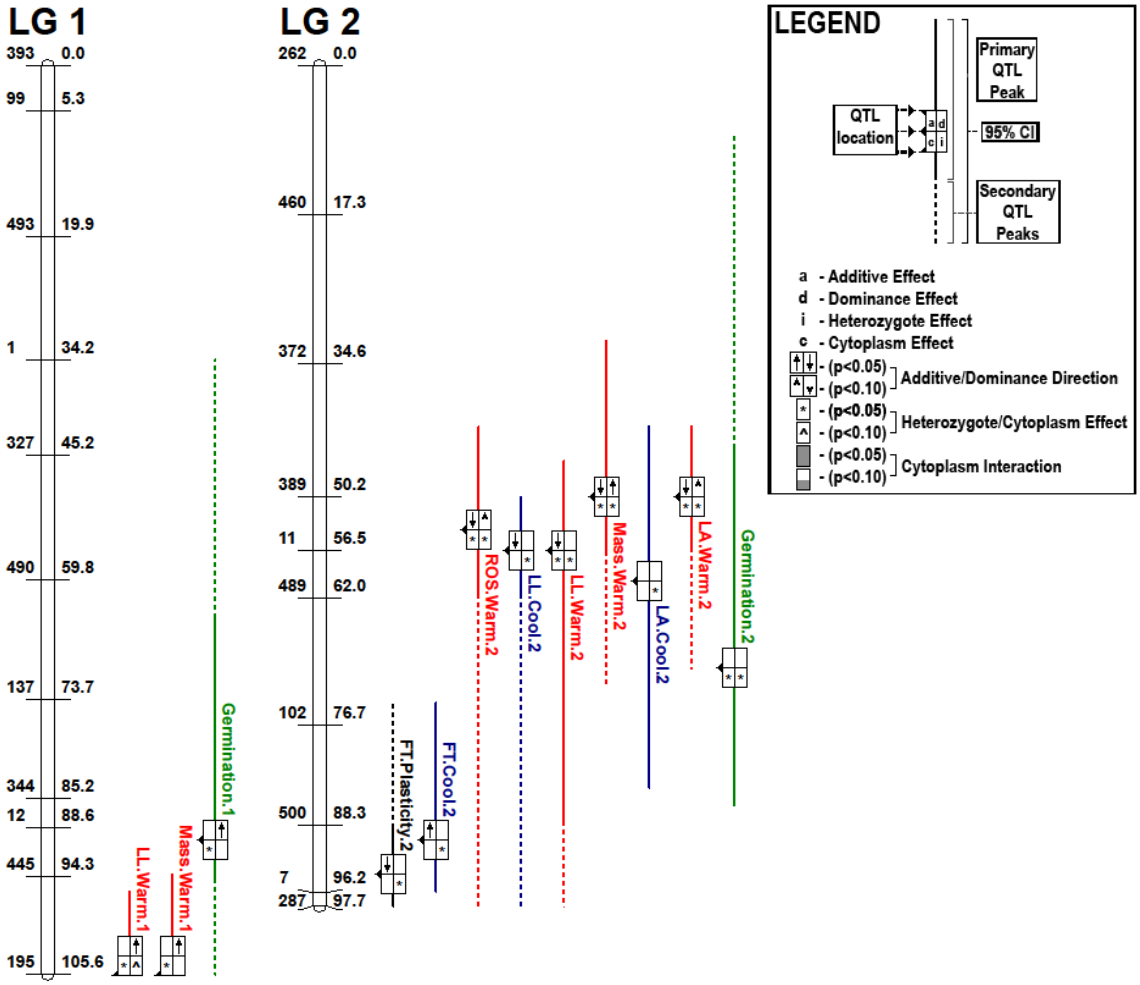


Figure 3.6 QTLs Displaying a Significant Effect on Trait Values in Cool and Warm Environments and Trait Plasticity for Flowering Time (FT), Floral Reflectance (REF), Rosette Diameter (ROS), Leaf Length (LL), Leaf Area (LA), and Leaf Fresh Mass (Mass), and in a Single Environment for Germination (Germination). QTL peak locations (solid lines) and Bayesian 95% credible intervals (dashed lines) are shown to the right, and genetic markers are shown to the left of each linkage group. Significant QTLs and interactions were identified using the fitqtl function in R/qtl. Each QTL was partitioned into additive (a), dominance (d), difference between heterozygous classes (i), cytoplasmic (c), and cytoplasmic interactions (shaded boxes) in separate generalized linear models. Arrows indicate the significance and direction of additive and dominance effects of alleles from northern populations (Denmark and Sweden). Asterisk (*) and hat (^) symbols indicate significance of difference between heterozygous classes and cytoplasmic effects. ~ p-value = 0.061 for LL.Cool.3 in full fitqtl model. $ Bayesian 95% credible interval for LL.Cool.6 is between 0-2 cM. QTLs are labeled as **[trait].[environment].[LG]**, **trait**: floral reflectance (REF), flowering time (FT), rosette diameter (ROS), leaf length (LL), leaf fresh mass (Mass), leaf area (LA), and germination time (Germination); **environment**: Cool, Warm, or Plasticity; and **LG**: numbered 1-6.



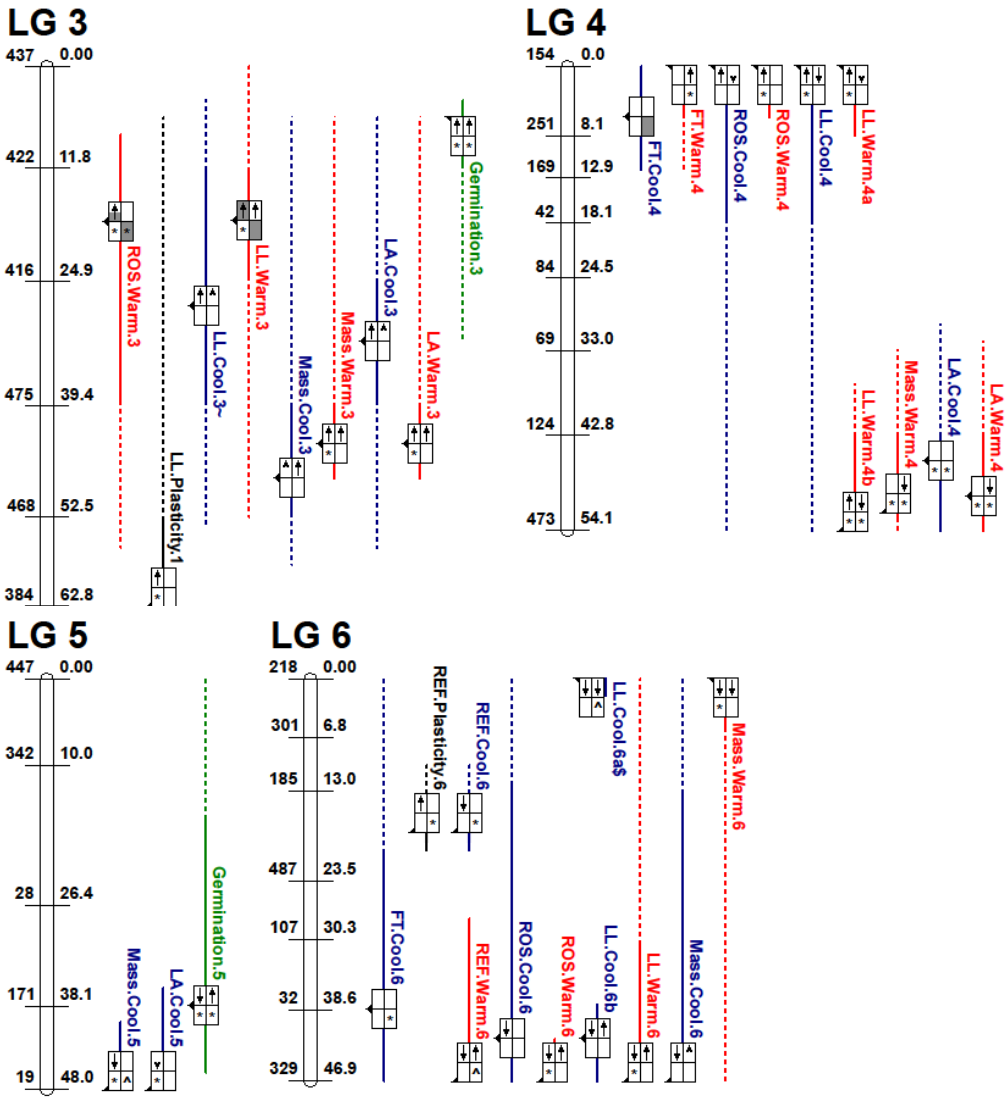
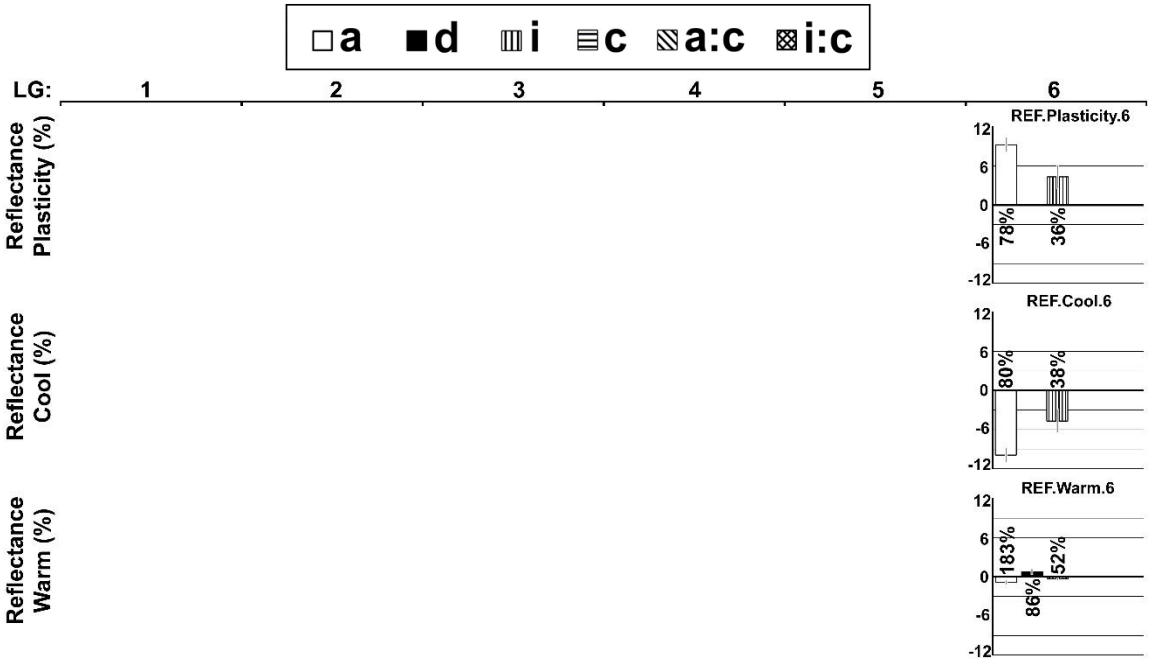
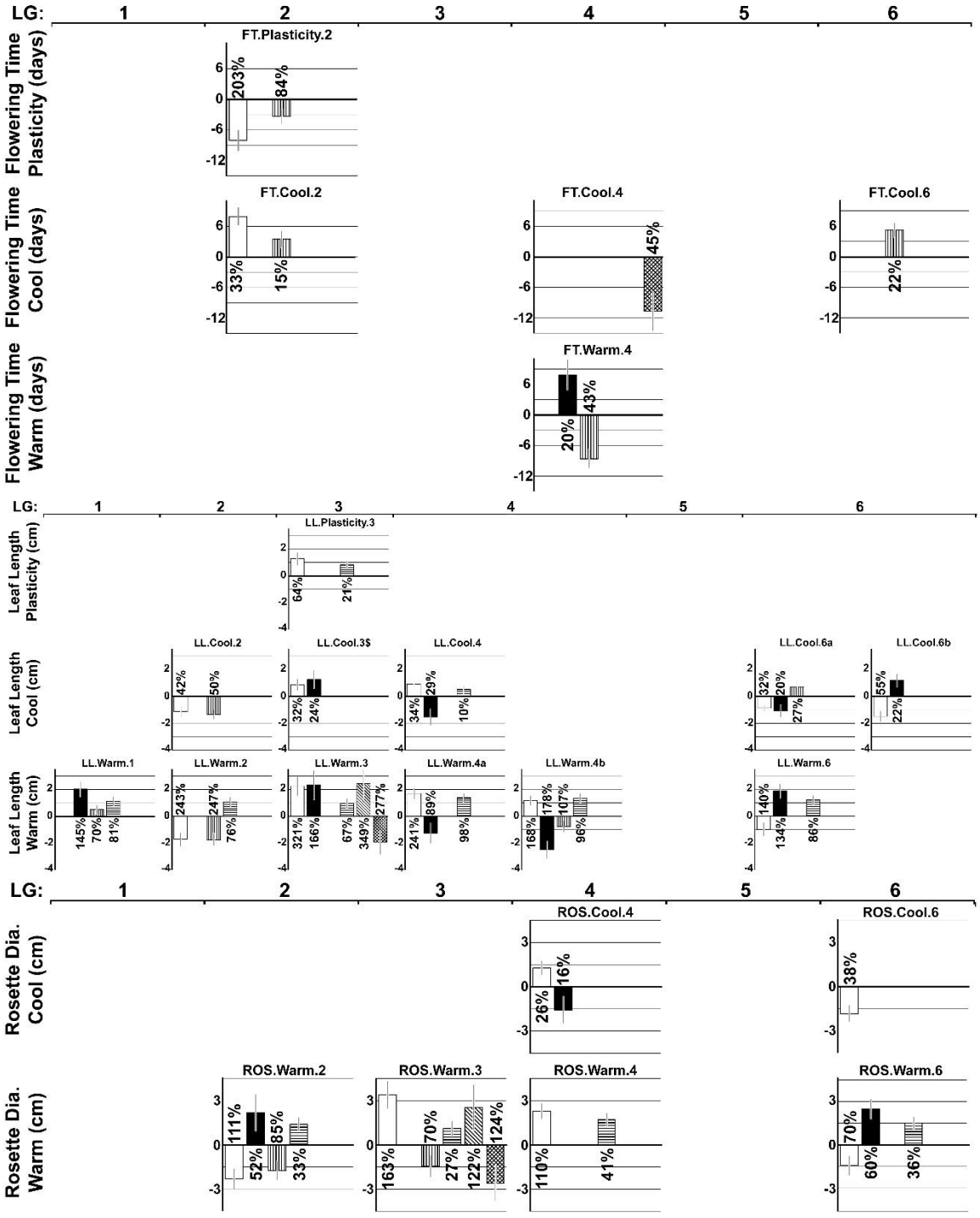

LG 3
437 0.00
422 11.8
416 24.9
475 39.4
468 52.5
384 62.8
ROS.Warm.3
LL.Plasticity.1
LL.Cool.3~
LL.Warm.3
Mass.Cool.3
Mass.Warm.3
LA.Cool.3
LA.Warm.3
Germination.3
LG 4
154 0.0
251 8.1
169 12.9
42 18.1
84 24.5
69 33.0
124 42.8
473 54.1
FT.Cool.4
FT.Warm.4
ROS.Cool.4
ROS.Warm.4
LL.Cool.4
LL.Warm.4a
LL.Warm.4b
Mass.Warm.4
LA.Cool.4
LA.Warm.4
LG 5
447 0.00
342 10.0
28 26.4
171 38.1
19 48.0
Mass.Cool.5
LA.Cool.5
Germination.5
LG 6
218 0.00
301 6.8
185 13.0
487 23.5
107 30.3
32 38.6
329 46.9
REF.Plasticity.6
REF.Cool.6
FT.Cool.6
REF.Warm.6
ROS.Cool.6
ROS.Warm.6
LL.Cool.6a$
LL.Cool.6b
LL.Warm.6
Mass.Cool.6
Mass.Warm.6

Figure 3.7 Bar Plots Displaying the Magnitude and Direction (± SE) of Significant QTL Effects on Trait Values. Each QTL was partitioned into additive (a), dominance (d), difference between heterozygous classes (i), cytoplasmic (c), and cytoplasmic interaction effects in separate generalized linear models. Additive and dominance direction display effects of nuclear alleles from northern (Danish and Swedish) populations. The difference between heterozygote classes was calculated by subtracting heterozygotes with Danish/French alleles from those with Swedish/Italian alleles. Cytoplasmic effects display direction of northern (Danish) cytoplasmic alleles. Percentages presented above/below each bar represent the difference between mean trait values of northern and southern parents explained by each QTL effect. Percentages associated with a, i, c:a and c:i were doubled to better reflect differences between F_2 genotypic classes. $ p-value = 0.061 for LL.Cool.3 in full fitqtl model.




LG: 1 2 3 4 5 6
Flowering Time Plasticity (days)
FT.Plasticity.2
203%
84%
Flowering Time Cool (days)
FT.Cool.2
33%
15%
FT.Cool.4
45%
FT.Cool.6
22%
Flowering Time Warm (days)
FT.Warm.4
20%
43%
LG: 1 2 3 4 5 6
Leaf Length Plasticity (cm)
LL.Plasticity.3
64%
21%
Leaf Length Cool (cm)
LL.Cool.2
42%
50%
LL.Cool.3$
32%
24%
LL.Cool.4
34%
29%
10%
LL.Cool.6a
32%
20%
27%
LL.Cool.6b
55%
22%
Leaf Length Warm (cm)
LL.Warm.1
145%
70%
81%
LL.Warm.2
243%
247%
76%
LL.Warm.3
321%
166%
67%
349%
277%
LL.Warm.4a
241%
89%
98%
LL.Warm.4b
168%
178%
107%
96%
LL.Warm.6
140%
134%
86%
LG: 1 2 3 4 5 6
Rosette Dia. Cool (cm)
ROS.Cool.4
26%
16%
ROS.Cool.6
38%
Rosette Dia. Warm (cm)
ROS.Warm.2
111%
52%
85%
33%
ROS.Warm.3
163%
70%
27%
122%
124%
ROS.Warm.4
110%
41%
ROS.Warm.6
70%
60%
36%

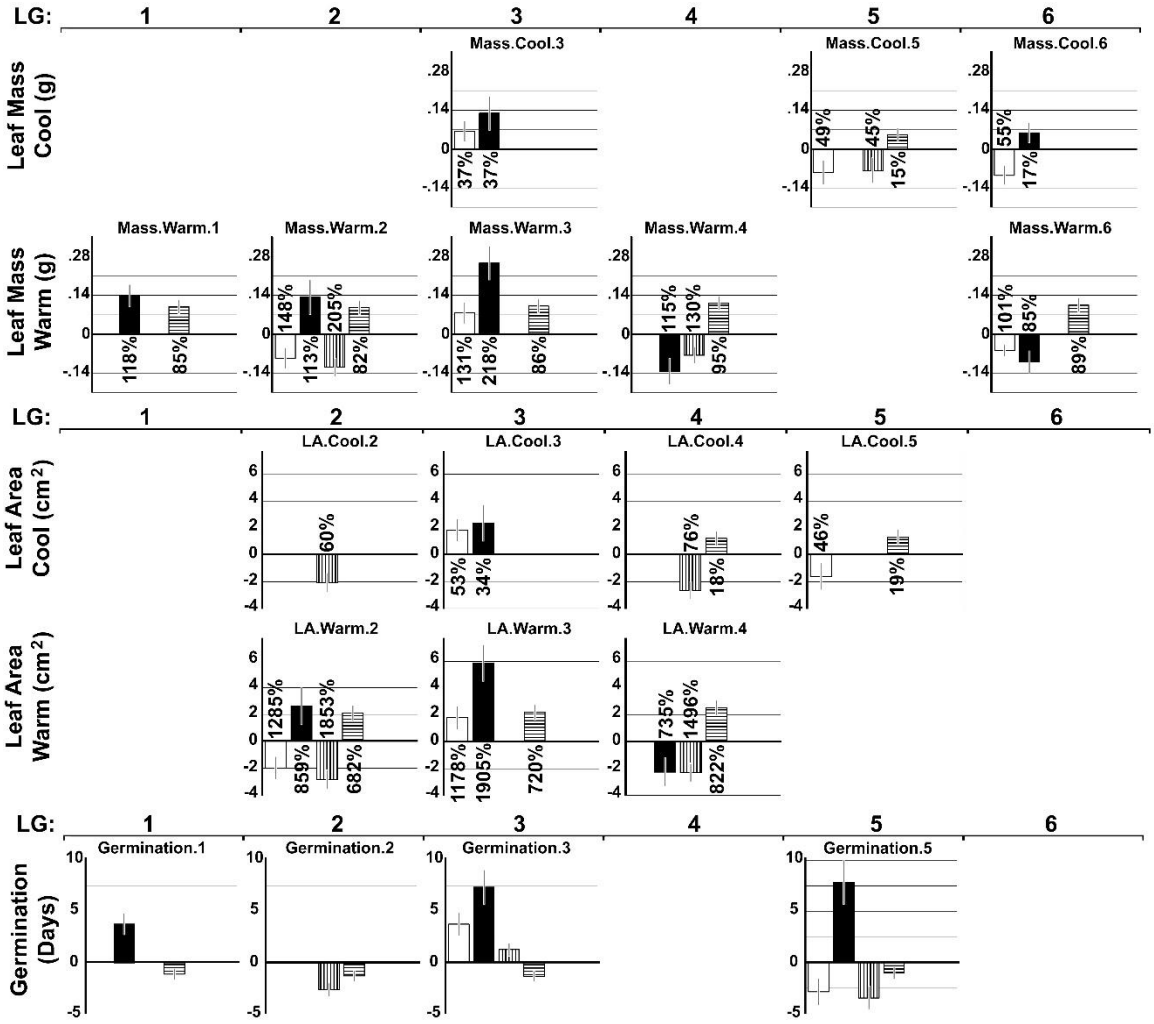

LG: 1 2 3 4 5 6
Leaf Mass Cool (g)
Mass.Cool.3
37%
37%
Mass.Cool.5
49%
45%
15%
Mass.Cool.6
55%
17%
Leaf Mass Warm (g)
Mass.Warm.1
118%
85%
Mass.Warm.2
148%
113%
205%
82%
Mass.Warm.3
131%
218%
86%
Mass.Warm.4
115%
130%
95%
Mass.Warm.6
101%
85%
89%
LG: 1 2 3 4 5 6
Leaf Area Cool (cm²)
LA.Cool.2
60%
LA.Cool.3
53%
34%
LA.Cool.4
76%
18%
LA.Cool.5
46%
19%
Leaf Area Warm (cm²)
LA.Warm.2
1285%
859%
1853%
682%
LA.Warm.3
1178%
1905%
720%
LA.Warm.4
735%
1496%
822%
LG: 1 2 3 4 5 6
Germination (Days)
Germination.1
Germination.2
Germination.3
Germination.5

Figure 3.8 Genotypic Means (± SE) of Reciprocal F2s at A. QTLs with Significant Nuclear by Cytoplasm Interactions Detected in Single QTL fitqtl Models, B. QTLs with Significant Nuclear by Cytoplasm Interactions Detected in a-d-i Models, and Shading Highlights FT.Cool.4, which was Significant in Both Models. Allele designations indicate inheritance from F_0 parent; N_1 = Danish, N_2 = Swedish, S_1 = Italian, S_2 = French. Symbols represent Danish (circles) and French (triangles) cytoplasmic genomes.

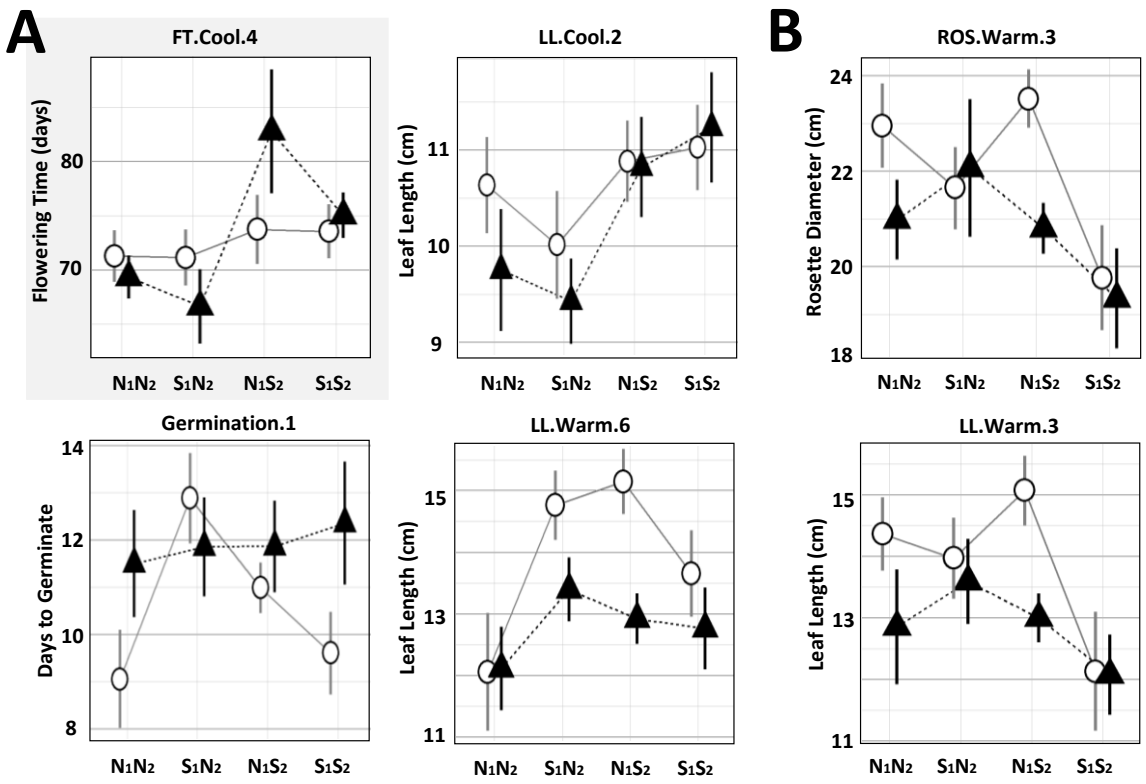
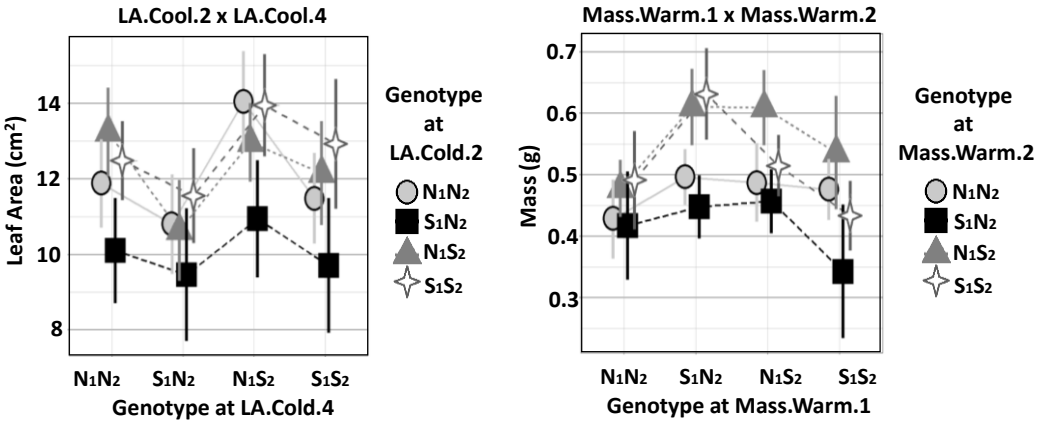


Figure 3.9 Genotypic Means (± SE) of all F_2s at QTLs with Significant Nuclear QTL x QTL Interactions. Allele designations indicate inheritance from F_0 parent; N_1 = Danish, N_2 = Swedish, S_1 = Italian, S_2 = French.



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Supplemental Materials

Laboratory Methods

Genomic DNA extraction

Total genomic DNA was extracted 100 mg of powdery lyophilized young leaf tissue using the MasterPure™ plant leaf DNA purification kit and suspended in 50µL low TE buffer. DNA was then treated with 1µL RNase (100mg/mL) for 30 minutes at 37°C, precipitated by ethanol based precipitation and resuspended in 100µL low TE buffer. Integrity of high molecular weight genomic DNA bands were verified visually on 1% agarose gels run in 1x TAE buffer, stained with 0.2µg/mL ethidium bromide and viewed with the Bio-Rad ChemiDoc XRS system.

Restriction enzyme selection

To determine which restriction enzymes would be appropriate for this project we selected four enzymes with an optimal reaction temperature of 37ºC that were not methylation-sensitive. Two were common cutters with 4-nucleotide recognition sites, MseI and MspI; and two were rare cutters with 6-nucleotide recognition sites, EcoRI and PstI.

Digestions were performed on genomic DNA from each of the F_0 parents as single digestions (each restriction enzyme alone), and double digestions (each combination of common + rare cutter). Digestions of 1µg genomic DNA were conducted at 37ºC for 1hr followed by heat inactivation at 80ºC for 20 min in 1x Buffer B (ThermoFisher Scientific™) with 2µg acetylated BSA, 5 units of each restriction enzyme, and diH_2O in a final volume of 20µL. Digested fragments were cleaned with AMPure XP beads (Agencourt) and resuspended in 40µL low TE buffer. Concentration of DNA was estimated with a nanodrop spectrophotometer (ThermoFisher Scientific™). DNA fragments were verified visually on 1% agarose gels run in 1x TAE buffer, stained with 0.2µg/mL ethidium bromide and viewed with the Bio-Rad ChemiDoc XRS system.

Digested DNA samples were subjected to a dilution series from 1.25ng/µL to 10ng/µL and run on an Agilent 2100 Bioanalyzer High Sensitivity chip. The number of sequencable fragments produced from each combination of restriction enzymes was estimated using the methods described in (Peterson et al. 2012). After digestion with EcoRI and MspI, a size selection window of 200-400bp was estimated to produce ~38,000 sequencable fragments per individual. Therefore, to capture gDNA fragments of 200-400bp ligated to 120bp of adapters, a size selection window of 320-520bp was used for library preparation.

ddRADseq library preparation

Genomic DNA was extracted from 465 individuals (4 F_0, 2 F_1, 459 F_2) as described above. Integrity of high molecular weight genomic DNA bands were verified visually on 1% agarose gels run in 1x TAE buffer, stained with 0.2µg/mL ethidium bromide and viewed with the Bio-Rad ChemiDoc XRS system.

DNA samples were sent to the genomics core lab at Texas A&M University Corpus Christi for library preparation. SPRI size selection was used to purify high molecular weight genomic DNA. Illumina library preparation was conducted using the restriction enzymes EcoRI and MspI with a size selection window of 320-520bp. For each run on a single Illumina lane, 196 individuals were pooled and 100bp PE sequencing was performed. This was estimated to produce ~38,000 reads per individual at 40x coverage.

Bioinformatic Processing

Bioinformatic processing of raw sequence reads was performed in STACKS v. 1.35-1.37 (Catchen et al. 2011; Catchen et al. 2013). Separately, forward and reverse reads were cleaned from erroneous and low-quality reads, and demultiplexed using the process_radtags script with the following options: -i gqfastq, -p, -b, -c, -q, -r, -D, --inline_null, --renz_1 ecoRI, renz_2 mspI. Reads from each individual were organized into sets of unique loci with a minimum number of 5

identical reads and a maximum number of 2 alleles per locus using the ustacks script with the following options: -m 5, -N 0, -H, --max_locus_stacks 2. A catalog of identical loci shared among parental F_0 and F_1 genotypes was constructed using the cstacks script with the -n 0 option. Stacks of reads produced for each individual in ustacks were searched against the catalog of shared loci using the sstacks script. The populations script was used to output reads found in ≥20% of F_2s (11,294 and 10,387 from forward and reverse reads, respectively) were exported as haplotypes.

Filtering of genetic markers and recombination mapping was conducted using the 118 F_2s (25.7%) with the highest sequence coverage. In Microsoft Excel the haplotypes output file was organized and genetic markers that did not contain allelic differences between F_1 genotypes, could not be traced to F0 genotypes, or were scored in fewer than 70% of F_2s were manually filtered. From the remaining markers (756 and 659 from forward and reverse reads, respectively) we then removed markers with extremely skewed segregation ratios ($p<0.0001$ from a chi-square test). The remaining 555 markers (327 and 228 from forward and reverse reads, respectively) were used to create the genetic recombination map.

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Table S3.1 Location of Origin of Parental Genotypes used in Experimental Cross.

Region	Location, Country	Latitude (ºN)	Longitude (ºE)	Altitude (m)
Northern	Uppsala, Sweden	59.94	17.39	20
Northern	Veno, Denmark	56.55	8.63	0
Southern	Castel Volturno, Italy	41.03	13.93	1
Southern	Hameau de St. Felix, France	43.58	3.97	35

Table S3.2 Estimates of Broad Sense Heritability, Shapiro-Wilk's W and Associated P-value, and Pearson's Kurtosis of Traits Measured in Cool and Warm Environments and Trait Thermal Plasticity. Plasticity values were calculated as the difference between mean phenotype in warm minus cool.

Trait	Environment	h_B^2	W	p-value	Pearson's Kurtosis
Number of Days to Flower	Cold	-0.38	0.966	1.06E-08	3.669
	Warm	0.64	0.752	< 2.2e-16	8.652
	Plasticity	0.57	0.973	3.38E-07	4.470
% Reflectance at 850nm	Cold	0.73	0.965	9.69E-09	2.170
	Warm	0.70	0.889	< 2.2e-16	6.685
	Plasticity	0.68	0.970	8.86E-08	2.270
Rosette Diameter (cm)	Cold	0.17	0.991	0.007	3.753
	Warm	0.56	0.999	0.975	3.126
	Plasticity	0.52	0.994	0.070	3.520
Leaf Length (cm)	Cold	0.57	0.985	1.60E-04	3.262
	Warm	0.51	0.985	1.45E-04	3.393
	Plasticity	0.25	0.983	5.82E-05	4.206
Fresh Mass (g)	Cold	0.57	0.929	1.22E-13	4.230
	Warm	0.64	0.929	1.06E-13	4.939
	Plasticity	0.64	0.975	7.70E-07	4.652
Leaf Area (cm^3)	Cold	0.41	0.948	2.50E-11	3.844
	Warm	0.60	0.945	1.03E-11	4.584
	Plasticity	0.41	0.983	5.97E-05	4.215
Number of Days to Germinate*		-	0.930	1.58E-13	3.005

* Germination occurred under 20ºC, 8H day/15ºC, 16H night

Table S3.3 Number of ddRAD Tag Sequences, Mean Coverage per Read, Standard Deviation and Total Number of Unique Reads for F_0 and F_1 Genotypes used to Produce F_2 Mapping Population, and Mean and Median Sequence Coverage Statistics for F_2 Population.

Sample	ddRAD-Tags	Mean coverage	Standard Deviation	Total Unique Reads
F_0 parent: Italy	2,692,546	24	280	87,882
F_0 parent: Denmark	1,233,993	28	168	32,839
F_1 parent: Denmark x Italy	1,021,866	29	189	28,290
F_0 parent: France	1,073,087	31	179	26,697
F_0 parent: Sweden	69,300	13	40	1,948
F_1 parent France x Sweden	23,666	13	31	676
F_2 mean	596,893	16	131	27,208
F_2 median	349,807	14	99	20,323

Table S3.4 Marker Density by Linkage Group.

LG	Number of Markers (m_i)	Map Length (cM)[a] (M_i)	Inferred LG length (cM) (G_i)	Expected number of markers (λ_i)	Poisson two-tailed p-value[b]
1	11	105.6	125.8	11.02	0.577
2	10	97.7	117.9	10.33	0.542
3	6	62.8	83	7.27	0.410
4	8	54.1	74.3	6.51	0.329
5	5	48	68.2	5.98	0.449
6	7	46.9	67.1	5.88	0.374

[a] Map Lengths are in centiMorgans (cM0, Kosambi function

[b] Poisson probability of having as many ($m_i \geq \lambda_i$) or as few ($m_i \leq \lambda_i$) markers as observed in linkage group *i*under the null hypothesis that true marker density does not differ between linkage groups. This test is two-tailed, so a p-value of 0.025 corresponds to a significance value of 0.05

Table S3.5 Magnitude and Direction of QTL Effects on Trait Values. Each QTL was Partitioned into Additive (a), Dominance (d), and Difference between Heterozygous Classes (i), Cytoplasmic (c), and Cytoplasmic Interaction Effects in Separate Generalized Linear Models. Additive and dominance direction display effects of nuclear alleles from northern (Danish and Swedish) populations. The difference between heterozygote classes was calculated by subtracting heterozygotes with Danish/French alleles from those with Swedish/Italian alleles. Cytoplasmic effects display direction of northern (Danish) cytoplasmic alleles. ~ LL.Cool.2 by cytoplasm interaction p-value < 0.05 in best *fitqtl* model. $ p-value = 0.061 for LL.Cool.3 in secondary *fitqtl* model.

A. REFLECTANCE PLASTICITY

	REF.Plasticity.6	SE	p	
Intercept	22.10	0.61	<2e-16	***
a	9.27	1.01	<2e-16	***
d	2.23	2.14	0.297	
i	4.33	1.63	0.008	**

B. REFLECTANCE COOL

	REF.Cool.6	**SE**	**p**	
Intercept	68.95	0.62	<2e-16	***
a	-9.92	1.02	<2e-16	***
d	2.27	2.17	0.298	
i	-4.70	1.66	0.005	**

C. REFLECTANCE WARM

	Ref.Warm.6	**SE**	**p**	
Intercept	90.88	0.10	<2e-16	***
a	-0.88	0.28	0.002	**
d	-0.83	0.29	0.005	**
i	-0.25	0.15	0.098	^

D. FLOWERING TIME PLASTICITY

	FT.Plasticity.2	SE	p	
Intercept	-48.39	0.75	< 2e-16	***
a	-8.08	1.91	2.82E-05	***
d	-0.97	2.72	0.722	
i	-3.35	1.41	0.018	*

E. FLOWERING TIME COOL

	FT.Cool.2	SE	p		FT.Cool.4	SE	p		FT.Cool.6	SE	p	
Intercept	73.11	0.69	< 2e-16	***	72.56	0.96	< 2e-16	***	72.55	0.71	< 2e-16	***
a	7.96	1.67	2.45E-06	***	-1.29	1.89	0.493		0.43	1.72	0.805	
d	-2.61	2.65	0.326		2.32	3.77	0.539		-1.27	2.48	0.608	
i	3.47	1.47	0.019	*	-1.52	2.40	0.526		5.21	1.32	9.67E-05	***
Cytoplasm	-	-	-	-	-1.25	1.42	0.380		-	-	-	-
a:Cyto.	-	-	-	-	-1.66	2.83	0.558		-	-	-	-
d:Cyto.	-	-	-	-	-2.10	5.78	0.716		-	-	-	-
i:Cyto.	-	-	-	-	-10.67	3.76	0.005	**	-	-	-	-

F. FLOWERING TIME WARM

	FT.Warm.4	SE	p	
Intercept	25.33	0.65	<2e-6	***
a	-1.99	1.40	0.156	
d	7.84	2.86	0.006	**
i	-8.57	1.74	1.23E-06	***

G. ROSETTE DIAMETER COOL

	ROS.Cool.4	SE	p		ROS.Cool.6	SE	p	
Intercept	17.13	0.20	<2e-16	***	17.28	0.20	<2e-16	***
a	1.27	0.44	0.005	**	-1.84	0.53	5.25E-04	***
d	-1.57	0.88	0.076	^	0.94	0.69	0.177	
i	-0.09	0.53	0.860		0.29	0.35	0.401	

H. ROSETTE DIAMETER WARM

	ROS.Warm.2	SE	p		ROS.Warm.3	SE	p		ROS.Warm.4	SE	p	
Intercept	22.07	0.30	< 2e-16	***	21.83	0.32	< 2e-16	***	22.14	0.29	< 2e-16	***
a	-2.32	0.67	6.09E-04	***	3.39	0.92	2.68E-04	***	2.28	0.49	4.08E-06	***
d	2.17	1.21	0.073	^	1.25	1.45	0.389		-1.12	0.98	0.254	
i	-1.76	0.59	0.003	**	-1.45	0.70	0.038	*	0.71	0.59	0.234	
Cytoplasm	1.39	0.44	0.002	**	1.11	0.47	0.018	*	1.70	0.43	7.64E-05	***
a:Cyto.	-	-	-	-	2.53	1.51	0.094	^	-	-	-	-
d:Cyto.	-	-	-	-	-2.27	2.52	0.368		-	-	-	-

i:Cyto.	-	-	-	-	-2.59	1.18	0.028	*	-	-	-	-

	ROS.Warm.6	**SE**	**p**	
Intercept	22.01	0.31	< 2e-16	***
a	-1.46	0.64	0.023	*
d	2.50	0.67	2.29E-04	***
i	0.49	0.35	0.161	
Cytoplasm	1.49	0.43	5.58E-04	***
a:Cyto.	-	-	-	-
d:Cyto.	-	-	-	-
i:Cyto.	-	-	-	-

I. LEAF LENGTH PLASTICITY

	LL.Plasticity.3	**SE**	**p**	
Intercept	3.34	0.20	<2e-16	***
a	1.27	0.43	0.003	**
d	-0.14	0.73	0.848	
i	0.06	0.38	0.876	
Cytoplasm	0.81	0.28	0.003	**

J. LEAF LENGTH COOL

	LL.Cool.2	**SE**	**p**		**LL.Cool.4**	**SE**	**p**		**LL.Cool.6a**	**SE**	**p**	
Intercept	10.39	0.13	<2e-16	***	10.64	0.17	<2e-16	***	10.42	0.14	<2e-16	***
a	-1.13	0.39	0.004	**	0.92	0.30	0.002	**	-0.87	0.21	3.55E-05	***
d	0.50	0.67	0.459		-1.55	0.59	0.008	**	-1.08	0.42	0.011	*
i	-1.35	0.35	1.06E-04	***	0.06	0.35	0.876		0.72	0.44	0.099	^
Cytoplasm	-	-	-	-	0.51	0.26	0.045	*	-	-	-	-
	LL.Cool.6b	**SE**	**p**		**LL.Cool.2$**	**SE**	**p**		**LL.Cool.3$**	**SE**	**p**	
Intercept	10.51	0.13	<2e-16	***	10.52	0.18	<2e-16	***	10.40	0.14	<2e-16	***
a	-1.49	0.35	2.43E-05	***	-1.09	0.40	0.006	**	0.87	0.41	0.033	*
d	1.20	0.46	0.009	**	0.46	0.67	0.493		1.27	0.66	0.055	^
i	0.21	0.23	0.361		-1.32	0.35	1.70E-04	***	-0.13	0.33	0.701	
Cytoplasm	-	-	-	-	0.28	0.26	0.277		-	-	-	-

K. LEAF LENGTH WARM

	LL.Warm.1	SE	p		LL.Warm.2	SE	p		LL.Warm.3	SE	p	
Intercept	13.85	0.23	< 2e-16	***	13.87	0.22	< 2e-16	***	13.80	0.23	< 2e-16	***
a	-0.07	0.53	0.894		-1.71	0.47	3.52E-04	***	2.25	0.68	9.36E-04	***
d	2.04	0.55	2.24E-04	***	1.25	0.81	0.125		2.33	1.07	0.029	*
i	0.49	0.28	0.085	^	-1.74	0.43	6.25E-05	***	-0.34	0.51	0.501	
Cytoplasm	1.14	0.32	4.23E-04	***	1.07	0.32	8.76E-04	***	0.94	0.34	0.006	**
a:Cyto.	-	-	-	-	-	-	-	-	2.46	1.11	0.027	*
d:Cyto.	-	-	-	-	-	-	-	-	-2.39	1.85	0.197	
i:Cyto.	-	-	-	-	-	-	-	-	-1.95	0.86	0.025	*
	LL.Warm.4a	**SE**	**p**		**LL.Warm.4b**	**SE**	**p**		**LL.Warm.6**	**SE**	**p**	
Intercept	14.03	0.22	< 2e-16	***	14.05	0.21	< 2e-16	***	13.90	0.23	< 2e-16	***
a	1.70	0.36	3.78E-06	***	1.18	0.34	6.24E-04	***	-0.98	0.47	0.039	*
d	-1.25	0.73	0.085	^	-2.50	0.62	6.87E-05	***	1.88	0.50	1.79E-04	***
i	-0.01	0.44	0.975		-0.75	0.38	0.048	*	-0.06	0.26	0.804	
Cytoplasm	1.37	0.32	1.71E-05	***	1.35	0.31	1.92E-05	***	1.21	0.32	1.70E-04	***
a:Cyto.	-	-	-	-	-	-	-	-	-	-	-	-
d:Cyto.	-	-	-	-	-	-	-	-	-	-	-	-
i:Cyto.	-	-	-	-	-	-	-	-	-	-	-	-

L. FRESH MASS COOL

	Mass.Cool.3	SE	p		Mass.Cool.5	SE	p		Mass.Cool.6	SE	p	
Intercept	0.43	0.01	<2e-16	***	0.46	0.01	<2e-16	***	0.44	0.01	<2e-16	***
a	0.06	0.04	0.070	^	-0.08	0.04	0.040	*	-0.09	0.03	0.003	**
d	0.13	0.06	0.029	*	-0.04	0.08	0.658		0.06	0.03	0.081	^
i	-0.01	0.03	0.634		-0.08	0.04	0.073	^	0.01	0.02	0.543	
Cytoplasm	-	-	-	-	0.05	0.02	0.019	*	-	-	-	-

M. FRESH MASS WARM

	Mass.Warm.1	SE	p		Mass.Warm.2	SE	p		Mass.Warm.3	SE	p	
Intercept	0.53	0.02	< 2e-16	***	0.54	0.02	< 2e-16	***	0.54	0.02	< 2e-16	***
a	0.00	0.04	0.895		-0.09	0.03	0.012	*	0.08	0.04	0.033	*
d	0.14	0.04	2.80E-04	***	0.13	0.06	0.026	*	0.26	0.06	1.16E-05	***
i	0.01	0.02	0.597		-0.12	0. 03	5.48E-05	***	0.03	0.03	0.229	
Cytoplasm	0.10	0.02	9.49E-06	***	0.10	0.02	1.93E-05	***	0.10	0.02	3.42E-06	***

	Mass.Warm.4	SE	p		Mass.Warm.6	SE	p	
Intercept	0.55	0.01	< 2e-16	***	0.54	0.02	< 2e-16	***
a	0.04	0.02	0.125		-0.06	0.02	0.001	**
d	-0.13	0.04	0.003	**	-0.10	0.04	0.007	**
i	-0.08	0.03	0.005	**	-0.01	0.04	0.738	
Cytoplasm	0.11	0.02	5.06E-07	***	0.10	0.02	2.81E-06	***

N. LEAF AREA COOL

	LA.Cool.2	SE	p		LA.Cool.3	SE	p		LA.Cool.4	SE	p	
Intercept	11.73	0.25	< 2e-16	***	11.69	0.26	< 2e-16	***	12.37	0.33	< 2e-16	***
a	-0.84	0.73	0.249		1.83	0.79	0.021	*	0.01	0.51	0.986	
d	-1.39	1.31	0.288		2.36	1.29	0.068	^	-0.15	0.97	0.876	
i	-2.08	0.66	0.002	**	-0.72	0.63	0.251		-2.66	0.61	1.52E-05	***
Cytoplasm	-	-	-	-	-	-	-	-	1.22	0.49	0.014	*

	LA.Cool.5	SE	p	
Intercept	12.50	0.33	< 2e-16	***
a	-1.61	0.95	0.092	^
d	-1.57	1.87	0.401	
i	-1.27	0.99	0.203	
Cytoplasm	1.31	0.50	0.009	**

O. LEAF AREA WARM

	LA.Warm.2	SE	p		LA.Warm.3	SE	p		LA.Warm.4	SE	p	
Intercept	13.83	0.35	< 2e-16	***	13.86	0.35	< 2e-16	***	14.10	0.34	< 2e-16	***
a	-1.98	0.79	0.012	*	1.81	0.81	0.026	*	0.80	0.53	0.130	
d	2.65	1.35	0.051	^	5.87	1.30	8.57E-06	***	-2.26	1.01	0.026	*
i	-2.85	0.67	2.31E-05	***	0.37	0.58	0.528		-2.31	0.61	1.86E-04	***
Cytoplasm	2.10	0.50	3.62E-05	***	2.22	0.49	6.87E-06	***	2.53	0.50	5.27E-07	***

P. GERMINATION

	Germination.1	SE	p		Germination.2	SE	p		Germination.3	SE	p	
Intercept	10.65	0.32	< 2e-16	***	10.45	0.32	2.00E-16	***	10.11	0.35	< 2e-16	***
a	-0.64	0.63	0.307		0.12	0.64	0.846		3.78	1.04	2.92E-04	***
d	3.80	0.96	9.29E-05	***	-	1.21	0.949		7.40	1.62	6.30E-06	***

i	0.28	0.50	0.579		-2.63	0.59	1.21E-05	***	1.31	0.58	0.024	*
Cytoplasm	-1.11	0.46	0.016	*	-1.28	0.46	0.006	**	-1.32	0.46	0.004	***

	Germination.5	**SE**	**p**	
Intercept	10.62	0.33	< 2e-16	***
a	-2.86	1.23	0.020	*
d	7.89	2.12	2.24E-04	***
i	-3.46	1.03	8.16E-04	***
Cytoplasm	-1.01	0.48	0.036	*

CHAPTER IV

CONCLUSIONS

My geographic study is, to the best of our knowledge, is the first to have provided genetic evidence that the geographic variation in the plasticity of a trait has resulted from contributions of adaptive divergence and neutral evolutionary forces. I found evidence that natural selection has significantly contributed to the latitudinal pattern of temperature-sensitive floral reflectance plasticity in *P. lanceolata.* My research provides strong evidence this trait has evolved as an adaptation to thermally variable cool and short environmental conditions. As between-population differences in these environmental variables increased, phenotypic differentiation of thermal plasticity increased more quickly than did neutral genetic differentiation. Genetic data did not support the hypothesis that the magnitude of thermal variation influenced geographic patterns of thermal plasticity.

My genetic mapping of thermal plasticity is, to the best of my knowledge, the first to report geographic patterns of genetic information that parallel phenotypic patterns of thermal plasticity. Phenotypic patterns and genetic architectures underlying thermal plasticities and single environment trait values were trait-specific. I found one single QTL underlying the thermal plasticities of three traits, floral reflectance, flowering time and leaf length, confirming that thermal plasticity in *P. lanceolata* has a genetic basis. I found evidence that plasticity QTLs of these traits were not pleiotropic, suggesting that plasticities of these traits are free to evolve independently. Additionally, genotypic differences at plasticity QTLs paralleled phenotypic patterns of plasticity along latitudinal clines. Northern genotypes increased the magnitude of

plasticity, while southern genotypes decreased plasticity. These findings provide genetic support that observed latitudinal clines of thermal plasticity of floral reflectance reflect adaptation to local conditions. These findings suggest thermal responses in a suite of different, unrelated traits are adaptive in thermally variable environments with cool and short growing seasons.

Genetic independence of thermal plasticities provides the potential for genetic manipulation of thermal plasticity in one, or a suite of selected traits. If crop species also display genetically independent thermal plasticities, then agricultural breeding programs may be able to select for specific plasticities in crops. My observation that greater plasticity improves fitness in cool environments with short growing seasons in *Plantago lanceolata* suggests that breeders may be able to improve crop yields in northern environments by selecting for greater thermal plasticity.

Finally, phenotypic plasticity provides organisms with the potential to respond rapidly to changes in their environment and has been proposed as a mechanism for coping with contemporary climate change. My dissertation research highlights some useful points when evaluating this idea. Global climate change is occurring via widespread temperature increases, regional changes in precipitation and local land-use changes. Whether or not plasticity, or the evolution of plasticity, can ameliorate the effects of these changes depends on several factors. Among these are the range and nature of phenotypic plasticity organisms display in response to environmental cues and the standing genetic variation in plasticity they possess.

At present clarity about the selective factors that have created current levels of plasticity is limited. Our data show geographic and genetic variation in thermal plasticity. Thus, as warming proceeds, these plastic individuals are likely to lead any pole-ward migration, given dispersal capability. If southern populations are genetically variable for thermal plasticity, as is true for *P. lanceolata* flowers, the reproductive organs, then they may also survive warming, but

thermal plasticity is likely to diminish or evolve in a direction toward greater tolerance to a warm climate. Given genetic recombination, populations should persist and perhaps evolve in response to some further lengthening of the reproductive season and more time at warmer temperatures. What is unknown, however, is where the upper thermal limit lies, a parameter that will be critical when evaluating the amount of environmental change that allows for long-term persistence. This represents a big gap in our understanding for most species.

CHAPTER V

FUTURE DIRECTIONS FOR THE STUDY OF THERMAL PLASTICITY

Future studies should validate the adaptive value of thermal plasticity in natural environments by determining whether individuals possessing local alleles at the plasticity QTLs I identified display higher fitness than those with foreign alleles in reciprocal transplant experiments between northern and southern populations. Ultimately we would like to identify the specific genes underlying thermal plasticity. With this information we could identify the nucleotide sequence differences among alleles of plasticity genes and the amino acid differences that influence function of plasticity proteins. The development of a genetic map with higher resolution and the sequencing of the *P. lanceolata* genome will assist researchers in fine mapping the thermal plasticity QTLs I identified in this dissertation, and in determining which genes lie at these loci. Temperature sensitive gene expression analyses can complement these approaches by confirming candidate genes display temperature sensitivity and identifying natural allelic variants.