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Cyanogenesis and the Genetics of Local Adaptation in White Clover (Trifolium repens L.)

Sara Jeanes Wright
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Ecology, Evolution and Population Biology

Dissertation Examination Committee:
Kenneth Olsen, Chair
Christine Edwards
Justin Fay
Allan Larson
Jonathan Myers
David Queller

Cyanogenesis and the Genetics of Local Adaptation in White Clover (*Trifolium repens L.*)

by

Sara Jeanes Wright

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Sara J. Wright
Washington University in St. Louis
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Dedicated to my “village”

ABSTRACT OF THE DISSERTATION

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by

Sara J. Wright

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Professor Kenneth M. Olsen, Chair

Geographically widespread species experience varied selection across their ranges, and adaptation to local environments plays a critical role in their ability to persist. Understanding the genetic basis of local adaptation is a longstanding goal in evolutionary biology and provides practical information for agriculture and conservation. However, the genetic architecture of local adaptation has been characterized in relatively few plant species, primarily those with short lifespans and high rates of self-fertilization. Moreover, for plants, chemical defenses are known to play an important role in adaptation, but the extent to which they contribute to local adaptation is less understood. This dissertation provides a genome-wide, multi-environment assessment of the importance of a well-studied chemical defense polymorphism for local adaptation, relative to other genetic factors, and addresses fundamental questions in evolutionary biology about the genetic architecture of local adaptation in an outcrossing plant.

White clover (*Trifolium repens L*.) is a perennial, obligately outcrossing legume and an important forage crop. Naturalized populations occur across a wide range of

climates, from subtropical to near arctic. White clover populations display adaptation related to a chemical defense polymorphism, cyanogenesis—the production of hydrogen cyanide upon tissue damage. Adaptive cyanogenesis clines have repeatedly evolved across the species range, such that higher proportions of cyanogenic plants are found in warmer climates. However, the relative adaptive importance of the cyanogenesis polymorphism for local adaptation, compared to other genetic factors, is unknown.

Chapter 1 in this dissertation provides evidence of local climatic adaptation in white clover by documenting correlations between fitness traits and home-site climate variation for 15 widespread populations grown in a central North American common garden experiment. Chapter 2 demonstrates that divergent life history strategies associated with early flowering versus multi-year persistence contribute to local adaptation across three common garden experiments in locations spanning the U.S. latitudinal range of white clover. It also suggests that allelic trade-offs at major-effect loci are common for local adaptation in this outcrossing species. We did not find significant fitness differences that were attributable to cyanogenesis in the experiments presented in Chapters 1 and 2, which focused on mature adult plants; however, Chapter 3 documents significant shifts in cyanogenesis frequencies from the seedling to adult life stages and also from benign greenhouse to field germination environments, specifically at the seedling stage, across three environments. These results suggest that cyanogenesis may be more important for local adaptation at the earliest life stages, thereby promoting clinal evolution in this chemical defense trait.

INTRODUCTION

Historical Context

Organisms tend to be well adapted to the environments where they are found — that is, they often possess traits that make them successful in their environment. Adaptation has long intrigued biologists and was originally thought to be the work of a creator. A typological paradigm was dominant, and minor variations among individuals within the same species were largely ignored. Lamarck was one of the first biologists to propose the idea of biological evolution, which shifted focus onto the importance of variation between individuals. Darwin's seminal book *On the Origin of Species* later proposed natural selection as a non-random evolutionary mechanism that leads to change in populations over time (1859). Darwin's theory of natural selection suggested that, due to competition for finite resources, individuals possessing the traits best suited to their environment will leave more offspring, thereby bringing the underlying heritable genetic variations to higher frequencies in future generations. Adaptation can thus be explained as the result of this process acting on variation over many generations.

By synthesizing Mendel's laws of genetic inheritance with Darwin's theory in the early 20th century, the new statistical field of population genetics began developing quantitative models of microevolution (Provine, 1971). Fisher, Wright and Haldane were major early contributors to this "Modern Synthesis" and the field of population genetics. Fisher first proposed the "Fundamental Theorem of Natural Selection," defining the mathematical relationship between genetic variance and rate of change in fitness due to natural selection (Fisher, 1930). Wright and Haldane emphasized the need to consider factors other than natural selection, which may affect the evolution of populations in the same or different directions as that of selection. Wright studied

interactions within and among demes, proposing ideas about mutation, mating system, and genetic drift (Wright, 1932, 1949). Among Haldane's many contributions, he wrote about the evolution of genetic clines that can result from the interaction between selection and gene flow across environmental gradients (Haldane, 1948, 1976).

Early studies of adaptation across heterogeneous environments in plant species employed common garden experiments, whereby many genotypes of the same species were grown in contrasting environments (J Clausen, Keck and Hiesey, 1941; Jens Clausen, Keck and Hiesey, 1941); these studies empirically demonstrated that populations are adapted to their local environments. Increased recognition that fitness depends on environmental context led to early models for the genetics of local adaptation (Levene, 1953). However, the lack of molecular technologies for assessing population-level heritable polymorphisms made it difficult to identify locally-adaptive genetic variation, as well as to test theoretical predictions in the field of population genetics.

Advances in molecular biology techniques in later decades of the 20th century and subsequent advances in the field of genomics have vastly accelerated empirical progress in population genetics (Nadeau and Jiggins, 2010; Stapley *et al.*, 2010; Visscher and Goddard, 2019), as well as our ability to study the molecular basis of adaptation across many study systems and heterogeneous environments (Savolainen, Lascoux and Merilä, 2013; Bragg *et al.*, 2015; Hoban *et al.*, 2016). The chapters presented in this dissertation leverage next-generation advances in sequencing technologies (Elshire *et al.*, 2011), in combination with classical common garden experiments and longstanding quantitative genetics approaches (Haley and Knott,

1992), to study the genetic basis of local adaptation in an herbaceous, perennial plant species (Anderson *et al.*, 2014).

Local Adaptation

Species that are broadly geographically distributed often experience large amounts of environmental heterogeneity among their populations. Natural selection resulting from differences in local climates or biotic communities can thus lead to adaptive divergence across populations. Local adaptation is said to have occurred when the fitness of local genotypes is highest in their respective local habitats, which produces a deme × habitat interaction (Kawecki and Ebert, 2004).

Local adaptation is most likely to occur in species with large population sizes, which have the ability to harbor large amounts of standing genetic variation and to generate high numbers of new mutations — the source of all evolutionary change and adaptation. In contrast, genetic drift and low additive genetic variation may prevent local adaptation in small populations (Kawecki and Ebert, 2004; Leimu and Fischer, 2008). Local adaptation helps to maintain genetic variation and polymorphism within a species (Hedrick, 1986). It can also serve as a route to speciation when gene flow is restricted or when ecological specialization in different habitats leads to reproductive isolation (Sobel *et al.*, 2010).

Local adaptation is not an inevitable path for widespread species with large populations; it is a possible result of the balance between selection and gene flow. Provided there is sufficient additive genetic variation, isolated populations with no gene flow are expected to undergo local adaptation and eventually speciation, whereas high

levels of gene flow may prevent differentiation and local adaptation (Barton, 1999; Lenormand, 2002; Savolainen, Pyhäjärvi and Knürr, 2007; Polechová and Barton, 2015). Furthermore, metapopulations with extinction-colonization dynamics are unlikely to undergo local adaptation (Hanski, 1999). In populations experiencing temporally varying selection, phenotypically plastic individuals may be favored over those that are locally adapted to a specific set of environmental conditions (Chevin, Lande and Mace, 2010; Nicotra *et al.*, 2010).

Despite the fact that many factors may oppose local adaptation, it has been frequently observed in herbaceous plant species with large population sizes (Leimu and Fischer, 2008). In the face of global climate change, local adaptation has therefore become increasingly invoked as an important potential mechanism for the evolutionary persistence of sessile plant species, which cannot rapidly migrate out of unfavorable locations (Anderson, 2016). Identifying locally adaptive traits and characterizing the underlying genetic architecture of local adaptation in plants is therefore a major goal in the fields of agriculture and conservation biology.

The genetic architecture of local adaptation

Despite many examples of local adaptation (Hereford, 2009), we know surprisingly little about its genetic basis due to experimental and technological limitations. Experimentally, measuring lifetime fitness for large numbers of individuals in multiple environments is simply a sizeable undertaking. Doing so with access to complementary genetic data is rare. Previous field studies in plants have therefore largely been limited to a small number of experimentally tractable species and their

close relatives (Rushworth *et al.*, 2011; Ågren *et al.*, 2013; Yant and Bomblies, 2017; Price *et al.*, 2018). Advances in sequencing technologies have allowed researchers to begin evaluating the genetic architecture of local adaptation in a broader number of plant species, including non-model organisms (Anderson, Willis and Mitchell-Olds, 2011). Developments in the field of statistical genomics have further increased the ability to identify locally-adaptive genetic variation with correlational and outlier methods that do not require empirical fitness data (Neale and Savolainen, 2004; De Mita *et al.*, 2013; Savolainen, Lascoux and Merilä, 2013; Hoban *et al.*, 2016).

Several basic questions still remain unanswered. For example, at the gene or locus level, is local adaptation due to a few genes of large effect, many genes of small effect, or some combination of effect sizes? In other words, what is the effect size distribution of loci underlying local adaptation? Fewer contributing loci with larger effect sizes are predicted, as selection can act strongly on them in the face of gene flow (Hedrick, 1986; Yeaman and Whitlock, 2011). However, local adaptation could also result from the combined action of many small-effect loci (Whitlock, 2015; Yeaman, 2015). In species studied to date, heterogeneous effect size patterns are seen, with a common trend of a few large-effect loci and many small-effect loci contributing to local adaptation (Ågren *et al.*, 2013; Savolainen, Lascoux and Merilä, 2013). Loci with large effects on locally-adaptive quantitative traits (e.g., flowering time) have been identified more often for the self-fertilizing *A. thaliana* than in outcrossing forest tree species (González-Martínez *et al.*, 2008; Ingvarsson *et al.*, 2008; Eckert *et al.*, 2009; Atwell *et al.*, 2010; Alberto *et al.*, 2013).

Related questions about the genetic architecture of local adaptation arise at the allelic level and are concerned with the prevalence of antagonistic pleiotropy at locally-adaptive loci (i.e., QTL × E interactions). At any given locus, an allele may be favored in one environment and selectively neutral in others, thus facilitating local adaptation. This conditional neutrality is predicted to lead to local adaptation when gene flow is limited between populations; however, it is expected always to be transient, as even modest amounts of gene flow would be expected to lead to the fixation of conditionally-neutral alleles across loci in all populations (Gavrilets and Gibson, 2002; Kawecki and Ebert, 2004). Instead, antagonistic pleiotropy — i.e., allelic trade-offs for fitness in contrasting environments — is expected to be the major cause of local adaptation (Anderson, Willis and Mitchell-Olds, 2011).

Previous studies in plant species have been mixed in their ability to detect antagonistic pleiotropy, with conditional neutrality often detected more readily (Price et al. 2018; reviewed in Savolainen, Lascoux, and Merilä 2013). Researchers have noted a statistical bias against detecting antagonistic pleiotropy, due to the difficulties of demonstrating significant genotypic fitness differences in two or more environments (a criterion not required for conditional neutrality) (Anderson *et al.*, 2013). This pattern may also be an artifact of the species and populations chosen for study. Most have focused on systems that contain inherently low levels of pollen-mediated gene flow, such as highly self-fertilizing species (*e.g., Arabidopsis thaliana, Boechera stricta*, *Avena barbata,* and *Hordeum spontaneum*). Low effective recombination in self-fertilizing species could slow the displacement of conditionally neutral alleles; antagonistic pleiotropy may therefore be more prevalent in outcrossing species, where

higher effective recombination creates lower levels of linkage disequilibrium and allows for more rapid introgression of adaptive alleles into a population.

This dissertation characterizes the genetic architecture of local adaptation in an outcrossing plant species and identifies optimal life history strategies in contrasting environments that span a North American latitudinal gradient. Furthermore, it assesses the contribution of a well-studied, adaptive chemical defense polymorphism for local adaptation by describing its importance in different life stages and also in comparison to other genetic polymorphisms that are associated with fitness trade-offs across environments.

Study System

White clover (*Trifolium repens* L., Fabaceae) is an obligately outcrossing, perennial, herbaceous plant species with large population sizes that would be expected to facilitate local adaptation. In addition to being pollinated by generalists and producing large quantities of seed, white clover spreads vegetatively by stolons and forms large clonal mats. The species exhibits little population structure across its global range (George *et al.*, 2006; Kooyers and Olsen, 2012, 2013). This feature facilities characterization of the genetics of local adaptation, since population structure can be a major confounding factor in distinguishing between locally-adapted alleles and neutral variants (De Mita *et al.*, 2013; de Villemereuil and Gaggiotti, 2015).

White clover is native to Europe and was important as a source of nitrogen fertilizer before the advent of synthetic fertilizers. It was introduced worldwide by humans and is now ubiquitous across mesic temperate and cool tropical regions

(Kjærgaard, 2003). In North America, it was widely and intentionally introduced and naturalized within the last 500 years. As a nitrogen-fixing legume, it remains important as a human commensal and forage crop for grazing livestock (USDA, 2002; Abberton and Thomas, 2010). Understanding the genetics of local adaptation in white clover is therefore valuable for clover breeders.

The cyanogenesis polymorphism in white clover

Cyanogenesis (the ability to produce hydrogen cyanide, HCN, following tissue damage) is a chemical defense that has evolved multiple times across the plant kingdom and can be found in >3,000 plant species (Møller, 2010). Two spatially separated biochemical components are necessary to produce HCN in white clover: 1) cyanogenic glucosides (CNglcs), specifically lotaustralin and linamarin, which are stored in the vacuoles of photosynthetic tissue, and 2) their hydrolyzing enzyme linamarase, a cyanogenic β-glucosidase present in the apoplast (Gleadow and Møller, 2014). Unlike most cyanogenic plant species, where all individuals within the species constitutively produce both CNglcs and their hydrolyzing enzymes, white clover is characterized by a genetic polymorphism for cyanogenesis, with both cyanogenic and acyanogenic plants occurring in natural populations.

The physiological, biochemical and genetic bases of the cyanogenesis polymorphism are well characterized in white clover. Cyanogenic plants readily release HCN if tissue damage causes cell rupture to bring the two cyanogenic precursors into contact (Armstrong, Armstrong and Horton, 1913; Rigg, Askew and Kidson, 1933). Acyanogenic plants may lack one or both of the required precursors. The

presence/absence of the two cyanogenic components is controlled by two independently segregating (unlinked) simple Mendelian genetic polymorphisms, where a dominant allele at each gene confers the presence of the component. Specifically, the *Ac/ac* polymorphism controls the presence/absence of CNglcs, and the *Li/li* polymorphism controls the presence/absence of linamarase (Williams, 1939; Coop, 1940; Melville and Doak, 1940; Corkill, 1942). At the molecular level, the *Li/li* polymorphism is a genomic presence/absence polymorphism for the locus encoding the protein precursor of the linamarase glycoprotein (Olsen, Sutherland and Small, 2007); similarly, at the molecular level the *Ac/ac* polymorphism locus corresponds to a genomic presence/absence polymorphism for the 138-kb genomic region that contains the three genes encoding the three-step CNglc biosynthetic pathway (*CYP79D15*, *CYP736A187*, *UGT85K17*) (Olsen and Small, 2018). For both the *Ac/ac* and *Li/li* polymorphisms, the recessive (gene-deletion) alleles have evolved repeatedly in white clover and in related *Trifolium* species through recurrent gene deletion events (Olsen, Kooyers and Small, 2013, 2014). Because both polymorphisms are widely distributed throughout the species range, four cyanogenesis phenotypes or 'cyanotypes' can be found in wild white clover populations: AcLi (cyanogenic, containing both precursors); and Acli, acLi, and acli (acyanogenic, lacking one or both precursors).

Cyanogenesis clines and selective factors that maintain them

The cyanogenesis polymorphism in white clover spurred many ecological-genetic studies beginning in the 1950s. Latitudinal, altitudinal, and drought-related cyanogenesis clines in white clover have since been documented worldwide. In these

clines, higher proportions of cyanogenic plants are found in warmer and drier climates, while acyanogenic plants dominate cooler, wetter environments (Daday, 1954a, 1954b, 1958; de Araújo, 1976; Till-Bottraud, Kakes and Dommée, 1988; Caradus *et al.*, 1990). Recent studies have demonstrated the rapid evolution of cyanogenesis clines in introduced North American populations that are associated most closely with two climatic variables — minimum winter temperature, and aridity index (a function of precipitation and evapotranspiration) (Ganders, 1990; Kooyers and Olsen, 2012, 2013; Kooyers *et al.*, 2014).

Selective mechanisms that lead to climate-associated cyanogenesis clines have been suggested and tested to some extent (reviewed in Hughes 1991; N. Kooyers et al. 2018). In the case of temperature-associated clines, higher herbivore pressure in warmer environments may favor cyanogenic plants. In support of this hypothesis, many studies have provided evidence that cyanogenic plants experience reduced herbivore damage compared to acyanogenic plants; herbivore deterrence has been demonstrated for diverse species including chewing insects, gastropods (slugs and snails), and small mammals (Whitman, 1973; Angseesing, 1974; Dritschilo *et al.*, 1979; Dirzo and Harper, 1982; R. Dirzo and Harper, 1982; Horrill and Richards, 1986; Burgess and Ennos, 1987; Kakes, 1989; Pederson and Brink, 1998; Saucy *et al.*, 1999; Viette, Tettamanti and Saucy, 2000). Two main hypotheses have been proposed to explain the prevalence of acyanogenic plants in cooler climates: 1) cell rupture due to freezing temperatures leads to “autotoxicity” in cyanogenic plants and thus low fitness in colder climates, or 2) fewer herbivores are present in cooler climates, which confers a competitive advantage to acyanogenic plants that invest energetically in growth and reproduction rather than in

costly and unnecessary anti-herbivore defenses. No evidence for the autotoxicity hypothesis has been found in controlled freezing experiments in *T. repens* (Olsen and Ungerer, 2008; Kooyers *et al.*, 2018). The growth-defense trade-off hypothesis is supported by evidence of greater and earlier reproductive output and higher competitive ability for plants lacking cyanogenesis components in controlled greenhouse conditions and under cooler, wetter conditions (Daday, 1965; Foulds and Grime, 1972; R. A. Ennos, 1981; R. Ennos, 1981; Kakes, 1989).

Besides functioning in the anti-herbivore defense response, CNglcs may also serve as nitrogen storage and transport compounds that may be particularly adaptive in drought-prone environments (since they can provide a readily-metabolized source of reduced nitrogen) (Møller, 2010; Burke *et al.*, 2013; Kooyers, 2015). Consistent with this function, higher frequencies of CNglc-producing plants (both AcLi and Acli cyanotypes) are found in white clover populations occurring in drier environments (Kooyers and Olsen, 2013; Kooyers *et al.*, 2014). This proposed adaptive function is further supported by growth chamber experiments, which have demonstrated a reproductive fitness advantage for CNglc-producing plants under conditions simulating a moderate, long-term drought (Kooyers *et al.*, 2014). Considered together with the extensive literature on temperature-associated clinal variation at the cyanogenesis loci, these studies suggest that multiple selective factors are likely at play in the evolution of the *Ac/ac* and *Li/li* polymorphisms.

Chapters of the Dissertation

While it is abundantly clear that the cyanogenesis polymorphism is adaptive in white clover and has repeatedly evolved clinal variation throughout the species range, it does not necessarily follow that populations of this species will exhibit strong evidence of local adaptation, since high levels of interpopulation gene flow could inhibit this evolutionary process (Savolainen, Pyhäjärvi and Knürr, 2007; Lenormand and Raymond, 2017). If populations are locally adapted, the cyanogenesis polymorphism may or may not contribute substantially to locally-adaptive fitness traits (Su, Lam and Bürger, 2019). Furthermore, little is known about when in a plant's life cycle the cyanogenesis polymorphism experiences the strongest selection in natural settings. Relatively few of the previous white clover studies that have aimed to assess cyanogenesis-related fitness effects were performed in natural environments; fewer still have directly assessed the fitness variation at the seedling life stage, and none have assessed the seedling stage in multiple natural environments. This dissertation aims to address all of these shortcomings using field experiments performed in multiple years, multiple environments, and across multiple life stages. This work also represents the first study in white clover to take advantage of next generation sequencing technology for genetic mapping of fitness traits; this approach can directly assess the relative contributions of the cyanogenesis genes vs. other genome-wide variants for local adaptation across highly contrasting climates.

Chapter 1 of the dissertation evaluates the relationships between growth and reproductive fitness traits, cyanogenesis variation, and continent-wide climatic variation for 15 widespread North American populations grown in a centrally located common garden environment. It assesses whether populations exhibit correlations between their

climate-of-origin and the common garden environment that are indicative of local adaptation, and it employs linear mixed modeling approaches to determine which climatic factors have the greatest effects on different aspects of fitness (i.e., growth vs. reproduction). It further assesses the relationship between cyanogenesis variation and fitness in a single field environment. This chapter has been published as part of a *Journal of Heredity* special issue on local adaptation (Wright *et al.*, 2017).

In Chapter 2, reciprocal common garden experiments in three field environments that span a United States latitudinal gradient were used to evaluate local adaptation across contrasting climates. These experiments assess the role of cyanogenesis variation in contributing to fitness trade-offs across environments, as would be predicted by clinal patterns. Experimental F_2 mapping populations were created and used for these experiments, so that the contribution of the cyanogenesis genes could be assessed in comparison to genome-wide variation that may also play a role in local adaptation. The use of genetic mapping populations in this study further provided the ability to characterize the overall genetic architecture of local adaptation in this outcrossing, perennial plant, thereby contributing valuable information for the broader evolutionary, conservation, and agricultural communities.

Finally, Chapter 3 utilizes greenhouse and field germination experiments, along with wild adult population sampling, to begin to evaluate the contribution of the seedling life stage for the evolution of cyanogenesis clines. Findings suggest that studying the adaptive role of cyanogenesis at juvenile life stages is a promising future direction for white clover research. A final Conclusions chapter summarizes and synthesizes findings of the three data chapters.

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CHAPTER 1

Continent-wide climatic variation drives local adaptation in North American white clover

ABSTRACT

Climate-associated clines in adaptive polymorphisms are commonly cited as evidence of local adaptation within species. However, the contribution of the clinally varying trait to overall fitness is often unknown. To address this question, we examined survival, vegetative growth and reproductive output in a central US common garden experiment using 161 genotypes of white clover (*Trifolium repens* L.) originating from 15 locations across North America. White clover is polymorphic for cyanogenesis (hydrogen cyanide release upon tissue damage), a chemical defense against generalist herbivores, and climate-associated cyanogenesis clines have evolved repeatedly across the species range. Over a 12 month experiment, we observed striking correlations between population of origin and plant performance in the common garden, with climatic distance from the common garden site predicting fitness more accurately than geographic distance. Assessments of herbivore leaf damage over the 2015 growing season indicated marginally lower herbivory on cyanogenic plants; however, this effect did not result in increased fitness in the common garden location. Linear mixed modeling suggested that while cyanogenesis variation had little predictive value for vegetative growth, it is as important as climatic variation for predicting reproductive output in the central US. Together, our findings suggest that knowledge of climate similarity, as well as knowledge of locally favored adaptive traits, will help to inform transplantation strategies for restoration ecology and other conservation efforts in the face of climate change.

INTRODUCTION

Geographically widespread species can experience substantial environmental heterogeneity across their ranges, and populations frequently adapt to their local climates (Jens Clausen, Keck and Hiesey, 1941; Hiesey, Clausen and Keck, 1942; Kawecki and Ebert, 2004). Local adaptation is thought to be common across all domains of life (Leimu and Fischer, 2008; Hereford, 2009). In the face of rapid climate change, the ability to adapt to local climate may be particularly important for plant species due to their sessile nature (Alberto *et al.*, 2013). Studies of local adaptation in plants are therefore informative for identifying species that can readily adapt to local climate, and for quantifying the relative importance of specific traits for local adaptation. Insights into local climatic adaptation also provide useful information for conservation and restoration efforts.

Home-site fitness advantage (*i.e.,* higher fitness for the local genotype in its local environment) is central to the concept of local adaptation and has been demonstrated in plant species for many years through common garden experiments (J Clausen, Keck and Hiesey, 1941; Leimu and Fischer, 2008). Kawecki and Ebert (2004) suggested that geographic distance and/or ecological distance, defined by quantitative environmental parameters, may be used as explanatory variables for fitness variation in common garden experiments. Previous studies comparing these two distance measures have suggested that geographic distance can be a good predictor of local adaptation at broad spatial scales (>200 km) (Galloway and Fenster, 2000; Becker *et al.*, 2006). In contrast, environmental distance has generally been found to be a better predictor of success in ecological restoration experiments (Montalvo and Ellstrand, 2000; Raabová,

Münzbergová and Fischer, 2007). More generally, different contributors to total fitness, including survival, vegetative growth and flowering phenology, have been found to show different responses to a variety of climatic factors across species ranges (Olsson and Ågren, 2002; Prieto *et al.*, 2008; Haggerty and Galloway, 2011; Samis *et al.*, 2012b; Moles *et al.*, 2014; Preite *et al.*, 2015; Siepielski *et al.*, 2017).

Many studies of local adaptation have focused on polymorphic traits with simple genetic underpinnings and identifiable locally adaptive functions (*e.g.,* Colosimo *et al.*, 2005; Kivimäki *et al.*, 2007; Linnen *et al.*, 2013; Savolainen, Lascoux and Merilä, 2013; Tiffin and Ross-Ibarra, 2014). Due to the challenges of directly measuring fitness in natural settings, the importance of these traits for overall fitness and local adaptation is commonly inferred indirectly, either from observations of correlations between environmental gradients and genotype (or phenotype) frequencies (*e.g.,* Baxter *et al.*, 2010), or from short-term experiments in controlled conditions that may not generalize to natural settings (Anderson, Lee and Mitchell-Olds, 2011; Jacobs and Latimer, 2012). Fewer studies have directly assessed the fitness impact of polymorphic traits in field experiments (e.g., Hall and Willis, 2006; Wadgymar, Daws and Anderson, 2017). In an attempt to add to our understanding of the process of local adaptation, this study uses fitness measures from an experimental field plot to assess the ability of a well-documented, locally adaptive chemical defense polymorphism to predict overall fitness variation. The relative predictive values of geographic vs. environmental distance (specifically, climatic distance) for local adaptation are also explicitly examined.

The white clover cyanogenesis polymorphism

Cyanogenesis, the production of hydrogen cyanide upon tissue damage, occurs in >3000 species across the plant kingdom (Gleadow and Møller, 2014). It is generally accepted to have evolved as a chemical defense against generalist herbivores. While this trait is typically universally present in individuals of cyanogenic species, white clover (*Trifolium repens* L.) is unusual in that both cyanogenic and acyanogenic plants can be found within populations (Armstrong, Armstrong and Horton, 1913). The cyanogenesis polymorphism is manifested geographically as climate-associated clines where the frequency of cyanogenic plants decreases with increasing latitude and altitude; thus, higher proportions of cyanogenic plants are found in warmer climates. Cyanogenesis clines have evolved in both the native European species range as well as in introduced white clover populations worldwide (Daday, 1954a, 1954b; de Araújo, 1976; Till-Bottraud, Kakes and Dommée, 1988; Caradus *et al.*, 1990; Kooyers and Olsen, 2012, 2013; Kooyers *et al.*, 2014; Thompson, Renaudin and Johnson, 2016a). These patterns of repeated cline evolution provide evidence for strong selection on the cyanogenesis polymorphism and for local climatic adaptation that is specifically related to this phenotype. Proposed selective factors for cyanogenesis cline evolution include climatically varying herbivore abundance, fitness costs of cyanogenesis in colder climates, and potential benefits of cyanogenic components for functions other than herbivore deterrence (*e.g.,* cyanogenic glucosides may serve a function in drought stress adaptation) (Hughes, 1991; Kooyers and Olsen, 2012, 2013; Kooyers *et al.*, 2014; Thompson, Renaudin and Johnson, 2016a).

At the biochemical level, cyanogenesis in white clover results from the interaction of two components that are spatially separated in intact tissue, cyanogenic glucosides

and their hydrolyzing enzyme, linamarase (Gleadow and Møller, 2014). Two unlinked Mendelian genetic polymorphisms control the presence/absence of the two components (*Ac/ac* and *Li/li* for cyanogenic glucosides and linamarase, respectively); the dominant allele of each gene confers the presence of the component, and homozygous recessive genotypes lack the component (Hughes, 1991; Olsen, Kooyers and Small, 2013). At the molecular level, both *Ac/ac* and *Li/li* are gene presence/absence polymorphisms, with recessive alleles corresponding to recurrently evolved gene deletions (Olsen, Sutherland and Small, 2007; Olsen, Hsu and Small, 2008; Olsen, Kooyers and Small, 2013). Thus, four 'cyanotypes' are present in white clover populations. Cyanogenic plants (AcLi) produce both components, whereas acyanogenic plants (Acli, acLi, and acli) lack one or both components and do not produce HCN.

In this study, we performed a white clover common garden experiment in a central US location to assess local adaptation across North American white clover populations. We used 161 wild genotypes sampled from 15 geographically widespread locations to examine fitness variation as it relates to population of origin and cyanogenesis variation. While the use of a single common garden site does not allow for documentation of reciprocal home-site advantage (see discussion below), it can nonetheless reveal fitness variation as related to population of origin (Rutter, Shaw and Fenster, 2010; Preite *et al.*, 2015; Peterson, 2016). We asked the following specific questions: 1) To what extent do geographic and/or climatic distance predict fitness variation across populations? 2) Do cyanogenic plants experience less herbivore leaf damage than acyanogenic plants, resulting in higher fitness regardless of population of

origin? And 3) Which combinations of climate parameters and cyanotype best predict growth and fecundity variation in the common garden location?

MATERIALS AND METHODS

Study system

Trifolium repens is a perennial allotetraploid herbaceous legume that is obligately outcrossing and primarily bee pollinated. In addition to reproduction by seed, it spreads vegetatively by stolons, allowing for the study of multiple clonal replicates per genotype in field experiments. White clover was an important source of soil nitrogen for agriculture before the advent of synthetic fertilizers and was therefore intentionally introduced across temperate and cool tropical regions worldwide with European colonization (Kjærgaard, 2003); it remains an important temperate forage crop. Due to its history of repeated, intentional introductions, non-native populations contain extensive standing genetic variation that natural selection has acted upon (Kooyers and Olsen, 2014). White clover has extremely large effective population sizes worldwide and displays minimal population structure on continental and global scales (George *et al.*, 2006; Olsen, Sutherland and Small, 2007; Kooyers and Olsen, 2012, 2013).

Sampling

Plant samples were collected from 15 North American populations ranging from central Florida to Vancouver, British Columbia during the 2014 growing season (Figure 1.1, Table 1.1, Table S1.1). Samples were collected as stolon cuttings (STL and GFL populations) or mature seeds (all other populations) from 9-11 plants per population, for

a total of 161 unique genotypes that were used in a common garden experiment near St. Louis, MO. Collections were spaced a minimum of 5 m apart to prevent sampling multiple ramets or seed heads from the same genet; GPS coordinates were recorded for each sample. To control for potential confounding effects of sampling stolon cuttings (which represent a subset of genotypes that survived to maturity in their local climate) rather than seeds at the STL and GFL sites, analyses of local adaptation were performed both with and without those populations. For three of the populations (AOK, GFL and STL), deeper sampling was performed largely from stolon cuttings to assess neutral genetic differentiation among populations (32-48 samples per population; Table S1.1, S1.2). To calculate the geographic distance between the common garden site and sampled populations, latitudes and longitudes were averaged across samples within each population, and the great circle distance was calculated using the haversine formula (Veness, 2012; Table 1.1, Table S1.2).

All samples were grown in the Washington University (WU) greenhouse in 4” round pots filled with Metro-Mix 360 soil prior to genetic analyses and the field experiment (Table S1.1). Rooting hormone was applied to plants collected as stolon cuttings to encourage establishment on mist benches. For samples collected as seed, 10 seeds per maternal parent were scarified using fine grit sandpaper and planted in a single pot on mist benches. Upon germination, one seedling was randomly selected for use in further analyses, and others were discarded. We were not able to reduce potential maternal effects by producing a second generation of seed in the greenhouse, as self-incompatibility in white clover makes this impractical when using wild population sample collections.

Population structure analyses

For the three intensively sampled populations (AOK, GFL, STL), genomic DNA was extracted for genotyping-by-sequencing (GBS) using a DNA extraction protocol modified from Whitlock (2008) with 120-150 mg young leaf tissue (Elshire *et al.*, 2011; Table S1.2). Leaf tissue for each sample was ground in liquid nitrogen using mortars and pestles. Columns from the *IBI Scientific* Genomic DNA Mini Kit (Plant) were used for filtration and binding steps.

DNA samples were submitted to Cornell University's Institute for Genomic Diversity for library preparation and GBS using the Illumina HiSeq 2000 platform. Quality control and SNP calling were performed on raw GBS data by Cornell using the UNEAK pipeline and TASSEL v3.0.166 (Lu *et al.*, 2013). UNEAK was developed for polyploid species that lack reference genomes and provides a stringent filtering system to account for highly repetitive sequences. Read depth was calculated with VCFtools v0.1.11 (Danecek *et al.*, 2011). SNPs were called using a minor allele frequency cutoff of 0.01.

To assess genetic differentiation between the AOK, GFL and STL populations, pairwise F_{ST} values were calculated using the filtered SNP dataset in GenAlEx 6.5 (Peakall and Smouse, 2006). For a comparison to this background genomic F_{ST}, pairwise F_{ST} was also calculated separately for the *Ac* and *Li* cyanogenesis genes using genotypes inferred from cyanogenesis phenotyping and genotyping. *Ac/ac* and *Li/li* allele frequencies were calculated with the Hardy-Weinberg assumption that the frequency of homozygous recessive genotypes is equal to q^2 within each population (see Kooyers and Olsen, 2012).

Cyanogenesis phenotyping and genotyping

For each genotype, phenotyping for the presence/absence of HCN production in leaf tissue was performed using Feigl-Anger tests, as described previously (Olsen, Sutherland and Small, 2007). For acyanogenic individuals, the presence/absence of each cyanogenic component (*i.e.,* cyanogenic glucosides or linamarase) was determined by exogenous addition of the complementary component. Negative reaction results were repeated at least twice to minimize false negatives. To confirm that cyanogenesis phenotyping results corresponded to *Ac/ac* and *Li/li* gene presence/absence, DNA was extracted with the Genomic DNA Mini Kit (Plant) kits (*IBI Scientific*) using 100 mg young leaf tissue, and PCR was performed for the *Ac* and *Li* loci using previously described primers (Olsen, Sutherland and Small, 2007; Olsen, Hsu and Small, 2008). The presence of a PCR product was taken as evidence of gene presence. Negative results were confirmed by repeating the reaction at least twice. Fewer than 3% of PCR assays did not match phenotyping results.

Common garden establishment

Three replicate cuttings were made from each of the 161 unique genotypes, for a total of 483 plants (Table S1.3). Care was taken to establish cuttings of the same size, including similar root masses and numbers of leaves. Rooting hormone was applied to encourage establishment. Cuttings were grown on mist benches in the WU greenhouse for one week, and then were allowed to become established for an additional week under standard greenhouse conditions before being planted in the field.

The common garden experiment was conducted from April 2015 through March 2016. Established cuttings were transplanted to an experimental research garden plot at the WU Tyson Research Center in Eureka, MO (Figure S1.1). The experimental plot was enclosed by an underground concrete barrier to exclude burrowing rodents and by a fence to exclude deer and other large mammals. The soil substrate consisted of local, native prairie soil. Planting occurred on April 11, 2015 to coincide with the spring leaf flush of local clover populations. Replicate cuttings were planted in a blocked design to account for environmental heterogeneity across the plot; one replicate per genotype was planted in a randomized design within each of three replicate blocks. Cuttings were watered only upon transplantation, after which they were left exposed to local environmental conditions for the remainder of the 12-month experiment.

All data collection in the field plot was performed blind with respect to the cyanotype and population origin of each plant. To prevent intermingling of genotypes that would lead to inaccurate fitness measurements, plants were trimmed to 12×12-inch squares, with 6-inch gaps on all sides (Figure S1.1). Trimming was performed by hand using scissors at 2-6 week intervals, depending on the rate of growth; plants were trimmed eight times in 2015. Weeds were also removed from the plot to allow for accurate fitness measurements. As white clover generally performs best in areas with regular grazing or mowing (Andrae, Hancock and Harmon, 2016), this trimming and weeding regime is not suspected to have unduly biased fitness measures.

Common garden fitness measurements

Growth and survival. Vegetative growth and tissue survival were assessed using digital photographs of each plant taken at four time points: April 30; May 24 (prior to first trim); October 18 (following last trim); and March 23, 2016. Photos were taken directly over each plant, using a red-painted penny for color contrast and scale. *Easy Leaf Area* software (Easlon and Bloom, 2014) was used to quantify total vegetative tissue surface area (Figure 1.2a). All output photos with highlighted quantified pixels were visually checked for quality. From these data, relative growth for the growing season was calculated as the difference in vegetative tissue area from April to October divided by the largest difference. In addition, biomass was collected with the first trim to verify that that vegetative area can serve as an accurate proxy for biomass production (see Supplementary Methods in Appendix I).

Fecundity. White clover inflorescences are composed of tens to hundreds of individual florets, each capable of producing 1-8 seeds. Therefore, inflorescence count was used to measure fecundity because it was found to be significantly correlated with both seed mass and dried floral mass (Supplementary Methods in Appendix I; Figure S1.2) (see also Kooyers *et al.*, 2014) and references therein for similar measures of white clover reproductive output). Inflorescences were counted and removed from each plant once the oldest (basal) florets began turning downward, an indication of successful pollination.

Herbivory. Herbivore leaf damage was assessed four times (May 29, July 2, July 18, and August 5) using a modified protocol of Kooyers *et al*. (2014) (see also Dirzo and Harper, 1982a, 1982b), in which leaf tissue damage was quantified in an ordinal fashion as 0%, 1-25%, 26-50%, 51-75% or >75% for all leaves on a randomly chosen stolon

(Figure S1.3). Data across the four sampling points were combined, and two herbivory metrics were calculated (Table S1.3). Total herbivore leaf damage was calculated as the number of leaves with any herbivore damage, regardless of damage category, divided by the total number of leaves. Weighted herbivore leaf damage was calculated as the sum of leaf damage categories (A=0, B=0.25, C=0.5, D=0.75, E=1), each multiplied by the number of leaves in their respective category.

Germination experiment. Because we used clonally replicated cuttings of greenhouse-grown plants in the common garden experiment, fitness measures for these plants do not capture selection that occurs at early life stages, when germinants might be particularly susceptible to mortality from herbivore damage. Therefore, to address whether germinant fitness in the field is affected by cyanotype variation, we performed a germination experiment at the common garden site using seeds that originated from the same maternal parents as the common garden genotypes (see Supplementary Methods in Appendix I). We compared the cyanotype frequencies of the common garden genotypes (germinated in the greenhouse) to the germinants that survived to the seedling stage at the common garden site using a chi-squared contingency test.

Climate Principal Components Analysis and Distance Calculations

To quantify home-site climate variation across the 15 populations used in this study, we downloaded 19 bioclimatic variables related to temperature and precipitation (BIOCLIM, Hijmans *et al.*, 2005), as well as annual potential evapotranspiration data (CGIAR; Trabucco and Zomer, 2009, using averaged latitudes and longitudes for each

population (Table 1.1). To evaluate the relationship between home-site climate and fitness performance specifically during the growing season, when most fitness data were collected, we removed three variables related exclusively to winter months (Bio 6=Min Temperature of Coldest Month, Bio 11=Mean Temperature of Coldest Quarter, and Bio 19=Precipitation of Coldest Quarter). To reduce multicollinearity among climatic variables (Farrar and Glauber, 1967), we performed a principal components analysis (PCA) using the *princomp()* function in *R* and utilized the top three PCs for subsequent analyses (R Core Team 2015). We calculated climatic distances between each population and the St. Louis common garden site for each PC as the Euclidean distances between the PC score of STL and each of the 14 "away" populations, generating *PC1_euc*, *PC2_euc* and *PC3_euc* parameters. We then calculated an overall Climate PC index as the sum of the three *PC_euc* values for each population. In these metrics, lower values indicate climates that are more similar to St. Louis.

Statistical analyses and linear modeling

All statistical analyses were performed using *R* statistical software (v. 3.3.0). Figures were generated with the *ggplot2* package (Wickham, 2009). The *reshape2* and *plyr* packages were used to format and summarize data for plots (Wickham, 2007, 2011). For all fitness measures, we averaged data across the three replicate cuttings for each genotype and used this dataset of 161 averaged genotypes for subsequent statistical analyses.

To determine whether geographic distance or climatic distance is a better predictor of plant fitness in the St. Louis common garden site, we tested for correlations

between distance measures (relative geographic distance to St. Louis and Climate PC index) and key fitness measures (relative growth in vegetative tissue and inflorescence count). For these four comparisons, we calculated mean fitness measures for each population and performed Pearson correlation tests using the resulting 15 data points. The analysis was also performed excluding the two locations where stolon cuttings rather than seeds were sampled. We then created linear models using the *lm()* function in *R* and utilized adjusted R^2 values of the lines of best fit to compare the predictive abilities of geographic and climatic distances.

Using the two herbivory metrics, we performed pairwise Wilcoxon signed-rank tests between cyanogenic plants (AcLi) and each of the three acyanogenic groups to test for preferential feeding on acyanogenic plants. If preferential feeding on acyanogenic plants were associated with reduced fitness, we would expect the cyanogenic group to have elevated growth or reproduction relative to the acyanogenic groups. We therefore compared fitness measures (relative growth and inflorescence count) of the cyanogenic and acyanogenic groups using additional pairwise Wilcoxon signed-rank tests. To compare the abilities of different combinations of climate parameters and cyanotype to predict fitness variation in the common garden location, we built sets of linear mixed models separately for two fitness response variables (relative growth in vegetative tissue and inflorescence count) using all combinations of *PC1_euc*, *PC2_euc*, *PC3_euc* and cyanotype as parameters. We then performed multimodel inference and model averaging and calculated parameter weights across models to identify the most relevant parameters for predicting each fitness measure

(Burnham and Anderson, 2002; Botero *et al.*, 2014). Details on model construction and averaging can be found in the Supplementary Methods in Appendix I.

RESULTS

Population structure and cyanogenesis variation across sampled populations

Genotyping by sequencing (GBS) was performed for 112 individuals from three of the sampled locations (AOK, GFL and STL) to assess neutral population differentiation across the sampled species range. Due to the high stringency of the UNEAK pipeline, which was designed for GBS data in polyploid species lacking reference genomes (Lu *et al.*, 2013), the raw data (>2 million Illumina sequence reads) were filtered to 62,372 reciprocal sequence pairs for SNP calling. The average read depth per site was 3.37x. From these filtered sequences, 843 bi-allelic SNPs were identified and utilized for pairwise population F_{ST} calculations. We found negligible population structure, with all pairwise F_{ST} values < 0.03 (Table S1.4). These results corroborate previous findings that white clover shows very little population structure on regional and continental scales (George *et al.*, 2006; Olsen, Sutherland and Small, 2007; Kooyers and Olsen, 2012, 2013).

Cyanotype frequencies varied widely among the 15 sampled populations, with the frequency of cyanogenic (AcLi) plants broadly corresponding to latitude and minimum winter temperature as in previously documented cyanogenesis clines (*e.g.,*Kooyers and Olsen, 2013) (Figure S1.4). Consistent with this pattern, pairwise F_{ST} values for the *Ac* and *Li* cyanogenesis loci, which are expected to be under selection in

cyanogenesis clines, were elevated by up to an order of magnitude between climatically distinct population pairs relative to the background genomic F_{ST} (Table S1.4).

Fitness variation

Growth and survival. Table S1.5 provides summary statistics for survivorship and total vegetative tissue area (cm^2) of the 483 common garden plants (triplicate clones of 161 genotypes) at four time points: April, May, October, and March. Mortality was very low throughout the experiment. Three plants from different source populations (CVA, PPA, BID) had died by the end of the growing season in October. From October to March, 24 additional plants died, with mortality overrepresented in a subset of the populations (DCO=4, DMN=4, GFL=4, LMT=3 and VBC=4), all of which are geographically distant and climatically distinct from the common garden site.

All populations increased in average vegetative tissue area during the establishment period from April to May (Figure 1.2b). However, populations varied widely in their vegetative growth from May to October, with some showing increased vegetative tissue area and others showing static or decreased tissue area. At the end of the growing season (October), the local St. Louis (STL) population had the highest mean vegetative tissue area remaining, followed closely by the geographically proximal Louisville population (LKY). Overall, the relative growth of plants in the common garden displayed a clear correlation with source population distance. Populations located closer to St. Louis had higher relative growth than those collected from more distant sites (R^2=0.54, p=0.001; Figure 1.3a). This pattern remained significant when population samples that were collected as stolon cuttings (STL, GFL) were excluded

from the analysis (R^2=0.45, p=0.007; Figure S1.5a). Thus, relative growth based on vegetative area indicated a home-site fitness advantage among white clover populations, with a gradation in fitness as a function of geographic distance from the source population to the common garden location.

As with vegetative growth during the growing season, changes in average vegetative tissue area over the winter (October to March) varied widely among populations in a pattern consistent with local adaptation. The two populations with the highest mean vegetative tissue area in both October and March were the same two populations that displayed the greatest relative growth during the main growing season: STL and LKY (red lines, Figure 1.2b). Two east coast US populations from similar latitudes to St. Louis (HDE and CVA) also performed well from October to March, despite the fact that they declined over the summer months (green lines).

Fecundity. Over the course of the growing season (April through October), the 483 plants produced 57,385 inflorescences, and the average floral production was 119 inflorescences. Only 15 plants produced zero flowers, 11 of which originated from five genotypes of the southernmost population (GFL). Additional summary statistics can be found in Table S1.5. Total inflorescence count was positively correlated with relative growth in vegetative tissue. This held true both at the level of genotype (R^2=0.06, p=0.0009) and population (R^2=0.66, p=0.0001) (Figure S1.2c, d).

Similar to relative growth in vegetative tissue, mean inflorescence production was correlated with distance of the source population from the experimental plot, with populations originating from sites nearer to St. Louis producing more inflorescences on average than those from more distant locations (R^2=0.24, p=0.036; Figure 1.3b); this

correlation remained marginally significant when the STL and GFL populations were removed (R^2=0.23, p=0.056; Figure S1.5b). The SCD, LKY, SFD and STL populations, all from the central US, had the highest mean inflorescence counts.

Floral production varied over the growing season, increasing in June and decreasing in September for all populations (Figure S1.6). Plants displayed collective bursts of flowering following rainfall events (Figure S1.7). The rate and magnitude of this flowering response varied across populations, with populations that produced the highest inflorescence counts over the season responding most strongly during flowering bursts (red lines, Figures S1.6, S1.7).

Herbivory. Total leaf damage was low overall compared to recent studies in white clover (*e.g.,* Kooyers *et al.*, 2014; Thompson and Johnson, 2016), with only 10-12% of leaves showing any sign of leaf herbivore damage and no clear patterns across populations (Figure S1.8a). Nonetheless, despite low herbivore leaf damage in the St. Louis common garden location, pairwise comparisons between cyanotypes revealed a non-significant trend, with cyanogenic (AcLi) plants showing less total herbivore leaf damage than all three classes of acyanogenic plants (Figure 1.4). Weighted herbivore leaf damage revealed the same trend (Figure S1.8b).

Although cyanogenic (AcLi) plants showed a trend towards lower herbivore leaf damage, this did not translate into increased fitness (Figure S1.8c, d). Rather, the cyanotype with both the highest relative growth and inflorescence count was Acli (cyanogenic glucosides present but linamarase absent). While these trends in increased fitness for Acli were not statistically significant, it bears noting that this cyanotype is the most common cyanotype in local populations in the STL region (Figure

S1.4; Kooyers and Olsen, 2012). These results provide the first empirical evidence that the most common local cyanotype shows marginally higher fitness than the other cyanotypes in the local climate.

Germination Experiment. Cyanotypes of surviving germinants in the common garden plot and greenhouse are presented in Table S1.6. There was no significant difference in cyanotype proportions under the two growing conditions ($\chi^2 = 0.71$, p=0.87). This result suggests that cyanotype does not affect fitness at early life stages, at least in the central US location and year of this study. Therefore, fitness measurements made from the clonal replicates in the common garden are apparently not missing a key component of cyanogenesis-related fitness variation at the germinant life stage.

Climate Principal Components Analysis and Distance Calculations

In a principal components analysis (PCA) utilizing 16 Bioclim variables and annual potential evapotranspiration (Apet) data, PC1 explained 44% of the variance in climate among the 15 populations studied (Table 1.2). PC1 is driven primarily by variables related to precipitation (*e.g.,* annual precipitation, precipitation in the driest month and quarter, but also annual mean temperature) (Figure S1.9a). PC2 explained 24% of the variance in climate and is driven primarily by variables related to maximum and mean summer temperatures, as well as Apet (Figure S1.9b). Lastly, PC3 explained 16% of the variance and corresponds to yearly temperature variability (*e.g.,* isothermality, temperature seasonality) (O'Donnell and Ignizio, 2012) (Figure S1.9c). Climatic distances, calculated as Euclidean distances between PC scores of STL and

the 14 “away” populations (*PC1_euc* = Precipitation, *PC2_euc* = Heat, and *PC3_euc* = Variability), as well as the overall PC index, are presented in Table S1.7. Smaller values indicate home-site climate that is similar to STL, while larger values indicate climatic dissimilarity.

Geographic distance and climate PC index were roughly equivalent predictors of fitness variation across populations for relative growth (Figure 1.3a, c). In contrast, climate PC index was a better predictor of variation in reproductive output than geographic distance; the R^2 value increased from 0.24 in the geographic distance model to 0.48 in the PC index model ($p= 0.003$; Figure 1.3b, d). Climate PC index was highly correlated with geographic distance ($R^2=0.66$, $p=0.0001$; Figure S1.10).

Linear mixed models

Models containing alternative parameters best predicted variation in the two fitness measures. Single-parameter models best explained relative growth in vegetative tissue, with home-site temperature variability (‘Variability’) containing the most predictive value, followed by maximum summer temperature (‘Heat’) (Table 1.3, Table S1.8). ‘Precipitation’ was the least important climatic parameter for relative growth, and adding ‘Cyanotype’ as a parameter did not improve relative growth models. Parameter weights across models paralleled model rankings: Variability (0.52), Heat (0.42), Precipitation (0.19), and Cyanotype (0.00).

Inflorescence count was best explained by the model including Precipitation + Heat + Cyanotype, and the addition of Cyanotype improved models in all cases (Table 1.3, Table S1.8). Parameter weights for Heat and Precipitation were 0.74 and 0.62

across inflorescence count models. In contrast to relative growth models, Variability had the lowest parameter weight (0.45) for predicting inflorescence count, and Cyanotype the highest (1.00), where Acli (the locally favored cyanotype) and AcLi cyanotypes were associated with increased fitness (Table S1.8). This suggests a reproductive fitness advantage in the St. Louis climate for plants that produce cyanogenic glucosides. For the most important climatic parameters in all models, slopes were negative, indicating that home-site climate dissimilarity along those axes has negative effects on vegetative survival in St. Louis (Table S1.8). Additional details for the top ranking models are presented in Table S1.8.

DISCUSSION

Local adaptation in white clover has long been apparent from observations of repeatedly evolved clines in cyanogenesis. Less understood is the importance of this particular phenotype for overall plant fitness across varied climates. In this study, we evaluated the extent to which North American white clover populations exhibit local adaptation with respect to geographic or climatic distance from a central US common garden site, and we assessed the importance of cyanogenesis for predicting vegetative growth and reproductive output. We detect clear correlations between source population location and both of these fitness measures, with climatic distance the better predictor of reproductive output (Figure 1.3b, d). While cyanogenic plants showed marginally lower herbivore leaf damage (Figure 1.4), this effect did not translate into a fitness advantage at the common garden site (Figure S1.8c, d). However, linear mixed modeling suggests that the cyanogenesis polymorphism may play some role in local

adaptation for reproductive output (Table 1.3). Below we discuss the implications of these findings for white clover local adaptation and more broadly in the context of local adaptation, climate change and restoration ecology research.

Rapid local climatic adaptation in white clover

Our data provide strong evidence that North American white clover has adapted to local climate on a continental scale, with this evolution having occurred in the 500 years since its introduction from Europe. Similar rates of evolved climatic adaptation have been noted in other systems, including annual plant species (Franks, Sim and Weis, 2007), invasive plants experiencing range expansion (Colautti and Barrett, 2013), and salmonid fishes (Fraser *et al.*, 2011). In white clover, rapid evolution is likely facilitated by its very large population sizes, with the species showing a near-continuous distribution in lawns, roadsides and pastures across much of mesic North America, as well as an abundance of standing genetic variation that reflects intentional, repeated introductions of this agriculturally important plant (Kjærgaard, 2003). Such rapid evolution is promising in the face of climate change; however, the rapidity by which clover is able to evolve may be less generalizable to rare or range-restricted species with smaller population sizes and less genetic variation for selection to act upon (Franks, Weber and Aitken, 2014).

While we find correlations between average population fitness and both geographic and climatic distance in the St. Louis common garden (Figure 1.3), climatic distance is a better predictor of fitness variation, particularly for reproductive output (Figure 1.3b,d). The strong correlation that we observe between geographic and

climatic distance (Figure S1.10) is likely largely a reflection of the central location of the common garden site in relation to population samples and the way Climate PC index was calculated (Figure 1.1, Table S1.7). Across this continental scale, the key climatic variables show relatively smooth gradations (Figure S1.9), and summing the *PC_euc* distances to calculate Climate PC index thus generated similar values for populations from similar geographic distances (Table S1.7). For example, Duluth, MN (DMN) and Gainesville, FL (GFL) had similar Climate PC indexes (8.039 and 8.437, respectively), but they differ climatically from STL in different ways. Duluth is wetter (*PC1_euc*) and colder (*PC2_euc*) than STL, while Gainesville is wetter (*PC1_euc*) and shows less variability in temperature (*PC3_euc*). Thus, the relationship between geographic and climatic distance is context-dependent, and geographic distance from the source population may not be the best predictor of fitness in general. These findings corroborate previous studies suggesting that restoration efforts are best advised to focus on environmental similarity when selecting individuals to transfer between habitats (Raabová, Münzbergová and Fischer, 2007; Lawrence and Kaye, 2011; Noël *et al.*, 2011; Forrester *et al.*, 2013).

A key limitation of this study is the lack of reciprocal common garden sites for fitness comparisons. Identification of “reciprocal home-site advantage” in two or more locations is often considered the definitive test for demonstrating local adaptation (Kawecki and Ebert, 2004). On the other hand, previous single-site studies have provided compelling evidence for local climatic adaptation in plants (*e.g.,*Rutter, Shaw and Fenster, 2010; Preite *et al.*, 2015; Peterson, 2016). The relatively large number of populations sampled in the present study and the clear evidence of climate-associated

fitness variation that we detect across populations (Figures 1.2, 1.3; Figures S1.6, S1.7; Table 1.3) lend further support to our conclusion that local climatic adaptation is pervasive in white clover. The results of this study are also entirely consistent with inferences from cyanogenesis cline studies indicating that the species repeatedly evolves local adaptation across climatic gradients. Nonetheless, future multi-site common garden experiments in white clover would undoubtedly be valuable and could be especially useful for examining fitness-related traits not considered here — for example, flowering phenology, a critical trait for local climatic adaptation in many plant species (*e.g.,* Weinig, Ungerer and Dorn, 2002; Verhoeven *et al.*, 2008; Buckler *et al.*, 2009; Anderson, Lee and Mitchell-Olds, 2011; Anderson *et al.*, 2013; Friedman and Willis, 2013).

Another limitation of the study is that we did not consider the potential impacts of competition on fitness variation. By trimming and weeding, we eliminated both conspecific and heterospecific plant competition. It is thus possible that regional variation in competitive ability exists that we did not capture in this study. Additionally, we did not consider the impact of soil nutrients or microbes on fitness, which may be particularly important for legumes such as white clover that interact with local soil *Rhizobia* (Macel *et al.*, 2017). Follow-up studies would be valuable for examining both of these factors.

The effects of cyanogenesis on fitness

While our data suggest that cyanogenic (AcLi) plants experience marginally less herbivore leaf damage than acyanogenic plants in St. Louis (Figure 1.4, Figure S1.8b),

this advantage did not result in higher fitness for either fitness measure examined here (Figure S1.8c, d). We detected very low herbivory overall in the common garden (10-12% of leaves on average showed some amount of discernible herbivore damage) (Figure S1.8a). By comparison, Kooyers *et al.* (2014) found that on average, 25-30% of individual leaflets displayed some amount of herbivore leaf damage across four natural populations located south of St. Louis in Tennessee, Arkansas, and Oklahoma. Thus, our results are potentially consistent with lower herbivore damage in the central US than in southern US populations, as would be expected if variation in herbivore abundance drives the evolution of cyanogenesis clines. However, the two studies may not be directly comparable given that the present study examined herbivory in non-local genotypes and in a different year. In contrast to our findings, Thompson & Johnson (2016) quantified mean herbivore leaf damage by estimating overall percent damage per leaf on plants in a more northern common garden (Toronto, Canada) and found leaves experienced 35.7% and 23.8% herbivore damage on average during early and late season surveys, respectively. That high level of herbivory is somewhat unexpected given low frequencies of cyanogenic plants in most northern populations (see Thompson, Renaudin and Johnson, 2016).

Interestingly, rather than detecting a fitness advantage for cyanogenic plants in the common garden location, we instead found that the cyanotype that is most common in local wild populations (Acli) showed the highest mean fitness for both relative growth and reproductive output, although this trend was not statistically significant (Figure S1.8c, d). To our knowledge, these are the first data to establish a relationship between high cyanotype frequency in local wild populations and high fitness of non-local plants of

the same cyanotype in that region. The Acli cyanotype produces cyanogenic glucosides but lacks the enzyme required for HCN release. Growth chamber experiments suggest that this cyanotype shows differentially high reproductive fitness under simulated drought conditions when nitrogen is limited (Kooyers *et al.*, 2014). Consistent with that finding, studies in sorghum and other species indicate that cyanogenic glucosides can be metabolized through non-cyanogenic pathways and are likely beneficial as a nitrogen reserve under drought stress conditions (Møller, 2010; Kooyers, 2015). Thus, the slightly elevated fitness of Acli that we detect in the common garden might be a reflection of differential success during the dry, hot days of the peak growing season in the central US.

Predictive abilities of cyanogenesis vs. climatic parameters for fitness

Model averaging indicated that alternative climatic parameters are the best predictors of different aspects of fitness in white clover. Our findings agree with previous studies showing that survival and growth-related traits respond more strongly to temperature than precipitation (Moles *et al.*, 2014; Preite *et al.*, 2015), whereas water availability and precipitation are particularly important for flowering, especially during the driest portions of the growing season (Prieto *et al.*, 2008; Samis *et al.*, 2012b). Additionally, we documented striking variation across populations in their rate and magnitude of flowering in response to bouts of precipitation during the reproductive season. This result suggests that it is important to consider not only the total reproductive output but also the tempo of output relative to periodic environmental cues when assessing local adaptation. Furthermore, the predictive nature of chemical

defense (in this case, cyanotype) for floral production suggests that knowledge of locally favored adaptive traits, in addition to climate similarity, can help to inform restoration ecologists in selecting the most appropriate individuals for transplantation efforts.

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TABLES

Table 1.1. Geographic coordinates and distances to the common garden site for the 15 populations used in this study.

Population[a]	ID	N	Latitude	Longitude	Distance (km)	Relative Distance
Ardmore, OK	AOK	48	34.16	-97.14	789.1	0.28
Boise, ID	BID	11	43.60	-116.22	2232	0.79
Chicago, IL	CIL	9	41.96	-87.63	433.1	0.15
Charlottesville, VA	CVA	11	38.01	-78.52	1028	0.36
Denver, CO	DCO	11	39.78	-104.97	1270	0.45
Duluth, MN	DMN	11	46.79	-92.15	919.7	0.32
Gainesville, FL	GFL	32	29.64	-82.36	1237	0.44
Harbeson, DE	HDE	10	38.73	-75.28	1301	0.46
Louisville, KY	LKY	10	38.28	-85.62	408.3	0.14
Livingston, MT	LMT	11	45.65	-110.56	1838	0.65
Pittsburgh, PA	PPA	11	40.45	-79.95	908.8	0.32
Sioux City, SD	SCD	11	42.54	-96.53	682.4	0.24
Sioux Falls, SD	SFD	11	43.51	-96.73	764.7	0.27
St. Louis, MO	STL	32	38.64	-90.29	0	0.00
Vancouver, BC	VBC	11	49.22	-122.82	2830	1.00

[a]Contributions to sample collections are acknowledged in Table S1.1. Collection dates are also given.

Table 1.2. Results of the principal components analysis (PCA) for home-site climate variables during the growing season.

Bioclim variable	PC1 (44%)	PC2 (24%)	PC3 (16%)
AnnMeanTemp	**-0.318**	-0.209	0.086
AnnPrecip	**-0.313**	0.217	-0.136
AnnTempRange	0.285	-0.198	-0.258
Apet	-0.222	**-0.375**	0.103
Isothermality	-0.212	-0.146	**0.401**
MaxT_warmM	-0.107	**-0.456**	0.035
MeanT_dryQ	-0.230	0.011	**0.345**
MeanT_warmQ	-0.221	**-0.349**	-0.113
MeanT_wetQ	-0.135	-0.194	**-0.405**
MeanTempRange	0.097	**-0.411**	0.196
Precip_dryM	**-0.303**	0.025	-0.217
Precip_dryQ	**-0.305**	0.048	-0.223
Precip_warmQ	-0.238	-0.031	-0.301
Precip_wetM	-0.272	0.254	0.025
Precip_wetQ	-0.264	0.284	0.005
PrecipSeasonality	0.190	0.103	0.261
TempSeasonality	0.253	-0.106	**-0.380**

Bold font indicates the four most highly correlated variables for each of the top three PCs. The underlined values correspond to maps in Figure S1.9.

Table 1.3. Linear mixed model comparisons for two fitness measures.

			Relative Growth Models		Inflorescence Count Models	
Model	**Parameters included**	**K**	**ΔAIC**	**Akaike weight**	**ΔAIC**	**Akaike weight**
1	Precipitation	1	**2.929**	**0.107**	316.729	0.000
2	Heat	1	**0.817**	**0.306**	316.059	0.000
3	Variability	1	**0.000**	**0.461**	315.929	0.000
4	Cyanotype	1	17.309	0.000	3.677	0.052
5	Precipitation + Heat	2	3.837	0.068	311.959	0.000
6	Heat + Variability	2	4.753	0.043	315.069	0.000
7	Precipitation +Variability	2	7.120	0.013	316.139	0.000
8	Precipitation + Cyanotype	2	22.808	0.000	3.484	0.061
9	Heat + Cyanotype	2	20.742	0.000	2.131	0.120
10	Variability + Cyanotype	2	19.967	0.000	2.576	0.096
11	Precipitation + Heat + Variability	3	9.961	0.003	312.839	0.000
12	Precipitation + Heat + Cyanotype	3	23.811	0.000	**0.000**	**0.348**
13	Precipitation + Variability + Cyanotype	3	27.060	0.000	3.490	0.061
14	Heat + Variability + Cyanotype	3	24.761	0.000	**1.981**	**0.129**
15	Precipitation + Heat + Variability + Cyanotype	4	29.983	0.000	**1.261**	**0.185**

Akaike Information Criterion (AIC)-related metrics are given for models with all combinations of four parameters. Bold values indicate the ΔAIC and Aikaike weights for the top three models in each set, with the top models underlined. Additional information for the top models is provided in Table S1.8.

FIGURES

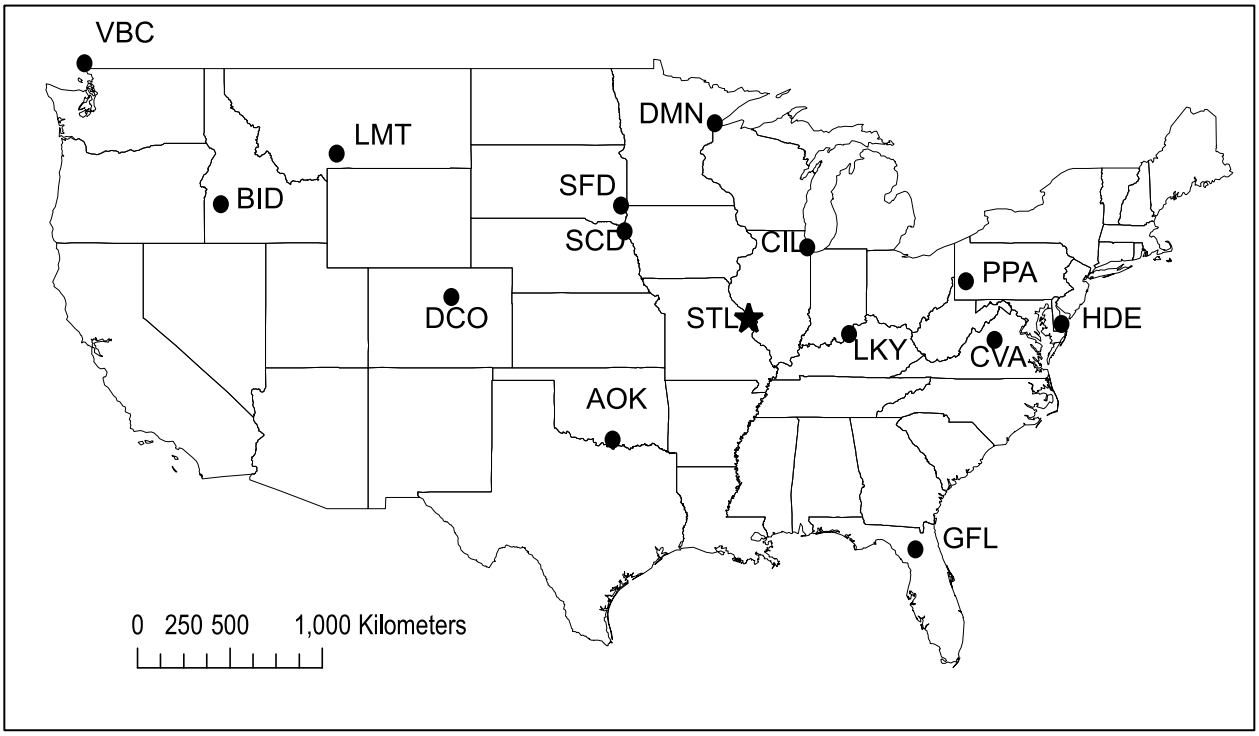

Figure 1.1. Sampling locations and abbreviations for the 15 populations used in the common garden experiment. The star indicates the location of the common garden experiment in St. Louis, MO (STL).

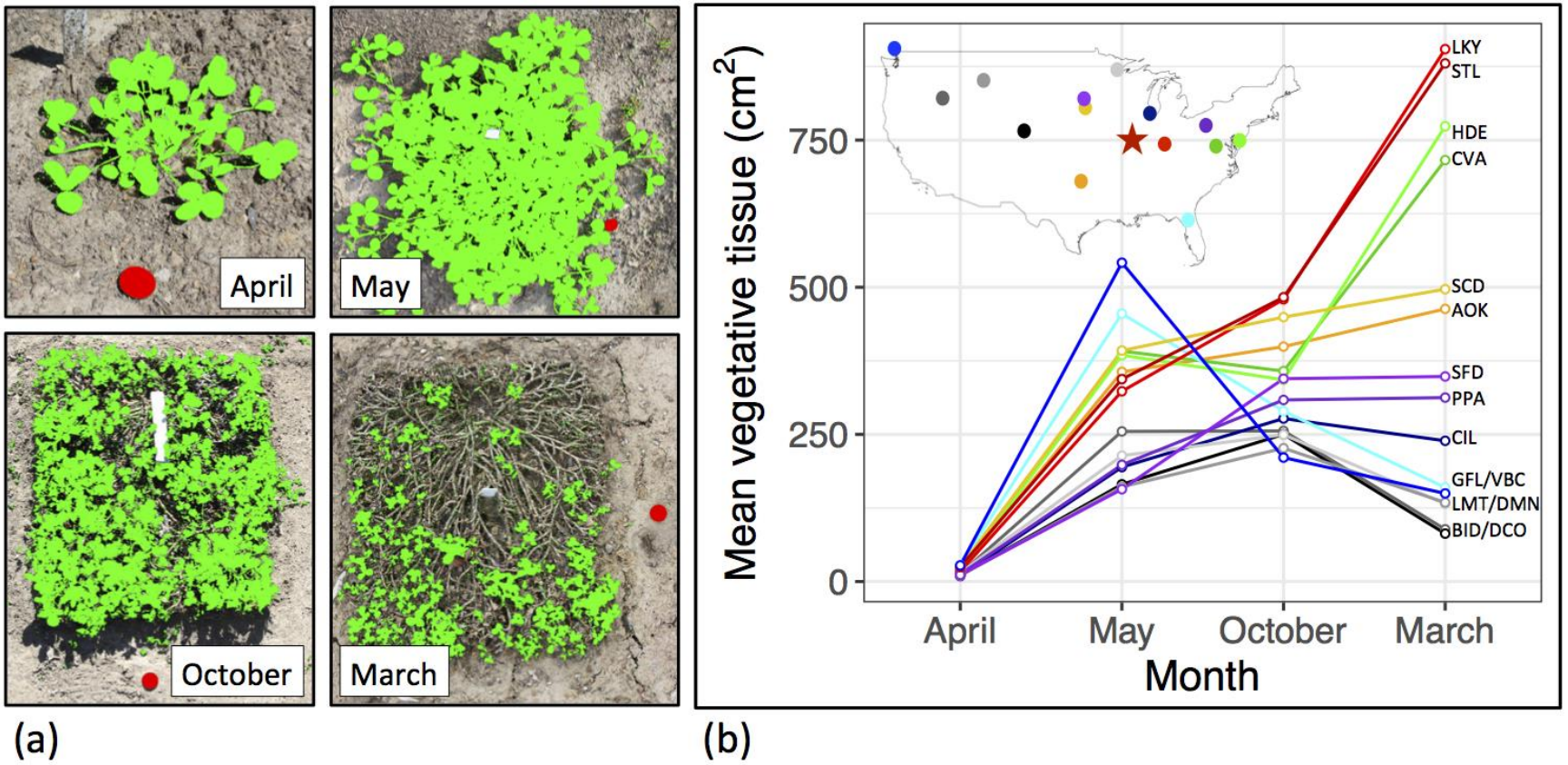

Figure 1.2. *Easy Leaf Area* output photos (a) are shown for a single plant at each photographic time point in the common garden experiment. Vegetative tissue is highlighted in green pixels, with a red-painted penny used for scale. The line graph (b) displays mean vegetative tissue across populations at each of the four time points. Lines that are similar in color represent populations that experienced similar trajectories over all four time points.

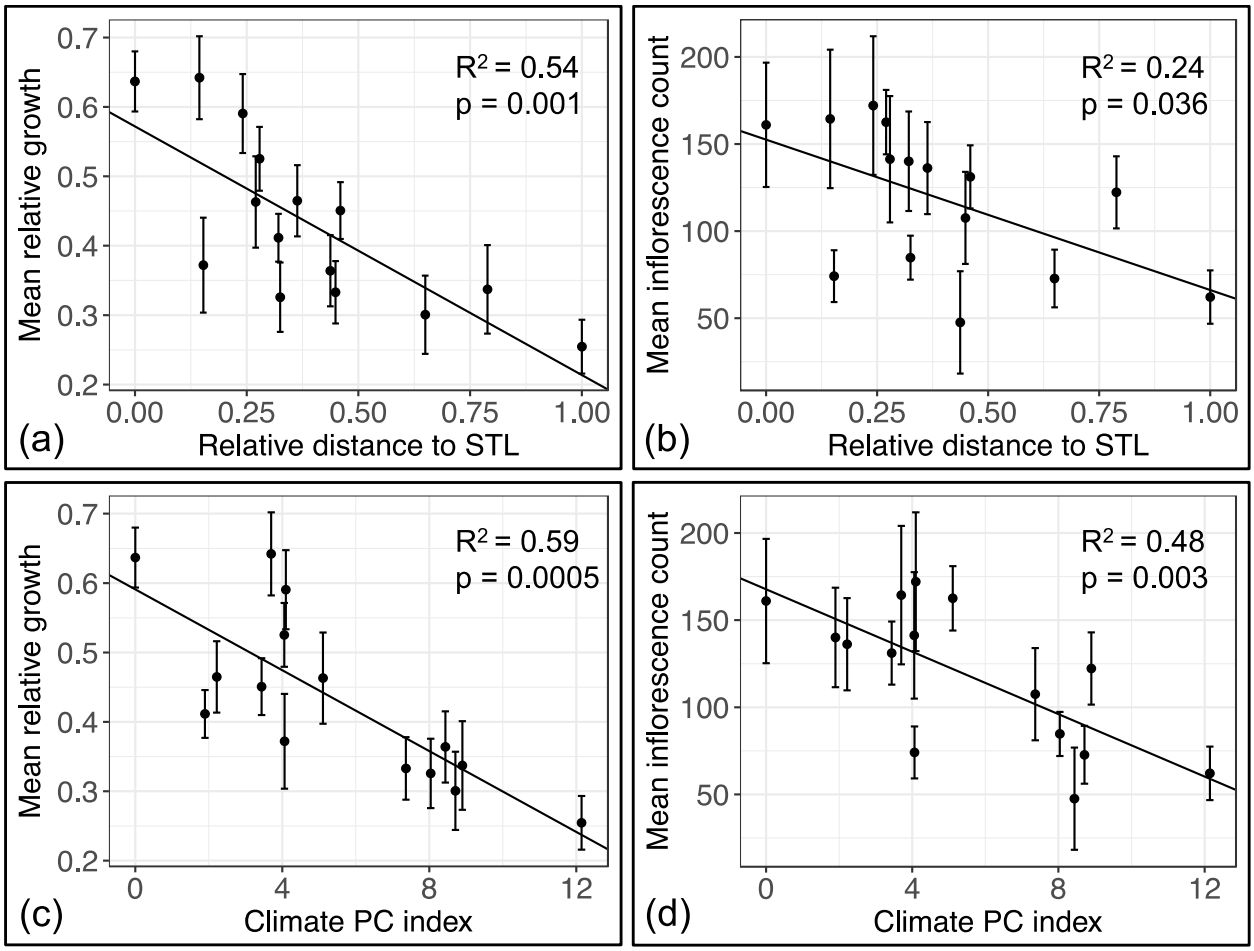

Figure 1.3. Linear relationships of two fitness measures (relative growth in vegetative tissue from April to October (a, c), and inflorescence count (b, d)) as a function of geographic distance (a, b) or climatic distance (PC index; c, d) across 15 populations. Data points show the mean value for each population with standard error bars. Adjusted R^2 values and p-values for lines of best fit are shown.

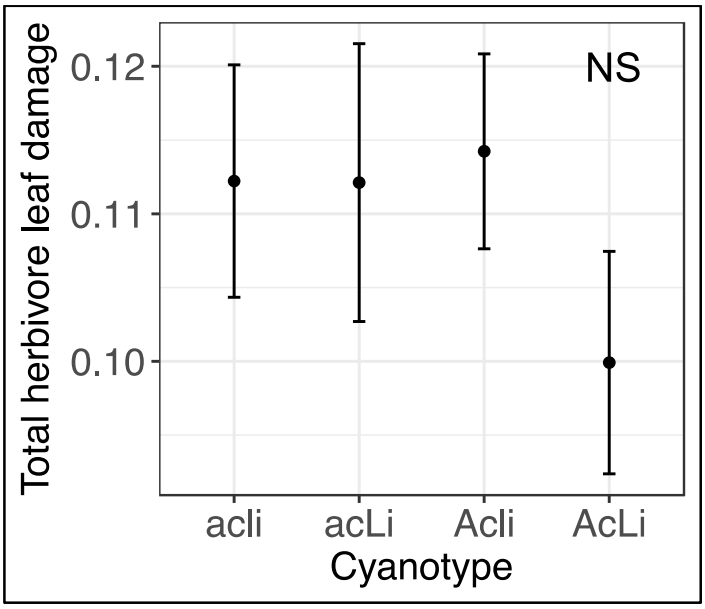

Figure 1.4. Variation in total herbivore leaf damage across cyanotypes. Mean values are shown for each cyanotype with standard error bars. Pairwise Wilcoxon signed-rank tests between the cyanogenic group (AcLi) and each of the three acyanogenic groups were not significant at the $p < 0.05$ level.

APPENDIX I

Chapter 1 Supplementary Material

SUPPLEMENTARY METHODS

Common garden fitness measurements

Growth. Trimmed biomass was collected from each plant during the first trimming. Samples were rinsed to remove soil and debris, then dried at 60°C for 48 hours before weighing. This measurement was compared to the total vegetative surface area of the plants in May, before the first trim, to verify that these two measures were correlated (R^2=0.54, p=2.2e-16), confirming that vegetative tissue area can serve as an accurate measure of vegetative biomass production.

Fecundity. We removed all inflorescences and buds from plants for the first 10 days after transplantation to the experimental plot, as these were produced during growth that had occurred in the greenhouse. From day 10 to the first trim (approximately 6 weeks), inflorescences were counted and tagged, and seeds were allowed to fully mature. Seed-filled infructescences were collected from each plant upon maturity and dried at 60 degrees C for a minimum of 48 hours. Seeds from 67 of the plants were harvested using 1 mm and 0.5 mm sieves in series and weighed to evaluate the correlation between inflorescence count and seed mass. Following the first trim, inflorescences were collected, dried and weighed for each plant to confirm a significant correlation between inflorescence counts and dried floral mass (Figure S1.2). Seed mass (R^2= 0.51, p=8.24e-12) and dried floral mass (R^2= 0.87, p=2.2e-16) were correlated with inflorescence count (Figure S1.2a, b), suggesting that inflorescence count serves as a good proxy for reproductive fitness in white clover.

Germination experiment

Ten seeds each from the maternal parents of the common garden genotypes were scarified and planted in an experimental plot immediately adjacent to common garden plot. We were unable to use seeds from STL and GFL plants in this germination experiment because those samples were collected as cuttings. Seeds were planted in 6” square pots with the bottoms removed. Pots were embedded in the soil of the experimental garden plot so as to be level with the surrounding ground surface. Seeds were watered only upon planting, and embedded pots were covered loosely with white Scrubbie Mesh (Jo-Ann Stores, Hudson, OH) for the duration of the experiment to mimic shading that would occur for seedlings that naturally germinate within a matrix of other plants. The mesh did not prevent small herbivores from accessing the germinant plants. After 30 days, we randomly selected one seedling per maternal parent for cyanogenesis phenotyping and genotyping, if a surviving seedling was present. All additional seedlings were discarded. This protocol paralleled the sampling process for the greenhouse-grown plants used in the common garden experiment, except no care was provided following the initial planting of the germinants in the experimental plot.

Linear mixed models and model averaging

We built linear mixed models with all combinations of the three *PC_euc* parameters and cyanotype to assess their relative predictive abilities for fitness variation, considering relative growth and inflorescence count separately. For the purposes of their use as parameters in linear mixed models, *PC1_euc*, *PC2_euc* and *PC3_euc* are referred to as Precipitation, Heat and Variability, respectively, to reflect the key bioclimatic variables underlying their variation (Table 1.2, Figure S1.9). Models

were constructed with the *lme4* package using the averaged data set of 161 genotypes (Table S1.3), with Population as a random effect (Bates *et al.*, 2015). In the case of October vegetative tissue area, the data were normally distributed, and the *lmer()* function was used. For inflorescence count, the *glmer()* function and a Poisson distribution were used. We assessed all models with and without interaction effects. The set of models that included interactions outranked the set without interactions; however, within each set, the rankings of models were qualitatively the same. Therefore, for simplicity we present models that do not include interaction effects.

Models for each fitness measure were compared using Akaike Information Criterion (AIC)-related metrics. We performed model averaging with ΔAIC and Akaike weights; because our sample size was quite large relative to the number of parameters, using AIC_c did not change our inferences. Parameter importance across models was assessed by calculating relative variable importance for each parameter as the sum of Akaike weights in all models in which the parameter occurs (Burnham and Anderson, 2002). Additionally, parameter slopes were compared, and the amount of variance contributed by parameters within models was assessed using *F* statistics calculated by the *lmerTest* package (Kuznetsova, Brockhoff and Christensen, 2017).

WORKS CITED

Bates, D. *et al.* (2015) 'Fitting Linear Mixed-Effects Models Using lme4', *Journal of Statistical Software*, 67(1). doi: 10.18637/jss.v067.i01.

Burnham, K. P. and Anderson, D. R. (2002) *Model Selection and Multimodel Inference*. New York, NY: Springer New York.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017) 'lmerTest Package: Tests in Linear Mixed Effects Models', *Journal of Statistical Software*, 82(13). doi: 10.18637/jss.v082.i13.

SUPPLEMENTARY TABLES

Table S1.1. Contributors and collection and planting dates for samples used in this study.

Population	Sample contributor(s)	Collected as	Collection Date	Planting Date[a]
AOK	Sara Wright, Meena Jeanes (AOK), Maria Monteros (Noble Foundation-NFWC)	seed (NFWC), cuttings (AOK)	2014 (NFWC); May 2014 (AOK)	January 2014 (NFWC)
BID	Sara Wright	seed	September 2014	February 2015
CIL	Sara Wright, Kenneth Wright, Devin Dobias, Madeline Keleher	seed	August 2014	February 2015
CVA	Nic Kooyers	seed	September 2014	January 2015
DCO	Rebecca Thomson	seed	September 2014	February 2015
DMN	Briana Gross, University of Minnesota-Duluth undergraduate class	seed	September 2014	December 2014
GFL	Patricio Munoz, Yolanda Lopez	cuttings	May 2014	—
HDE	John McDonald	seed	September 2014	February 2015
LKY	Sunita Crittenden	seed	September 2014	February 2015
LMT	Sara Wright	seed	September 2014	February 2015
PPA	Lisa Limeri	seed	August 2014	February 2015
SCD	Sara Wright	seed	September 2014	February 2015
SFD	Sara Wright	seed	September 2014	February 2015
STL	Sara Wright	cuttings	May 2014	—
VBC	Kathryn Turner, University of British Columbia undergraduate class	seed	September 2014	February 2015

[a]Refers to date samples were scarified and planted as seeds in greenhouse facilities. Cuttings were planted in the greenhouse immediately following collection.

Table S1.2. Sample coordinates, geographic distance to the common garden site and cyanotype information for all genotypes used in this study.

ID	Collected as	Pop	Latitude	Longitude	Distance	Cyanotype
AOK_001	cutting	AOK	34.1725	-97.160278	789.1	AcLi
AOK_004	cutting	AOK	34.173889	-97.135556	789.1	Acli
AOK_005	cutting	AOK	34.173889	-97.135556	789.1	Acli
AOK_006	cutting	AOK	34.173889	-97.135556	789.1	Acli
AOK_008	cutting	AOK	34.175833	-97.126667	789.1	Acli
AOK_009	cutting	AOK	34.176111	-97.126389	789.1	AcLi
AOK_012	cutting	AOK	34.185556	-97.119444	789.1	AcLi
AOK_014	cutting	AOK	34.185556	-97.119444	789.1	AcLi
AOK_017	cutting	AOK	34.163889	-97.148333	789.1	AcLi
AOK_018	cutting	AOK	34.164444	-97.145556	789.1	AcLi
AOK_021	cutting	AOK	34.164167	-97.144722	789.1	AcLi
AOK_022	cutting	AOK	34.164167	-97.144722	789.1	AcLi
AOK_023	cutting	AOK	34.164167	-97.144722	789.1	AcLi
AOK_024	cutting	AOK	34.164167	-97.144722	789.1	AcLi
AOK_025	cutting	AOK	34.164167	-97.144722	789.1	Acli
AOK_026	cutting	AOK	34.159167	-97.143611	789.1	Acli
AOK_027	cutting	AOK	34.155	-97.145833	789.1	AcLi
AOK_029	cutting	AOK	34.155	-97.145833	789.1	AcLi
AOK_030	cutting	AOK	34.155	-97.145833	789.1	AcLi
AOK_032	cutting	AOK	34.155278	-97.149167	789.1	AcLi
AOK_034	cutting	AOK	34.155278	-97.149167	789.1	AcLi
AOK_037	cutting	AOK	34.158056	-97.149167	789.1	AcLi
AOK_040	cutting	AOK	34.158056	-97.149167	789.1	Acli
AOK_042	cutting	AOK	34.158611	-97.139444	789.1	AcLi
AOK_044	cutting	AOK	34.158611	-97.139444	789.1	AcLi
AOK_048	cutting	AOK	34.161111	-97.14	789.1	AcLi
AOK_051	cutting	AOK	34.161944	-97.139722	789.1	Acli
AOK_055	cutting	AOK	34.161944	-97.139722	789.1	Acli
AOK_058	cutting	AOK	34.161944	-97.136389	789.1	AcLi
NFWC_04_4	seed	AOK	NA	NA	789.1	AcLi
NFWC_04_5	seed	AOK	NA	NA	789.1	AcLi
NFWC04_1	seed	AOK	NA	NA	789.1	Acli
NFWC04_2	seed	AOK	NA	NA	789.1	Acli
NFWC04_3	seed	AOK	NA	NA	789.1	Acli
NFWC04_6	seed	AOK	NA	NA	789.1	AcLi
NFWC04_7	seed	AOK	NA	NA	789.1	AcLi
NFWC04_8	seed	AOK	NA	NA	789.1	AcLi
NFWC05_117	seed	AOK	NA	NA	789.1	Acli
NFWC05_118	seed	AOK	NA	NA	789.1	AcLi
NFWC05_120	seed	AOK	NA	NA	789.1	Acli
NFWC05_121	seed	AOK	NA	NA	789.1	AcLi
NFWC05_122	seed	AOK	NA	NA	789.1	AcLi
NFWC05_123	seed	AOK	NA	NA	789.1	acLi
NFWC05_124	seed	AOK	NA	NA	789.1	AcLi
NFWC05_128	seed	AOK	NA	NA	789.1	AcLi
NFWC05_129	seed	AOK	NA	NA	789.1	AcLi
NFWC05_131	seed	AOK	NA	NA	789.1	Acli
NFWC05_132	seed	AOK	NA	NA	789.1	AcLi
BID_001	seed	BID	43.5959	-116.2222	2232	acli
BID_002	seed	BID	43.5959	-116.2222	2232	acLi

BID_003	seed	BID	43.5959	-116.2222	2232	acLi
BID_004	seed	BID	43.5959	-116.2222	2232	Acli
BID_005	seed	BID	43.5959	-116.2222	2232	acli
BID_006	seed	BID	43.5959	-116.2222	2232	acli
BID_007	seed	BID	43.5959	-116.2222	2232	acli
BID_008	seed	BID	43.5959	-116.2222	2232	acli
BID_009	seed	BID	43.5959	-116.2222	2232	acli
BID_010	seed	BID	43.5959	-116.2222	2232	acLi
BID_011	seed	BID	43.5959	-116.2222	2232	acli
CIL_001	seed	CIL	41.9632	-87.6334	433.1	acli
CIL_003	seed	CIL	41.9632	-87.6334	433.1	acli
CIL_004	seed	CIL	41.9632	-87.6334	433.1	AcLi
CIL_005	seed	CIL	41.9632	-87.6334	433.1	AcLi
CIL_006	seed	CIL	41.9632	-87.6334	433.1	acli
CIL_007	seed	CIL	41.9632	-87.6334	433.1	acli
CIL_008	seed	CIL	41.9632	-87.6334	433.1	AcLi
CIL_009	seed	CIL	41.9632	-87.6334	433.1	Acli
CIL_011	seed	CIL	41.9632	-87.6334	433.1	AcLi
CVA_001	seed	CVA	38.0102	-78.5171	1028	AcLi
CVA_002	seed	CVA	38.0102	-78.5171	1028	acLi
CVA_003	seed	CVA	38.0102	-78.5171	1028	acli
CVA_004	seed	CVA	38.0102	-78.5171	1028	acli
CVA_005	seed	CVA	38.0102	-78.5171	1028	Acli
CVA_006	seed	CVA	38.0102	-78.5171	1028	acli
CVA_007	seed	CVA	38.0102	-78.5171	1028	acLi
CVA_009	seed	CVA	38.0102	-78.5171	1028	Acli
CVA_010	seed	CVA	38.0102	-78.5171	1028	acli
CVA_011	seed	CVA	38.0102	-78.5171	1028	acLi
CVA_012	seed	CVA	38.0102	-78.5171	1028	acli
DCO_001	seed	DCO	39.7767	-104.9729	1270	Acli
DCO_002	seed	DCO	39.7767	-104.9729	1270	Acli
DCO_003	seed	DCO	39.7767	-104.9729	1270	acli
DCO_004	seed	DCO	39.7767	-104.9729	1270	Acli
DCO_005	seed	DCO	39.7767	-104.9729	1270	Acli
DCO_006	seed	DCO	39.7767	-104.9729	1270	acLi
DCO_007	seed	DCO	39.7767	-104.9729	1270	acLi
DCO_008	seed	DCO	39.7767	-104.9729	1270	acLi
DCO_009	seed	DCO	39.7767	-104.9729	1270	acLi
DCO_011	seed	DCO	39.7767	-104.9729	1270	Acli
DCO_012	seed	DCO	39.7767	-104.9729	1270	Acli
DMN_001	seed	DMN	46.792	-92.1546	919.7	acli
DMN_004	seed	DMN	46.792	-92.1546	919.7	Acli
DMN_005	seed	DMN	46.792	-92.1546	919.7	AcLi
DMN_007	seed	DMN	46.792	-92.1546	919.7	acli
DMN_008	seed	DMN	46.792	-92.1546	919.7	AcLi
DMN_013	seed	DMN	46.792	-92.1546	919.7	Acli
DMN_014	seed	DMN	46.792	-92.1546	919.7	acLi
DMN_015	seed	DMN	46.792	-92.1546	919.7	acLi
DMN_016	seed	DMN	46.792	-92.1546	919.7	acli
DMN_017	seed	DMN	46.792	-92.1546	919.7	Acli
DMN_018	seed	DMN	46.792	-92.1546	919.7	Acli
GFL_002	cutting	GFL	29.632973	-82.355522	1237	AcLi
GFL_004	cutting	GFL	29.633026	-82.355148	1237	AcLi

GFL_006	cutting	GFL	29.638927	-82.358749	1237	AcLi
GFL_007	cutting	GFL	29.640671	-82.362297	1237	AcLi
GFL_008	cutting	GFL	29.640635	-82.362282	1237	AcLi
GFL_010	cutting	GFL	29.640596	-82.363647	1237	AcLi
GFL_012	cutting	GFL	29.640257	-82.363968	1237	AcLi
GFL_013	cutting	GFL	29.638119	-82.362885	1237	AcLi
GFL_014	cutting	GFL	29.638138	-82.362923	1237	AcLi
GFL_016	cutting	GFL	29.638201	-82.362419	1237	AcLi
GFL_019	cutting	GFL	29.63517	-82.36142	1237	AcLi
GFL_021	cutting	GFL	29.636271	-82.364983	1237	AcLi
GFL_022	cutting	GFL	29.636152	-82.365402	1237	AcLi
GFL_024	cutting	GFL	29.635914	-82.36554	1237	AcLi
GFL_025	cutting	GFL	29.636101	-82.366623	1237	AcLi
GFL_029	cutting	GFL	29.648767	-82.359688	1237	Acli
GFL_038	cutting	GFL	29.637688	-82.363439	1237	acLi
GFL_039	cutting	GFL	29.63953	-82.364118	1237	AcLi
GFL_040	cutting	GFL	29.63952	-82.364205	1237	AcLi
GFL_044	cutting	GFL	29.650312	-82.371029	1237	AcLi
GFL_045	cutting	GFL	29.649031	-82.360259	1237	AcLi
GFL_047	cutting	GFL	29.64835	-82.350968	1237	AcLi
GFL_049	cutting	GFL	29.648228	-82.357699	1237	AcLi
GFL_051	cutting	GFL	29.649751	-82.343013	1237	acLi
GFL_052	cutting	GFL	29.649855	-82.341013	1237	acLi
GFL_054	cutting	GFL	29.643378	-82.332271	1237	AcLi
GFL_056	cutting	GFL	29.64356	-82.341192	1237	Acli
GFL_057	cutting	GFL	29.643654	-82.340297	1237	AcLi
GFL_058	cutting	GFL	29.64169	-82.342354	1237	AcLi
GFL_059	cutting	GFL	29.641976	-82.342825	1237	AcLi
GFL_060	cutting	GFL	29.642019	-82.342811	1237	AcLi
GFL_062	cutting	GFL	29.641224	-82.343284	1237	AcLi
HDE_001	seed	HDE	38.7252	-75.2837	1301	Acli
HDE_004	seed	HDE	38.7252	-75.2837	1301	acLi
HDE_007	seed	HDE	38.7252	-75.2837	1301	acLi
HDE_008	seed	HDE	38.7252	-75.2837	1301	acLi
HDE_009	seed	HDE	38.7252	-75.2837	1301	AcLi
HDE_010	seed	HDE	38.7252	-75.2837	1301	acLi
HDE_011	seed	HDE	38.7252	-75.2837	1301	Acli
HDE_012	seed	HDE	38.7252	-75.2837	1301	Acli
HDE_013	seed	HDE	38.7252	-75.2837	1301	acli
HDE_014	seed	HDE	38.7252	-75.2837	1301	acli
LKY_001	seed	LKY	38.2817	-85.6192	408.3	acli
LKY_002	seed	LKY	38.2817	-85.6192	408.3	AcLi
LKY_003	seed	LKY	38.2817	-85.6192	408.3	acli
LKY_004	seed	LKY	38.2817	-85.6192	408.3	Acli
LKY_005	seed	LKY	38.2817	-85.6192	408.3	acli
LKY_006	seed	LKY	38.2817	-85.6192	408.3	Acli
LKY_007	seed	LKY	38.2817	-85.6192	408.3	Acli
LKY_008	seed	LKY	38.2817	-85.6192	408.3	acli
LKY_010	seed	LKY	38.2817	-85.6192	408.3	Acli
LKY_011	seed	LKY	38.2817	-85.6192	408.3	AcLi
LMT_001	seed	LMT	45.6509	-110.562	1838	acli
LMT_002	seed	LMT	45.6509	-110.562	1838	AcLi
LMT_004	seed	LMT	45.6509	-110.562	1838	AcLi
LMT_005	seed	LMT	45.6509	-110.562	1838	AcLi
LMT_007	seed	LMT	45.6509	-110.562	1838	acLi

LMT_008	seed	LMT	45.6509	-110.562	1838	Acli
LMT_010	seed	LMT	45.6509	-110.562	1838	Acli
LMT_011	seed	LMT	45.6509	-110.562	1838	acli
LMT_012	seed	LMT	45.6509	-110.562	1838	acli
LMT_013	seed	LMT	45.6509	-110.562	1838	Acli
LMT_014	seed	LMT	45.6509	-110.562	1838	acli
PPA_001	seed	PPA	40.4526	-79.9476	908.8	AcLi
PPA_002	seed	PPA	40.4526	-79.9476	908.8	Acli
PPA_003	seed	PPA	40.4526	-79.9476	908.8	AcLi
PPA_004	seed	PPA	40.4526	-79.9476	908.8	Acli
PPA_006	seed	PPA	40.4526	-79.9476	908.8	AcLi
PPA_007	seed	PPA	40.4526	-79.9476	908.8	acli
PPA_008	seed	PPA	40.4526	-79.9476	908.8	Acli
PPA_009	seed	PPA	40.4526	-79.9476	908.8	Acli
PPA_010	seed	PPA	40.4526	-79.9476	908.8	AcLi
PPA_012	seed	PPA	40.4526	-79.9476	908.8	Acli
PPA_013	seed	PPA	40.4526	-79.9476	908.8	Acli
SCD_001	seed	SCD	42.5399	-96.5267	682.4	Acli
SCD_002	seed	SCD	42.5399	-96.5267	682.4	acli
SCD_003	seed	SCD	42.5399	-96.5267	682.4	acli
SCD_004	seed	SCD	42.5399	-96.5267	682.4	acLi
SCD_005	seed	SCD	42.5399	-96.5267	682.4	acLi
SCD_006	seed	SCD	42.5399	-96.5267	682.4	Acli
SCD_007	seed	SCD	42.5399	-96.5267	682.4	Acli
SCD_008	seed	SCD	42.5399	-96.5267	682.4	acli
SCD_009	seed	SCD	42.5399	-96.5267	682.4	acli
SCD_010	seed	SCD	42.5399	-96.5267	682.4	acLi
SCD_011	seed	SCD	42.5399	-96.5267	682.4	acli
SFD_002	seed	SFD	43.508	-96.7327	764.7	acli
SFD_003	seed	SFD	43.508	-96.7327	764.7	Acli
SFD_004	seed	SFD	43.508	-96.7327	764.7	acLi
SFD_005	seed	SFD	43.508	-96.7327	764.7	acli
SFD_006	seed	SFD	43.508	-96.7327	764.7	Acli
SFD_007	seed	SFD	43.508	-96.7327	764.7	Acli
SFD_008	seed	SFD	43.508	-96.7327	764.7	Acli
SFD_009	seed	SFD	43.508	-96.7327	764.7	acli
SFD_010	seed	SFD	43.508	-96.7327	764.7	acli
SFD_011	seed	SFD	43.508	-96.7327	764.7	acLi
SFD_012	seed	SFD	43.508	-96.7327	764.7	AcLi
STL_0108	cutting	STL	NA	NA	0	acli
STL_0701	cutting	STL	NA	NA	0	acli
STL_1010	cutting	STL	NA	NA	0	Acli
STL_5000	cutting	STL	38.648333	-90.288056	0	acli
STL_5002	cutting	STL	38.640556	-90.331667	0	Acli
STL_5003	cutting	STL	38.638611	-90.3575	0	AcLi
STL_5005	cutting	STL	38.655556	-90.406389	0	Acli
STL_5006	cutting	STL	38.666389	-90.395556	0	Acli
STL_5009	cutting	STL	38.674722	-90.359722	0	Acli
STL_5011	cutting	STL	38.672778	-90.342778	0	Acli
STL_5017	cutting	STL	38.645278	-90.280278	0	acli
STL_5019	cutting	STL	38.639722	-90.251111	0	Acli
STL_5020	cutting	STL	38.640278	-90.243889	0	Acli
STL_5022	cutting	STL	38.638889	-90.238889	0	Acli
STL_5023	cutting	STL	38.643611	-90.237778	0	acLi
STL_5024	cutting	STL	38.646389	-90.245833	0	Acli

STL_5026	cutting	STL	38.649722	-90.260833	0	Acli
STL_5029	cutting	STL	38.653056	-90.283889	0	Acli
STL_5030	cutting	STL	38.65	-90.285556	0	Acli
STL_5031	cutting	STL	38.622778	-90.351667	0	Acli
STL_5033	cutting	STL	38.617222	-90.394722	0	acLi
STL_5034	cutting	STL	38.607222	-90.393611	0	AcLi
STL_5035	cutting	STL	38.603056	-90.385278	0	Acli
STL_5036	cutting	STL	38.597222	-90.38	0	acli
STL_5038	cutting	STL	38.592222	-90.357222	0	AcLi
STL_5041	cutting	STL	38.603889	-90.332778	0	AcLi
STL_5042	cutting	STL	38.6125	-90.323333	0	AcLi
STL_5043	cutting	STL	38.623056	-90.286667	0	AcLi
STL_5044	cutting	STL	38.607778	-90.290278	0	AcLi
STL_5045	cutting	STL	38.614722	-90.284167	0	Acli
STL_5046	cutting	STL	38.6275	-90.291667	0	AcLi
STL_5049	cutting	STL	38.623056	-90.325278	0	AcLi
VBC_001	seed	VBC	49.2202	-122.8163	2830	AcLi
VBC_002	seed	VBC	49.2202	-122.8163	2830	acli
VBC_004	seed	VBC	49.2202	-122.8163	2830	AcLi
VBC_008	seed	VBC	49.2202	-122.8163	2830	acLi
VBC_009	seed	VBC	49.2202	-122.8163	2830	Acli
VBC_010	seed	VBC	49.2202	-122.8163	2830	AcLi
VBC_012	seed	VBC	49.2202	-122.8163	2830	acli
VBC_013	seed	VBC	49.2202	-122.8163	2830	AcLi
VBC_014	seed	VBC	49.2202	-122.8163	2830	Acli
VBC_015	seed	VBC	49.2202	-122.8163	2830	acLi
VBC_016	seed	VBC	49.2202	-122.8163	2830	Acli

Table S1.3 is too large to include here. It would be > 50 pages and difficult to read. It is essentially the full set of raw data used in analyses:

> **Table S1.3.** All information for the 483 plants studied in the common garden experiment. Garden block and position assignments, source population, cyanotype, fitness, and climate data are included. Fitness data include vegetative area, total inflorescence counts, daily inflorescence counts, and leaf herbivore damage measurements.

The table is published in the Supplementary Materials associated with:

Wright, S. J. *et al.* (2017) 'Continent-Wide Climatic Variation Drives Local Adaptation in North American White Clover', *Journal of Heredity*, 109(1), pp. 78–89. doi: 10.1093/jhered/esx060.

Table S1.4. Pairwise population F_{ST} comparisons for three populations genome-wide (GBS) and for the two cyanogenesis loci.

Comparison	GBS	Ac	Li
AOK-GFL	0.008	0.038	0.093
AOK-STL	0.019	0.126	0.038
GFL-STL	0.020	0.027	0.250

Table S1.5. Summary statistics for growth, survival and reproductive fitness measures across 483 plants studied in the common garden experiment.

	Vegetative tissue (cm^2)				Inflorescence count
(N = 483)	**April**	**May**	**October**	**March**	
Min	0.08	0.00	0.00	0.00	0
25th percentile	9.61	156.50	188.30	52.19	43
50th percentile	14.80	251.10	322.80	232.20	97
75th percentile	23.45	398.30	448.5	653.10	172
95th percentile	38.83	697.05	653.60	1193.94	321
Max	77.28	1609.00	1004.00	1687.00	601
Mean	17.80	303.40	327.80	388.40	119
# dead plants	0	2	3	27	—

Table S1.6. Cyanotypes of germinants in two locations.

Maternal_ID	Pop	Greenhouse	Tyson[a]
NFWC0401	AOK	Acli	Acli
NFWC0407	AOK	AcLi	Acli
NFWC0408	AOK	AcLi	acLi
NFWC050120	AOK	Acli	Acli
NFWC050121	AOK	AcLi	AcLi
NFWC050122	AOK	AcLi	AcLi
NFWC050124	AOK	AcLi	AcLi
NFWC050128	AOK	AcLi	AcLi
NFWC050129	AOK	AcLi	AcLi
NFWC050131	AOK	Acli	AcLi
NFWC050132	AOK	AcLi	Acli
BID_001	BID	acli	acli
BID_002	BID	acLi	acli
BID_003	BID	acLi	Acli
BID_004	BID	Acli	AcLi
BID_005	BID	acli	acLi
BID_006	BID	acli	acli
BID_007	BID	acli	acli
BID_008	BID	acli	acli
BID_009	BID	acli	acli
BID_010	BID	acLi	NA
BID_011	BID	acli	acli
CIL_001	CIL	acli	NA
CIL_003	CIL	acli	NA
CIL_004	CIL	AcLi	AcLi
CIL_005	CIL	AcLi	NA
CIL_006	CIL	acli	AcLi
CIL_007	CIL	acli	NA
CIL_008	CIL	AcLi	acli
CIL_009	CIL	Acli	NA
CIL_011	CIL	AcLi	Acli
CVA_001	CVA	AcLi	Acli
CVA_002	CVA	acLi	acli
CVA_003	CVA	acli	acLi
CVA_004	CVA	acli	NA
CVA_005	CVA	Acli	NA
CVA_006	CVA	acli	NA
CVA_007	CVA	acLi	AcLi
CVA_009	CVA	Acli	NA
CVA_010	CVA	acli	NA
CVA_011	CVA	acLi	Acli
CVA_012	CVA	acli	acli
DCO_001	DCO	Acli	Acli
DCO_002	DCO	Acli	Acli
DCO_003	DCO	acli	NA
DCO_004	DCO	Acli	Acli
DCO_005	DCO	Acli	NA
DCO_006	DCO	acLi	acLi
DCO_007	DCO	acLi	acli
DCO_008	DCO	acLi	AcLi

DCO_009	DCO	acLi	acli
DCO_011	DCO	Acli	NA
DCO_012	DCO	Acli	AcLi
DMN_001	DMN	acli	NA
DMN_004	DMN	Acli	NA
DMN_005	DMN	AcLi	acli
DMN_007	DMN	acli	NA
DMN_008	DMN	AcLi	NA
DMN_013	DMN	Acli	acli
DMN_014	DMN	acLi	NA
DMN_015	DMN	acLi	NA
DMN_016	DMN	acli	NA
DMN_017	DMN	Acli	NA
DMN_018	DMN	Acli	NA
HDE_001	HDE	Acli	Acli
HDE_004	HDE	acLi	NA
HDE_007	HDE	acLi	NA
HDE_008	HDE	acLi	NA
HDE_009	HDE	AcLi	acLi
HDE_010	HDE	acLi	NA
HDE_011	HDE	Acli	AcLi
HDE_012	HDE	Acli	NA
HDE_013	HDE	acli	NA
HDE_014	HDE	acli	NA
LKY_001	LKY	acli	acli
LKY_002	LKY	AcLi	Acli
LKY_003	LKY	acli	NA
LKY_004	LKY	Acli	acli
LKY_005	LKY	acli	NA
LKY_006	LKY	Acli	AcLi
LKY_007	LKY	Acli	acli
LKY_008	LKY	acli	Acli
LKY_010	LKY	Acli	NA
LKY_011	LKY	AcLi	acLi
LMT_001	LMT	acli	acLi
LMT_002	LMT	AcLi	NA
LMT_004	LMT	AcLi	AcLi
LMT_005	LMT	AcLi	AcLi
LMT_007	LMT	acLi	acli
LMT_008	LMT	Acli	Acli
LMT_010	LMT	Acli	NA
LMT_011	LMT	acli	Acli
LMT_012	LMT	acli	AcLi
LMT_013	LMT	Acli	acli
LMT_014	LMT	acli	Acli
PPA_001	PPA	AcLi	AcLi
PPA_002	PPA	Acli	Acli
PPA_003	PPA	AcLi	acLi
PPA_004	PPA	Acli	Acli
PPA_006	PPA	AcLi	acli
PPA_007	PPA	acli	NA
PPA_008	PPA	Acli	Acli

PPA_009	PPA	Acli	NA
PPA_010	PPA	AcLi	Acli
PPA_012	PPA	Acli	acli
PPA_013	PPA	Acli	NA
SCD_001	SCD	Acli	Acli
SCD_002	SCD	acli	acLi
SCD_003	SCD	acli	AcLi
SCD_004	SCD	acLi	acli
SCD_005	SCD	acLi	NA
SCD_006	SCD	Acli	acLi
SCD_007	SCD	Acli	acLi
SCD_008	SCD	acli	Acli
SCD_009	SCD	acli	acLi
SCD_010	SCD	acLi	acLi
SCD_011	SCD	acli	NA
SFD_002	SFD	acli	Acli
SFD_003	SFD	Acli	acLi
SFD_004	SFD	acLi	AcLi
SFD_005	SFD	acli	NA
SFD_006	SFD	Acli	acli
SFD_007	SFD	Acli	acli
SFD_008	SFD	Acli	acli
SFD_009	SFD	acli	NA
SFD_010	SFD	acli	acli
SFD_011	SFD	acLi	Acli
SFD_012	SFD	AcLi	acLi
VBC_001	VBC	AcLi	AcLi
VBC_002	VBC	acli	AcLi
VBC_004	VBC	AcLi	NA
VBC_008	VBC	acLi	NA
VBC_009	VBC	Acli	Acli
VBC_010	VBC	AcLi	AcLi
VBC_012	VBC	acli	AcLi
VBC_013	VBC	AcLi	NA
VBC_014	VBC	Acli	NA
VBC_015	VBC	acLi	NA
VBC_016	VBC	Acli	NA

[a]Missing values (NA) occurred at Tyson when no seeds germinated, or when all germinants died before the end of the 30-day experiment.

Table S1.7. Climatic distances (Euclidean) across populations for each of the top three principal components.

Population	PC1_euc (Precipitation)	PC2_euc (Heat)	PC3_euc (Variability)	Climate PC index*
AOK	0.988	1.182	1.884	4.055
BID	3.283	0.707	4.910	8.900
CIL	1.450	2.077	0.535	4.062
CVA	1.545	0.193	0.479	2.217
DCO	3.047	1.062	3.256	7.365
DMN	4.265	3.562	0.211	8.039
GFL	5.315	0.153	2.969	8.437
HDE	1.660	1.462	0.316	3.437
LKY	1.473	0.869	1.356	3.699
LMT	4.142	1.072	3.499	8.712
PPA	0.264	1.182	0.451	1.897
SCD	3.121	0.299	0.676	4.097
SFD	3.863	0.704	0.542	5.109
STL	0.000	0.000	0.000	0.000
VBC	1.030	7.051	4.063	12.144

*Climate PC index is the sum of each of the individual PC distances.

Table S1.8. Parameter summary statistics for the top three models for each of the two fitness measures.

Top 3 Growth Rate Models					
Model	**Parameter**	**Slope**	**Slope C.I. (±)**	**F**	**P-value**
1	Precipitation	-0.03	0.02	2.65	0.13
2	Heat*	-0.04	0.02	5.72	0.03
3	Variability*	-0.04	0.02	6.68	0.02
Top 3 Inflorescence Count Models					
Model	**Parameter**	**Slope**	**Slope C.I. (±)**	**F**	**P-value**
12	Precipitation*	-0.12	0.05	4.75	0.03
	Heat*	-0.13	0.05	6.63	0.01
	Cyanotype**	(relative to acli)			
	acLi	0.12	0.02	22.65	<0.001
	Acli	0.32	0.02	246.65	<0.001
	AcLi	0.37	0.02	229.10	<0.001
14	Heat	-0.09	0.05	2.82	0.09
	Variability	-0.09	0.06	2.31	0.13
	Cyanotype**	(relative to acli)			
	acLi	0.12	0.02	22.56	<0.001
	Acli	0.32	0.02	247.12	<0.001
	AcLi	0.37	0.02	230.13	<0.001
15	Precipitation	-0.10	0.06	2.98	0.08
	Heat*	-0.11	0.05	4.89	0.03
	Variability	-0.05	0.06	0.76	0.38
	Cyanotype**	(relative to acli)			
	acLi	0.12	0.02	22.66	<0.001
	Acli	0.32	0.02	246.43	<0.001
	AcLi	0.37	0.02	228.83	<0.001

* indicates significance at P ≤ 0.05, ** indicates significance at < 0.001.

SUPPLEMENTARY FIGURES

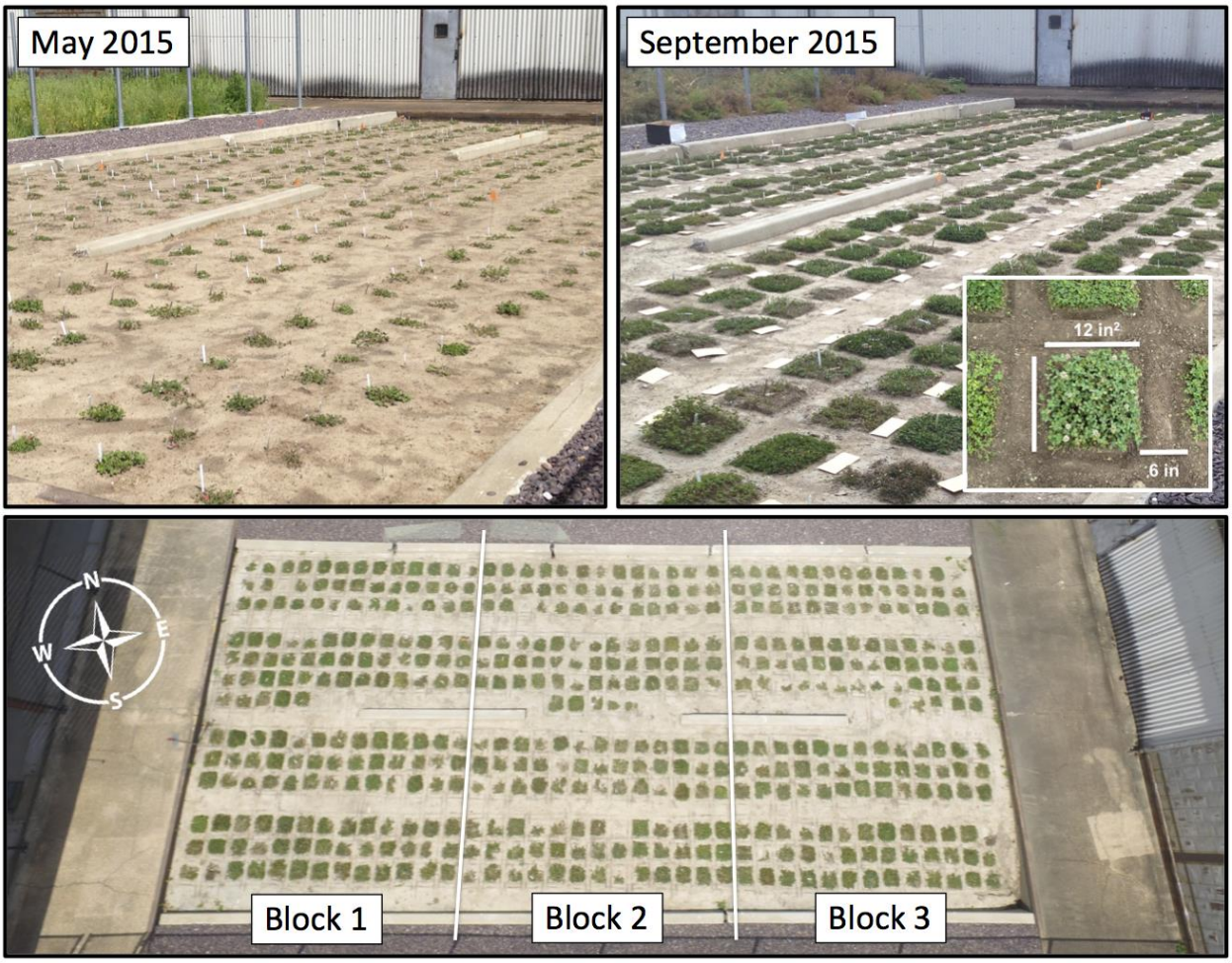

Figure S1.1. The experimental garden plot at the Washington University Tyson Research Center shown at two stages of the common garden experiment during the growing season (May and September). The plot is situated with buildings on the East and West sides. The aerial photo is labeled to delineate the three blocks. The inset depicts the spacing of plants following trimming.

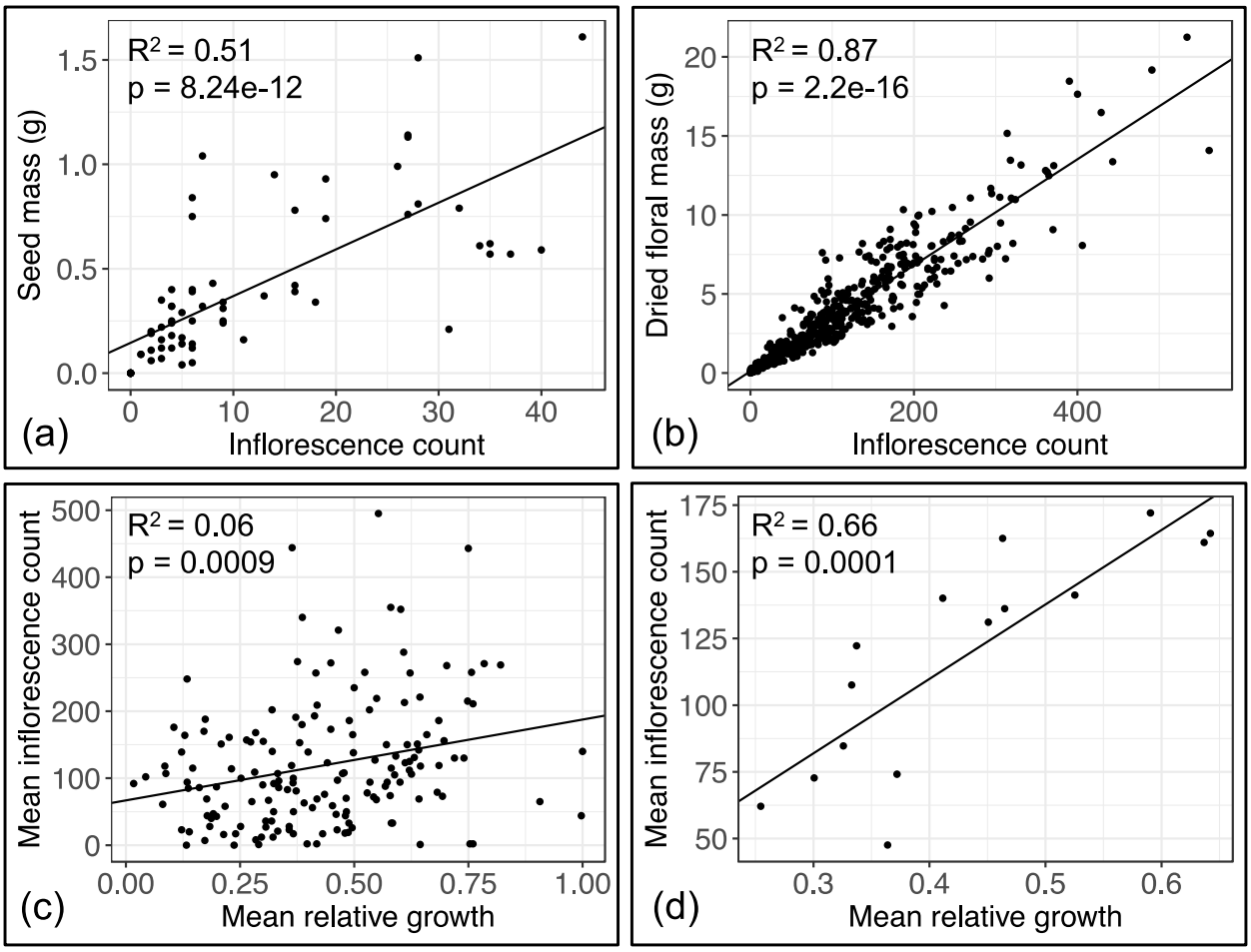

Figure S1.2. Linear correlations related to inflorescence count. Seed mass (a) and dried floral mass (b) were both correlated with inflorescence count. Inflorescence count was correlated with relative growth when averaged across the three replicate plants of a given genotype (c) and also when averaged across genotypes from the same population (d). Adjusted R^2 values and p-values for lines of best fit are shown.

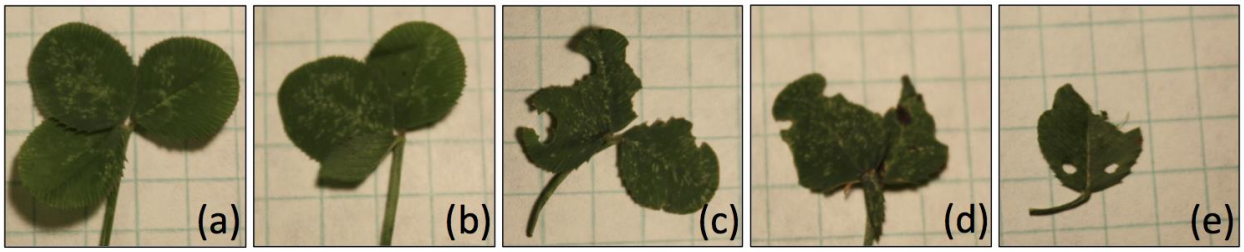

Figure S1.3. Examples of leaf herbivore damage categories: 0% (a), 1-25% (b), 26-50% (c), 51-75% (d), and >75% (e).

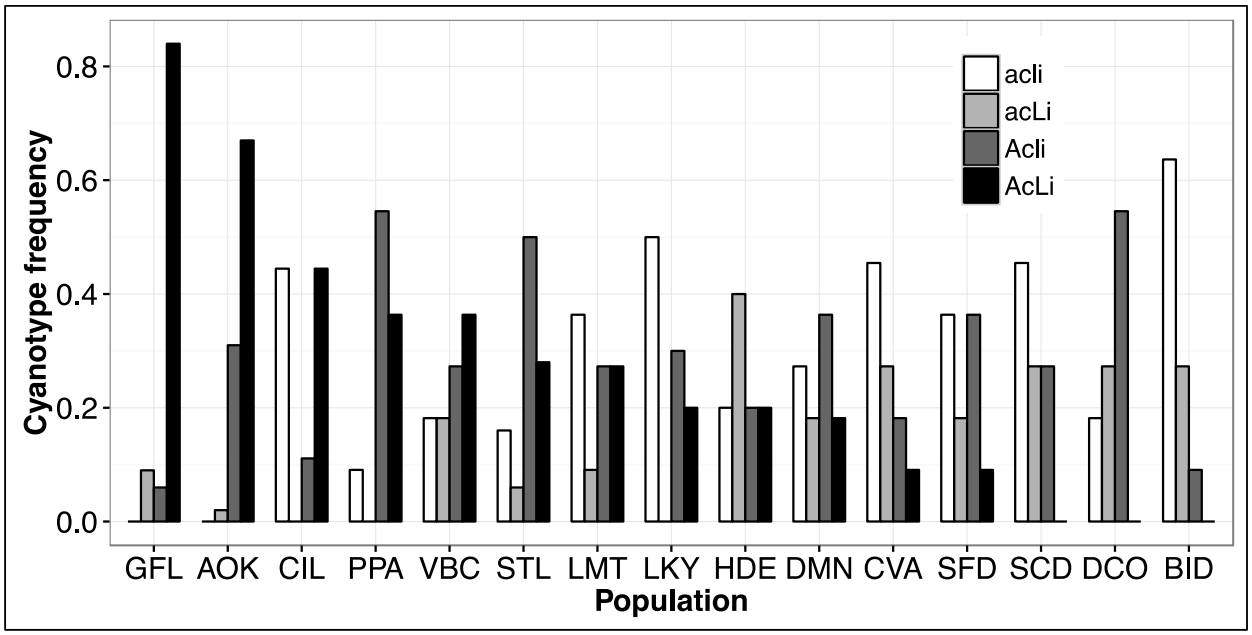

Figure S1.4. Cyanotype frequencies across populations used in the common garden experiment, including deeper sampling in the AOK, GFL and STL populations that were used for population structure analyses.

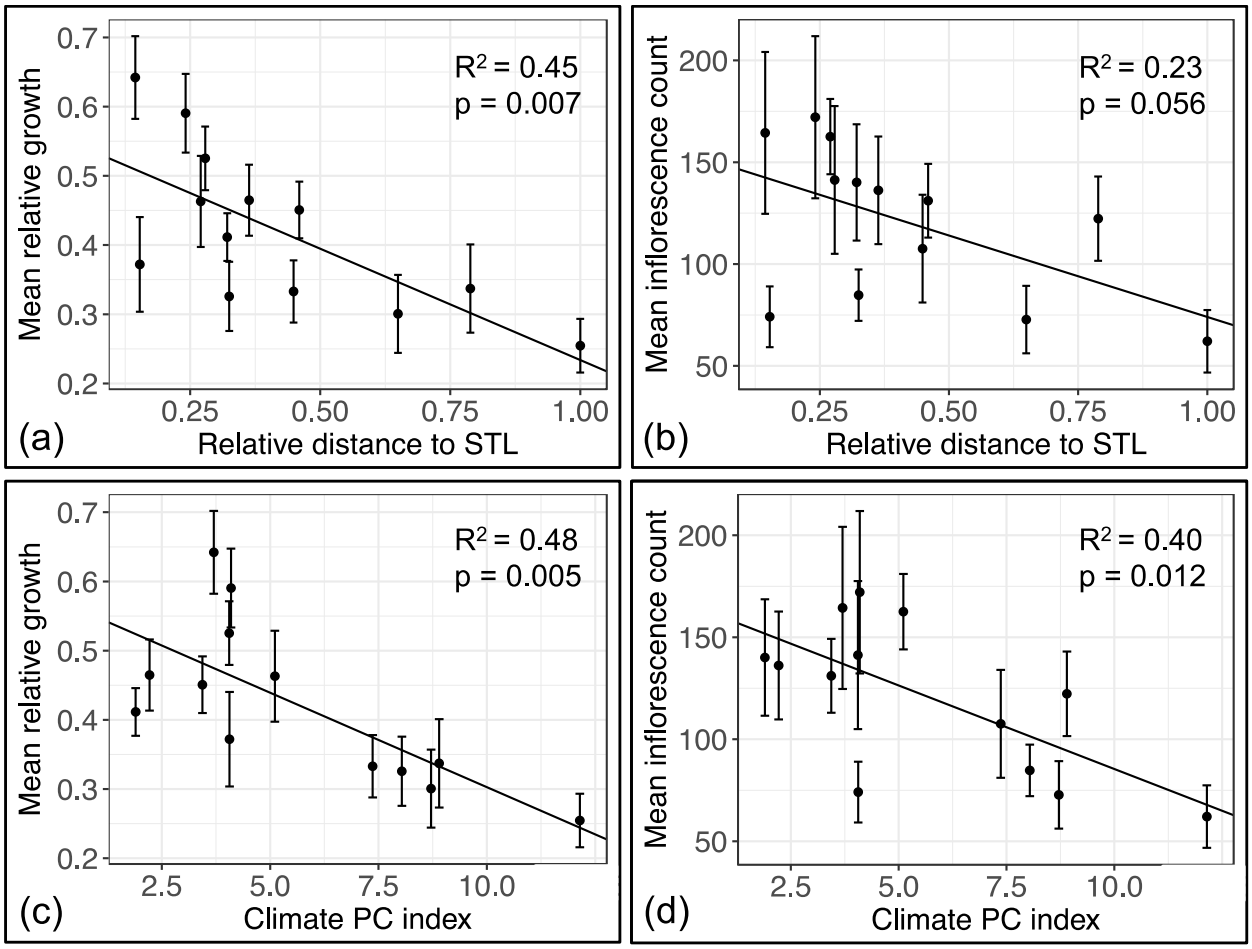

Figure S1.5. Linear relationships of two fitness measures (relative growth in vegetative tissue from April to October (a, c) and inflorescence count (b, d)) as a function of geographic distance (a, b) or climatic distance (PC index; c, d) across 13 populations. This figure presents the same data as Figure 3 but excludes populations collected as stolon cuttings (STL and GFL). Data points show the mean value for each population with standard error bars. Adjusted R^2 values and p-values for lines of best fit are shown.

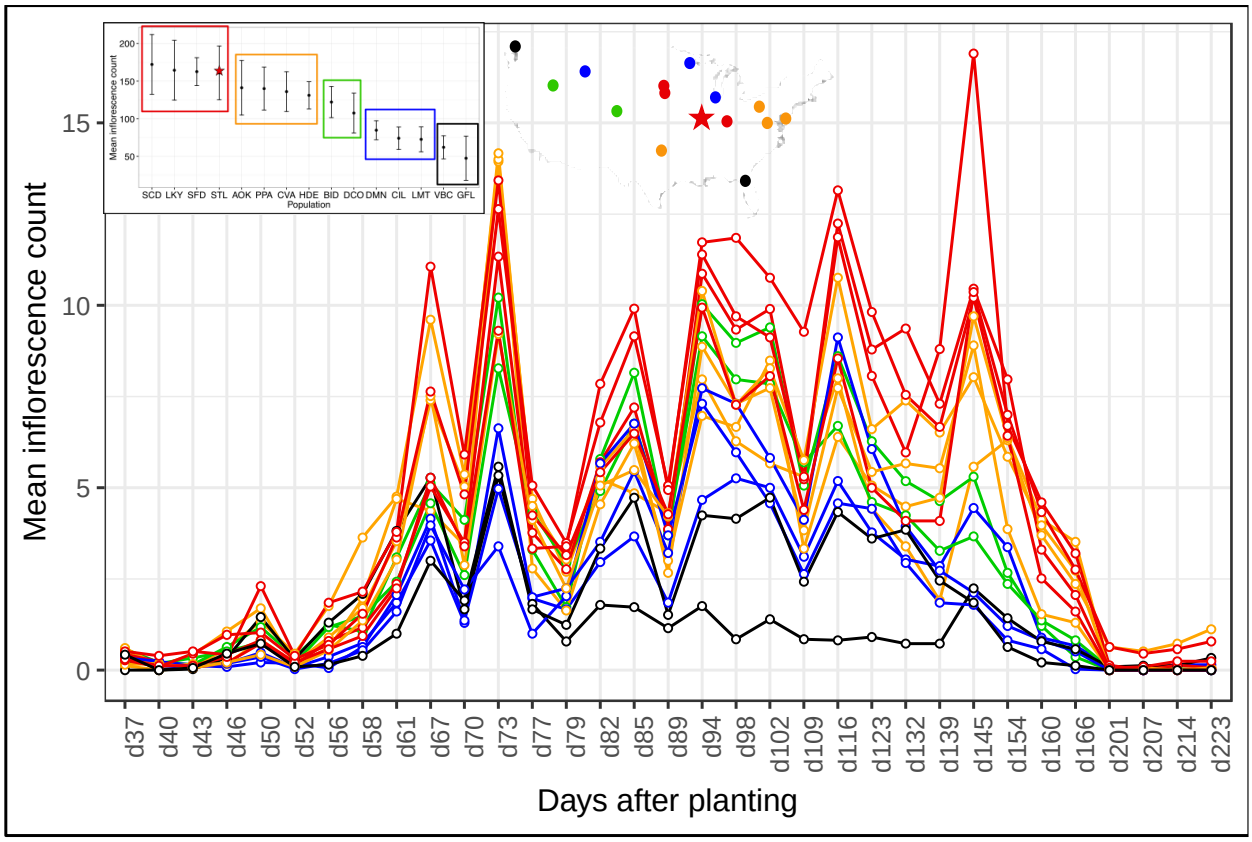

Figure S1.6. Mean inflorescence count across populations over the growing season. The four populations with red lines had the highest mean inflorescence count when individual inflorescence counts were summed over the entire season, followed successively by populations in orange, green, blue and black. Colors correspond to the insets.

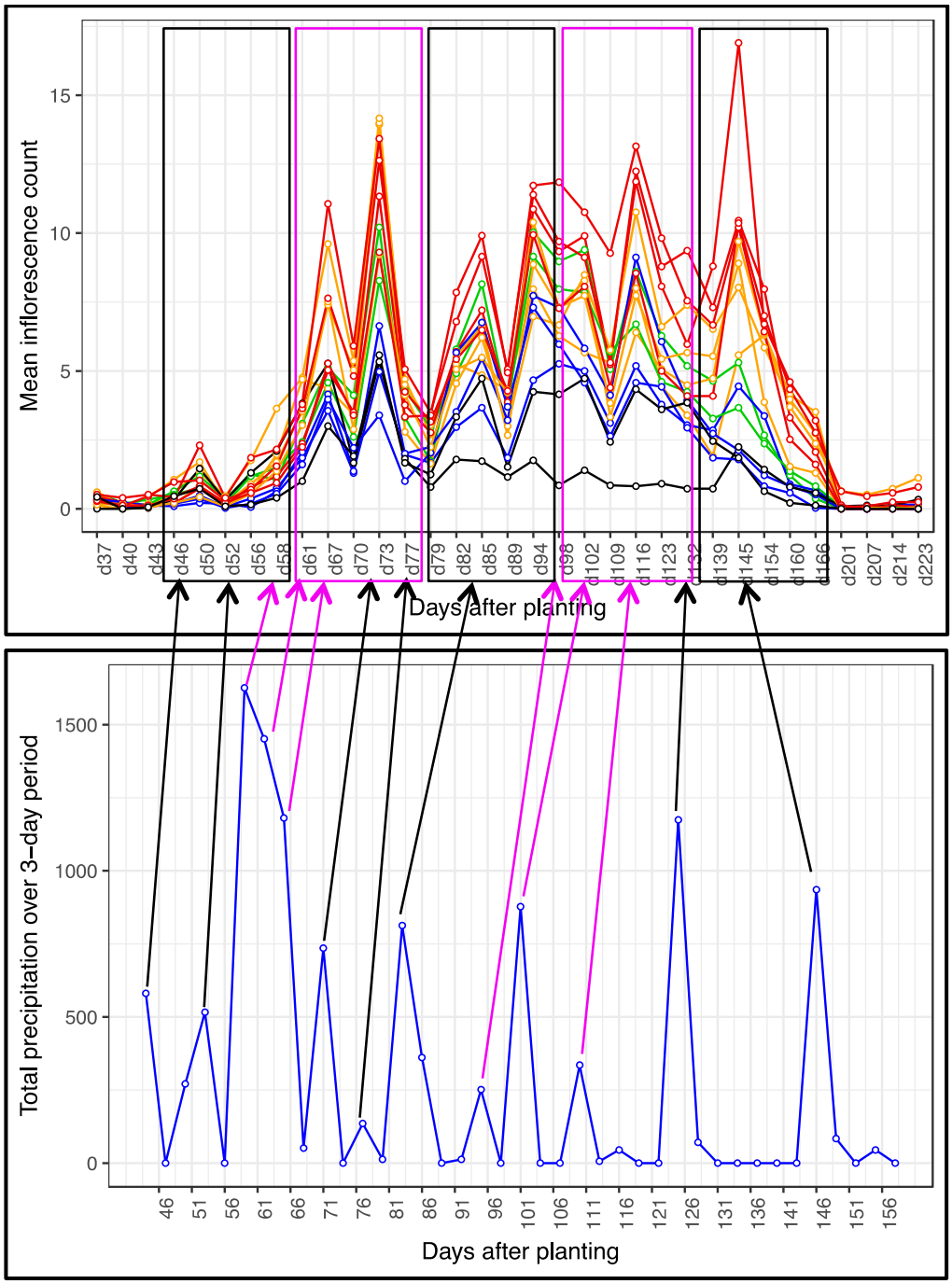

Figure S1.7. Mean inflorescence count across populations over the growing season as related to three-day precipitation windows.

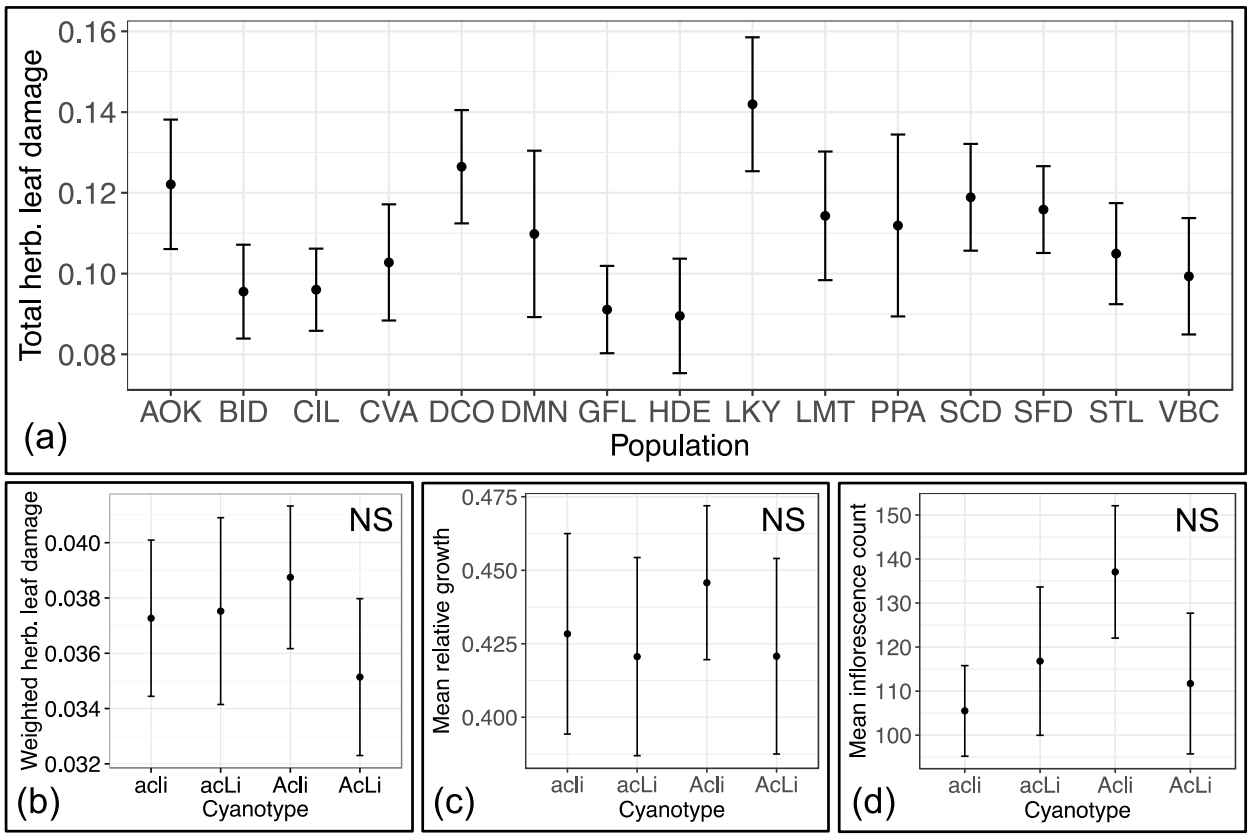

Figure S1.8. Total herbivore damage across populations (a), weighted herbivore leaf damage across cyanotypes (b), and two measures of fitness across cyanotypes (relative growth in vegetative tissue from April to October, (c) and inflorescence count, (d)). Data points show the mean value for each group with standard error bars. Pairwise Wilcoxon signed-rank tests between cyanotypes were not significant at the $p < 0.05$ level for any comparisons in (b), (c), and (d).

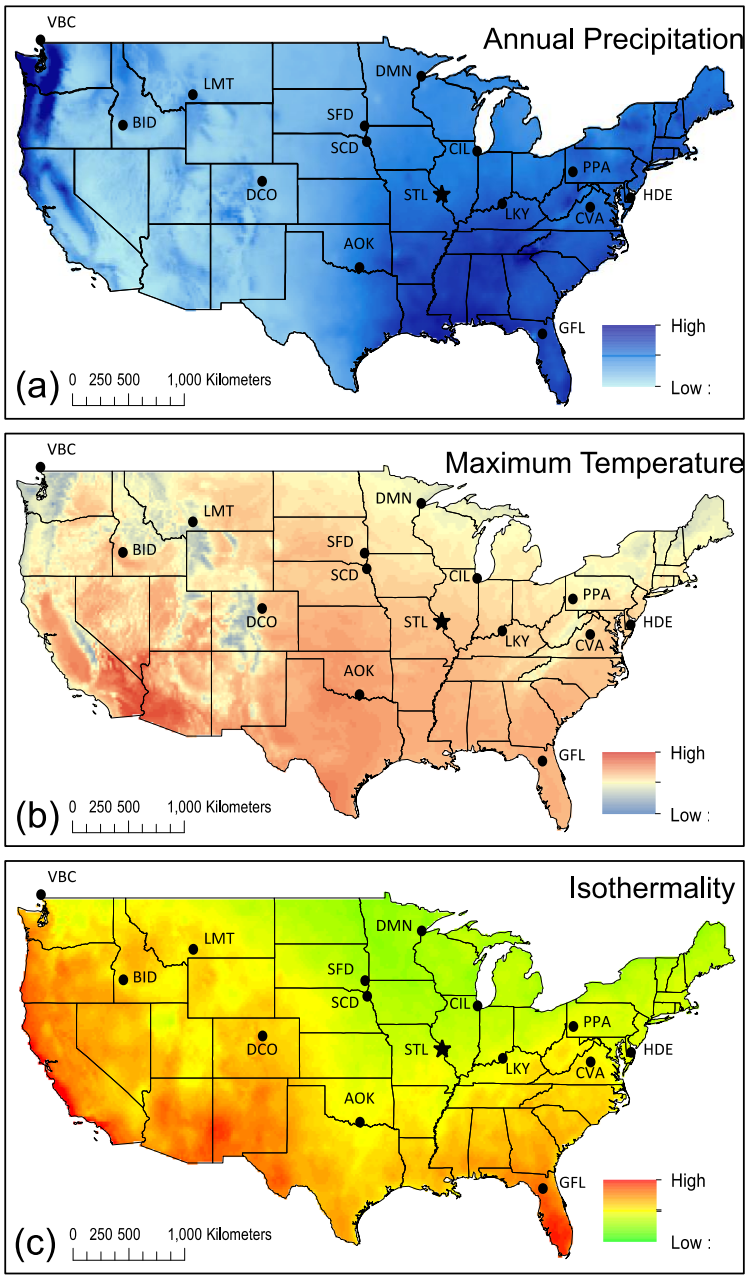

Figure S1.9. Sampling locations and abbreviations for the 15 populations used in the common garden experiment, with the star indicating the location of the common garden experiment in St. Louis, MO. Maps were created in ArcGIS 10 (ESRI 2011) and are shaded according to climatic variables downloaded from BIOCLIM (Hijmans *et al.* 2005). Annual precipitation (a) is highly correlated with PC1, which explains 44% of the climatic variation across the 15 populations. Maximum temperature of the warmest month (b) and isothermality (c) are highly correlated with PC2 and PC3, respectively. High isothermality denotes high day-to-night temperature oscillations relative to summer-to-winter oscillations.

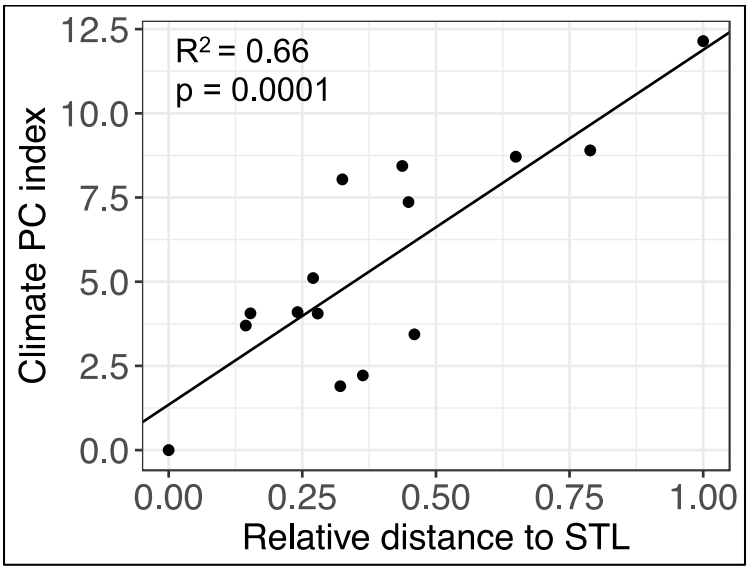

Figure S1.10. Correlation between climatic distance (PC index) and geographic distance. Adjusted R^2 and p-values for the line of best fit are shown.

CHAPTER 2

Divergent life history strategies and genetic trade-offs underlie local adaptation in white clover

ABSTRACT

Local adaptation is common in plants, and while understanding its genetic basis is an important goal of evolutionary biology, it has rarely been examined in herbaceous perennial species, which constitute a major component of many of the world's ecosystems. Moreover, while many plants are characterized by intraspecific chemical defense polymorphisms, the importance of this adaptive variation for local adaptation is poorly understood. We examined the genetic architecture of local adaptation in a perennial, obligately-outcrossing herbaceous legume, white clover. This species is characterized by a well-studied chemical defense polymorphism for cyanogenesis (HCN release following tissue damage) that has repeatedly evolved climate-associated clines. We generated two biparental F_2 mapping populations from plants collected in three environments that span the U.S. latitudinal species range (Duluth, MN, St. Louis, MO and Gainesville, FL); genome-wide markers were generated with genotyping-by-sequencing, and plants were grown for two years in reciprocal common garden experiments in the three parental environments. Fitness-related traits displayed clear evidence for local adaptation, and genetic mapping revealed an underlying genetic architecture characterized by multiple loci with allelic tradeoffs across environments (antagonistic pleiotropy). We found little evidence that the cyanogenesis polymorphism contributes to local adaptation. Instead, divergent life history strategies in reciprocal environments — specifically, early investment in flowering in the southernmost site vs. delayed flowering and multi-year persistence in the cooler northern environments — were major fitness determinants. These findings suggest that multi-locus allelic tradeoffs for life history traits may be a common mechanism for local adaptation in outcrossing herbaceous perennials.

INTRODUCTION

Population differentiation and adaptation to local conditions often occur when species have broad geographic distributions and experience spatially-heterogeneous environments (Hereford, 2009). Local adaptation maintains genetic polymorphism in the face of gene flow when selection is strong (Hedrick, 1986), providing a pool of adaptive variation that can allow species to persist through periods of environmental change; it may be a particularly important adaptive strategy for plants and other sessile species currently facing rapidly changing climatic conditions (Bradshaw and Holzapfel, 2001; Leimu and Fischer, 2008; Thompson *et al.*, 2013). Identifying the most relevant traits and the underlying genetic polymorphisms that contribute to local adaptation are therefore key goals in evolutionary, agricultural, and conservation biology research programs (Anderson, Willis and Mitchell-Olds, 2011).

Plant species encounter myriad selective pressures across their ranges, involving both biotic and abiotic stressors. In response, they have evolved adaptations for many of these demands, including chemical defenses against herbivores. Chemical defenses are pervasive across the plant kingdom, which strongly suggests that they play important adaptive roles (Mithöfer and Boland, 2012). However, defenses can be energetically costly, and producing them may or may not outweigh their benefits in a given environment (Züst and Agrawal, 2017). When a chemical defense is maintained as a polymorphism across a species range, it is often assumed that trade-offs exist across populations, such that the benefit of the defense for overall fitness varies depending upon environmental context; in these cases, chemical defense polymorphisms may be important for local adaptation (e.g., Prasad *et al.*, 2012; Kerwin

et al., 2015). However, the assumption that chemical defense polymorphisms contribute substantially to fitness differences within or across environments is rarely empirically tested (Erb, 2018). Thus, the relative contribution of chemical defense polymorphisms for local adaptation, compared to other factors, is not well understood.

A classic approach to test for local adaptation in plant species is through reciprocal common garden experiments, whereby the same set of genotypes is grown under different environmental conditions (Jens Clausen, Keck and Hiesey, 1941). Local adaptation is demonstrated when native genotypes exhibit high relative fitness in local environments and reduced fitness in foreign environments (i.e., genotypic fitness trade-offs across environments) (Kawecki and Ebert, 2004). When these experiments are performed using genetic mapping populations derived from parents that originated in the reciprocal environments, the genetic architecture of local adaptation can be characterized by mapping fitness-related quantitative trait loci (QTLs) in each environment (Ågren *et al.*, 2013; Anderson *et al.*, 2014). This combined field-experiment and QTL-mapping approach allows researchers to identify genetic trade-offs at the level of QTLs (Rausher and Delph, 2015). Moreover, for species with geographically structured chemical defense polymorphisms, the fitness contribution of a chemical defense trait to local adaptation can be directly assessed by determining the extent to which chemical defense loci are associated with fitness-related QTLs.

For a given fitness-related QTL, alternate alleles that show fitness trade-offs across reciprocal environments are said to show a pattern of antagonistic pleiotropy (also referred to as QTL×E interactions) (Anderson, Willis and Mitchell-Olds, 2011; Lowry *et al.*, 2019). Despite the prediction that antagonistic pleiotropy should be

common for locally-adapted QTLs, empirical evidence for this trade-off pattern has been mixed in plants (Wadgymar *et al.*, 2017). This may partly reflect statistical limitations in detecting significant fitness effects in two environments (Anderson *et al.*, 2013). However, it may also reflect a bias in study systems, as work to date has largely focused on annual species with high rates of self-fertilization owing to their experimental tractability (Anderson, Willis and Mitchell-Olds, 2011; Savolainen, Lascoux and Merilä, 2013). Antagonistic pleiotropy may be more commonly detected in outcrossing species, which display higher effective recombination and associated reductions in linkage disequilibrium (Wadgymar *et al.*, 2017).

In this study, we use QTL mapping of fitness-related traits to assess the genetic architecture of local adaptation in a geographically widespread, obligately-outcrossing species that is characterized by a well-documented chemical defense polymorphism. Our focal species, white clover (*Trifolium repens* L.), is an herbaceous legume that is native to Europe but naturalized in mesic temperate regions worldwide. In North America, where it was introduced within the last 500 years, it is widely distributed across much of the continent and can be found in climates ranging from boreal to subtropical (USDA, 2002). White clover shows evidence of local adaptation across this range. A recent fitness study using genotypes sampled from across North America demonstrated strong associations between climate-of-origin and plant performance in a central U.S. common garden (Wright *et al.*, 2017). In addition, white clover populations have recurrently evolved climate-associated clines in cyanogenesis (the production of HCN following tissue damage; described below), which suggests that this chemical defense polymorphism could play an important role in local adaptation. As a common plant of

lawns, roadsides and fields, white clover is characterized by very large effective population sizes and little population structure on continental or global scales (George *et al.*, 2006; Kooyers and Olsen, 2012, 2013; Wright *et al.*, 2017); these features could promote local adaptation via antagonistic pleiotropy. This species thus provides an ideal study system to complement existing studies on the genetic architecture of local adaptation and explicitly to assess the relative importance of chemical defense polymorphisms vs. other genetic factors in this process.

Cyanogenesis in white clover

Cyanogenesis is an anti-herbivore defense that has evolved convergently across the plant kingdom and occurs in >3,000 species (Gleadow and Møller, 2014). The cyanogenic response occurs when tissue damage triggers the mixing of two chemical precursors, cyanogenic glucosides (CNglcs) and their hydrolyzing enzymes, leading to the liberation of toxic HCN. This chemical defense is polymorphic in white clover, with both cyanogenic and acyanogenic plants found in natural populations. At the genetic level, the polymorphism is controlled by two unlinked simple Mendelian polymorphisms, *Ac/ac* and *Li/li*; these control the presence/absence of CNglcs and the hydrolyzing enzyme, linamarase, respectively (Olsen, Sutherland and Small, 2007; Olsen, Hsu and Small, 2008). For each gene, recessive alleles correspond to gene deletions, and homozygous recessive genotypes lack the corresponding precursor (Olsen, Kooyers and Small, 2013; Olsen and Small, 2018). Thus, four cyanogenesis phenotypes or 'cyanotypes' occur in wild populations: AcLi (cyanogenic, containing both precursors); and Acli, acLi, and acli (acyanogenic, lacking one or both precursors).

The potential adaptive function of the white clover cyanogenesis polymorphism has been studied for more than 60 years (Daday, 1954a, 1954b). Latitudinal and altitudinal clines in the frequency of cyanogenesis have been documented worldwide; higher frequencies of cyanogenic (AcLi) plants are consistently found in warmer climates, while higher frequencies of acyanogenic plants are maintained in cold climates (Daday, 1954b, 1958; de Araújo, 1976; Till-Bottraud, Kakes and Dommée, 1988; Caradus *et al.*, 1990; Kooyers and Olsen, 2012, 2013; Kooyers *et al.*, 2014). Cyanogenic plants may be favored in warm environments due to higher herbivore abundance. In line with this hypothesis, there is abundant evidence that AcLi plants are differentially protected against small herbivores (reviewed in Hughes, 1991; Kooyers and Olsen, 2013; Kooyers *et al.*, 2018). In colder environments, where herbivore pressure is likely lower, natural selection may favor acyanogenic plants that do not devote energy to producing costly and unnecessary cyanogenesis components (Kakes, 1989; Kooyers *et al.*, 2018). In addition to functioning in herbivore deterrence, the biochemical precursors of cyanogenesis (specifically, CNglcs) have also been proposed to function as nitrogen storage and transport compounds, which could be particularly adaptive in drought-prone environments (Gleadow and Møller, 2014; Kooyers *et al.*, 2014; Kooyers, 2015).

While this extensive body of accumulated research provides strong evidence that natural selection acts on the cyanogenesis polymorphism, the importance of this variation for local adaptation, relative to other fitness-related genetic factors, has not been assessed. In this study we used two white clover F_2 mapping populations in reciprocal common garden experiments spanning the latitudinal climatic gradient across

the United States to address the following questions: 1) Does white clover display local adaptation, as evidenced by genotypic fitness trade-offs across contrasting environments? 2) To what extent is local adaptation attributable to variation at the *Ac/ac* and *Li/li* cyanogenesis loci, relative to the overall genotypic effect? And 3) What is the genetic architecture of local adaptation in white clover, and to what extent does it occur through allelic trade-offs across environments (antagonistic pleiotropy)?

MATERIALS AND METHODS

Study system

Native to southern Europe, *Trifolium repens* was broadly introduced throughout temperate regions worldwide for soil enrichment prior to the invention of synthetic nitrogen fertilizers in the 20th Century (Kjærgaard, 2003). It remains one of the most important temperate forage crops and is commonly grown in mixed pastures with grasses (Abberton and Thomas, 2010; Andrae, Hancock and Harmon, 2016). White clover is primarily bee-pollinated; it also spreads vegetatively with lateral stolons, allowing it to form dense mats and clonal patches, as well as providing the ability to replicate genotypes clonally for field experiments. The species is allotetraploid (2n=4X=16) with a genome size of 1174 Mb (Griffiths *et al.*, 2013), 841 Mb of which has been assembled into a draft reference genome (Griffiths *et al.*, 2019). Two diploid congeners, *T. occidentale* and *T. pallescens*, have been identified as the closest extant relatives of *T. repens* and the contributors of its two subgenomes (Griffiths *et al.*, 2013, 2019).

Generation of F_2 mapping populations

The common garden experiments used two biparental F_2 mapping populations generated from three wild North American plants originating from geographical locations that span the latitudinal and temperature range of white clover populations in the United States: Duluth, MN (DMN), St. Louis, MO (STL), and Gainesville, FL (GFL). Duluth (USDA climate zone 4b) sits on the shores of Lake Superior near the U.S.-Canadian border and experiences some of the coldest winter temperatures in the contiguous 48 states. Gainesville (USDA zone 9a) is located near the transition from temperate to subtropical climate, near the southern limit of naturalized U.S. white clover populations. St. Louis (USDA zone 6b) is centrally located in the U.S., midway latitudinally between Duluth and Gainesville, and experiences a continental climate marked by cold winters and hot, humid summers.

The three parental genotypes were selected such that the *Ac/ac* and *Li/li* genetic polymorphisms would be segregating to create all four cyanotypes in the F_2 mapping populations: DMN_010 (*ac/ac*, *li/li*); STL_0701 (*ac/ac*, *li/li*); and GFL_007 (*Ac/Ac*, *Li/Li*). GFL_007 served as a parent in both mapping populations, with one population having DMN_010 as a parent (DMN $\times$ GFL, referred to below as the DG population) and the other STL_0701 (STL $\times$ GFL, referred to below as the SG population). Hand crosses were performed between parents in both directions to generate 50-100 F_1 genotypes per population. Within each F_1 population, random cross-pollinations were performed by hand or using bee cages to generate 502 and 500 F_2 genotypes in the DG and SG populations, respectively (see Supplementary Methods in Appendix II). All F_1 and F_2

genotypes were planted from seed and grown in the Washington University (WU) greenhouse facilities.

Genotyping

Genome-wide SNP markers for the two mapping populations (including the three parents and all F_1 and F_2 progeny) were generated with genotyping-by-sequencing (GBS) using the ApeKI restriction enzyme for digestion (Elshire *et al.*, 2011; Huang *et al.*, 2014) (Olsen et al., *in prep*). Cyanotypes for all plants were determined using Feigl-Anger cyanogenesis assays on fresh leaf tissue and by PCR-genotyping the *Ac/ac* and *Li/li* polymorphisms using established protocols (Olsen, Sutherland and Small, 2007; Olsen, Hsu and Small, 2008).

Linkage maps for genetic mapping were constructed independently for each F_2 population, using SNPs called only for markers that were homozygous in both parents and that had > 0.7 heterozygosity in the F_1 population. SNPs were filtered if they did not meet any of the following criteria: a minor allele frequency (MAF) > 0.05, missing data < 0.1, average read depth > 5X, or a p-value < 0.01 in a genotype frequency test (indicating deviations from 1:2:1 segregation in the F_2 generation). Final genetic linkage maps included 2,575 and 2,437 SNPs for the DG and SG populations, respectively (Olsen et al., *in prep*).

Reciprocal common garden experiments

Common garden experiments were performed for each mapping population in the two regions where the parent plants originated, with all F_2 genotypes grown in both

of the parental environments. Thus, both the DG and SG populations were planted at the GFL site, with the DG population also grown at the DMN site and the SG population also grown at the STL site. Planting dates were selected such that plants would become established during the main growth season at each site; the DMN and STL common gardens were planted in the late spring and early summer, while the GFL site was established in the fall. Specifically, the DG mapping population was planted in Duluth, MN at the University of Minnesota-Duluth's Research and Field Studies Center (46.866 °N, -92.048 °W) on June 14, 2016, the SG population was planted in Eureka, MO at WU's Tyson Research Center (38.527 °N, -90.562 °W) on June 11, 2016, and both mapping populations were planted at the University of Florida-Gainesville's Plant Science Research and Educational Unit (PSREU) in Citra, FL (29.409 °N, -82.171 °W) on October 12, 2016.

Three replicate stolon cuttings of each F_2 genotype were made 2-4 weeks prior to planting in each common garden. All stolon cuttings were initially ~10 cm in length, with 5-15 leaves and nodulated roots present at one or more nodes; rooting hormone was applied to encourage additional root formation. Cuttings were planted in Metro-Mix 360 soil in 2-inch square pots (Hummert International, Earth City, MO) and were grown in WU greenhouses for 3-4 days on mist benches, then for 1-2 weeks under standard greenhouse conditions to allow for further establishment before being transplanted in the field.

Full sets of F_2 genotypes were planted within three fully randomized blocks for each mapping population at each site. Supplemental watering, fertilizer and *Rhizobium* inoculum, as determined by each site's field coordinator (see Acknowledgements)

depending on the condition of the plants, was provided to prevent high mortality from transplantation in the first two months of each common garden experiment. Some plants that were suspected to have died primarily from transplanting stress were re-planted from new stolon cuttings within the first two months; cuttings needing re-planting were random with respect to genotype and constituted less than 1%, 15%, and 10% of all plants at the DMN, STL, and GFL sites, respectively (See Supplementary Results in Appendix II). Because white clover spreads laterally through stolon growth, it was important to keep individual plants from intermingling throughout the experiments for accurate fitness measurements. Thus, plants at all gardens were kept trimmed to 930 cm^2 (1 ft^2). Removal of weeds from the common garden plots was also performed for the duration of the experiments (see Supplementary Methods in Appendix II).

Fitness measurements

Vegetative area, survival, and reproductive fitness measurements were recorded for all plants in each common garden over a period of two years (2016-2018) (Table S2.1). Data collection was performed blind with respect to the genotype and cyanotype of each plant. Trait measurement procedures followed protocols used in a previous white clover common garden experiment at the STL site (Wright *et al.*, 2017), as described below.

Vegetative surface area. Digital photos were taken directly over individual plants once per month, with red-painted pennies used for scale and color contrast. Photos were not taken at DMN or STL during winter months when plants were dormant and

frequently covered in snow. After the experiments concluded, time points that were comparable across reciprocal sites in terms of days into the experiment, or that reflected key seasonal and mortality events, were selected for further digital analysis, in which vegetative surface area (cm^2) was estimated for individual plants using Easy Leaf Area (ELA) software and previously described methods (Table S2.1) (Easlon and Bloom, 2014; Wright *et al.*, 2017).

Survival. For the DMN and STL gardens, Year 1 survivor counts were assessed in the spring following the first winter. Mortality was low throughout the experiment at these sites, but genotypes exhibited variation in their response to winter; final survival measurements following the second winter season were therefore recorded in an ordinal fashion to capture additional variation (0 = dead; 1 = < 25% of allotted 930 cm^2 space filled with living plant material; 2 = between 25-50% of allotted space filled; 3 = 50-90% filled; 4 = > 90% filled). In GFL, where mortality was high, the presence/absence of living plant material was assessed monthly beginning in the second month of the experiment; total lifespan was calculated by summing the number of months each plant was recorded alive.

Reproductive output. Maturing inflorescences (identifiable as those with senescent, downturned basal florets) were counted every ~3-7 days throughout the flowering seasons and then removed to prevent seed dispersal and seedling recruitment within the common gardens. Inflorescence counts were used as a proxy for seed set, as the two are strongly correlated in white clover (Wright *et al.*, 2017). Flowering duration was calculated by subtracting the dates of the first and final recorded inflorescences for each plant at each site and for each growing season.

Quantitative genetic analyses

Prior to statistical analysis, all fitness measurements within each common garden site and year were evaluated for normality using a Shapiro-Wilk test and by visual assessment with histograms and Q-Q plots. Square root transformations were applied, and all subsequent analyses were completed using both transformed and non-transformed (raw) data to verify that results did not qualitatively change. All analyses were carried out using R statistical software (R Core Team, 2017).

We generated genotypic estimates and partitioned variance for fitness traits using both within-site and across-site linear models that were constructed using restricted maximum likelihood (REML) with *lme4* (Bates *et al.*, 2015). We first evaluated the extent to which fitness variation was heritable. For each trait within each common garden site and in each year, we constructed within-site models to partition variance among genotype and block, which were included as random effects. Variance estimates from within-site models were then used to calculate broad-sense trait heritability ($H^2=V_G/V_P$).

To assess the extent to which the different fitness measurements were correlated with each other within sites, we used genotypic trait estimates (i.e., best linear unbiased predictors (BLUPs) added to least squares trait means) from within-site trait models and calculated pairwise trait correlations among all fitness traits within each common garden site (Pearson correlation coefficients, *r*), correcting for multiple comparisons by controlling the false discovery rate (FDR) (Benjamini and Hochberg, 1995). We also performed complementary principal components analyses (PCA) to identify major axes of fitness variation within each site.

To evaluate whether white clover displayed local adaptation, we assessed genotypic fitness trade-offs for all traits that were comparably measured across reciprocal environments in each mapping population. To do so, we constructed across-site mixed models for comparable traits; models included the fixed effect of environment (E; common garden site) and the random effects of genotype (G), block nested within site, and G×E. Using genotypic estimates from these models, we evaluated genotype-environment correlations (r_{GE}), again with correction for multiple comparisons (FDR). In these tests, negative r_{GE} indicates genotypic trade-offs across reciprocal sites that correspond to local adaptation (i.e., genotypes with high fitness in one environment experience reduced fitness in the reciprocal comparison).

To evaluate fitness variation further, we constructed additional multi-year across-site trait models, but only for reproductive output traits; these models included an added fixed effect of year (Y) and random effects of G×Y and G×E×Y. For all across-site trait models (with and without year effects), we tested the significance of fixed effects using a mixed-model analysis of variance (ANOVA), and the significance of random effects was evaluated using likelihood ratio tests with *lmerTest* (Kuznetsova, Brockhoff and Christensen, 2017).

To assess the extent to which local adaptation in white clover was attributable to variation at the *Ac/ac* and *Li/li* cyanogenesis loci, relative to the overall genotypic effect, we re-constructed all within- and across-site trait models, and we replaced G with cyanotype for all random effects. For within-site models, we calculated the proportion of phenotypic variance explained by variation among cyanotypes (V_C/V_P), which we

compared to H^2. For across-site models, we again evaluated significant fixed and random effects using mixed-model ANOVAs and likelihood ratio tests.

QTL mapping and QTL×E analysis

To characterize the genetic architecture of local adaptation, we performed genetic mapping and identified quantitative trait loci (QTLs) associated with fitness trait variation in each common garden site. For our mapping analysis, we used genotypic fitness trait estimates from across-site trait models without year effects; we also included genotypic estimates from within-site models for traits that were not comparable across sites (e.g., survival traits). We did not perform genetic mapping for the earliest vegetative area measurements at any site because these measurements occurred during the acclimation period. Genotypes with >75% missing SNP data were removed from each mapping population prior to QTL mapping analysis; the DG sample size was reduced from 502 to 423, while all 500 SG F_2 genotypes remained.

QTL mapping analysis was performed with *R/qtl* using the *scanone* function and the Haley-Knott algorithm (Haley and Knott, 1992; Broman *et al.*, 2003). QTLs were considered significant if their LOD score exceeded a p=0.05 confidence threshold that was determined independently for each trait from 1000 permutations. Significant QTLs for each trait were then incorporated into a multiple QTL model and their positions were refined using the *refineqtl* function. The refined LOD score and effect size of each QTL were calculated using a drop-one analysis within the *fitqtl* function. The 1-LOD Drop support intervals for fitness trait QTLs were calculated and visualized with

jtlovell/qtlTools using the *calcCis* and *segmentsOnMap* functions, respectively (Campitelli *et al.*, 2018).

To evaluate the extent to which white clover exhibits antagonistic pleiotropy for local adaptation, we performed a post-hoc QTL×E analysis for QTLs associated with traits that were comparably measured in both reciprocal sites. For the highest LOD markers within each QTL, we compared the fitness of homozygote genotypes across environments by constructing linear mixed-models with genotype and environment as fixed effects, and with QTL×E (i.e., G×E at a single marker) as a random effect. Again, we evaluated the significance of effects in the models using ANOVAs and likelihood ratio tests. Lastly, we identified genomic regions where QTLs for two or more fitness traits co-localized, identified based on overlap in their refined 1-LOD Drop intervals, and we evaluated the direction of allelic effects in those QTLs to assess potential allelic trade-offs that may emerge for different aspects of fitness (e.g., growth vs. reproduction).

RESULTS

By all fitness measures (vegetative area, survival and reproductive output), plants grown in the two more northerly locations (DMN and STL) showed higher fitness over the duration of the common garden experiments than those in the southernmost location (GFL). This was largely due to differences in survival across reciprocal environments, which led to more pronounced differences in reproductive output over the full two-year experiment (Figure 2.1, Figure S2.1, Supplementary Results in Appendix

II). In DMN and STL, 99.5% of the plants (100% of the DG F_2 genotypes) and 97.1% of the plants (99% of the SG F_2 genotypes) survived the first year, respectively. In contrast, 45.5% of the SG plants (84% of genotypes) and 16.3% of DG plants (39% of genotypes) survived the first year in GFL (Figure 2.1B, Table S2.2). Lower survival rates among DG genotypes relative to SG genotypes in GFL are potentially consistent with a greater selective disadvantage for alleles from the northernmost DMN parent in the subtropical GFL environment.

For all quantitative genetic analyses, results were qualitatively the same and quantitatively very similar for raw and square root transformed data (see Tables S2.3-S2.9). Within each environment, a larger proportion of trait variance was always more attributable to genotypic variance than to replicate block. The average broad-sense heritability (H^2) across all traits was ~0.3, suggesting a substantial heritable component to the observed fitness variation (Table S2.3).

Within all common gardens, the different measurements of fitness were broadly positively correlated with one another. This was apparent in the first principal component (PC1) in the PCA results for each site, which explained 32.4-44.3% of the overall fitness variation in common gardens. Different measurements within the same fitness trait category (e.g., vegetative area at different time points) were positively correlated. Measurements of vegetative area and survival were also consistently positively correlated at all sites; however, the sign and magnitude of the coefficient of correlation between these persistence traits and reproductive output traits varied depending on the location and the year (Figures S2.2-S2.5). This pattern potentially

suggests that different environments favored different optimal life history strategies related to investment in vegetative growth vs. reproduction (see Discussion).

White clover displays local adaptation across reciprocal environments

We found strong evidence for genotypic fitness trade-offs across environments, consistent with local adaptation in white clover. Genotype-environment correlations (r_{GE}) were all negative and almost always highly significant ($p < 0.0001$) for all comparable traits in both populations, indicating that both vegetative and reproductive output fitness traits exhibit environmental trade-offs (Figure 2.2, Table S2.4). Consistent with negative r_{GE} findings, the effects of genotype (G) and G×E interactions were both highly significant for nearly all fitness traits in across-site models, while the fixed effect of common garden site (E) was rarely significant (Table S2.5). Multi-year analyses further identified highly significant Y and G×E×Y effects for reproductive output traits; these effects reflect strong differences in flowering across years in reciprocal environments, which were largely attributable to differences in mortality across sites (Figure S2.1).

Local adaptation is not attributable to cyanogenesis variation

Variance analyses indicated that the *Ac/ac* and *Li/li* cyanogenesis loci accounted for essentially none of the variation in fitness in the common gardens relative to overall genotypic effects. For both mapping populations and within all three common garden sites, cyanotype explained <3% of the variance for all fitness traits (i.e., $V_C/V_P < 0.03$) (Table S2.6). Cyanotype was never a significant effect in across-site comparisons

(Figure 2.2). In a small number of cases, cyanotype ×E was marginally significant, but this was far exceeded by the significance of the G×E interaction. Cyanotype ×E×Y was the only significant random effect for multi-year floral trait models; however, this was likely driven by the highly significant year effect, or an E×Y effect that was not included in the model, rather than by cyanotype (Table S2.7, Table S2.8).

Abundant evidence for genetic trade-offs related to local adaptation

Genetic mapping analysis identified many fitness-associated QTLs in each common garden. QTL effect sizes, as measured by the percent of trait variation explained (PVE), ranged from small (1.9%) to large (24.7%). Reproductive output traits tended to have more complex genetic architectures, with multiple QTLs detected in all sites, whereas single QTLs were more often identified for vegetative area and survival traits (Table 2.1, Table S2.9). None of the fitness QTLs co-localized with either of the cyanogenesis loci in either population. Specifically, there were no significant fitness QTLs on the entire linkage group containing the *Ac* locus in either population (Linkage group 2, Figure 2.3). In the DG population only, QTLs for reproductive output in GFL were located near, but did not overlap with, the *Li* locus on linkage group 12; consistent with this negative result, a direct comparison of reproductive output between plants with and without the functional *Li* allele confirmed no significant difference (Figure S2.6b).

Notably, for traits that were comparable across reciprocal sites, the majority of associated fitness QTLs exhibited patterns indicating antagonistic pleiotropy. In post-hoc QTL×E analyses of the DG and SG comparisons, 14 of 16 markers (88%) and 9 of 15 markers (60%) tested displayed significant QTL×E interactions, respectively (Table

S2.10). The higher proportion of significant QTL×E interactions in the DG comparison is consistent with greater numbers of genetic trade-offs between the two more extreme environments (Figure S2.6, Figure S2.7).

The strongest example of antagonistic pleiotropy was identified in the DG comparison, where QTLs for four different fitness measurements co-localized to the same region on linkage group 10 (Figure 2.4). Strikingly, genotypes homozygous for the DMN parental allele (DD) at this QTL produced 20.8 more inflorescences in Year 1 (22.2% above the mean) and were 86.7 cm^2 (12%) larger than GG genotypes in Year 2 in DMN (Day 339). Meanwhile, in the GFL environment, GG genotypes flowered for 20.7 (20.5%) more days and produced 38.7 (21.3%) more inflorescences than DD genotypes in Year 1 (Figure S2.6a). QTL×E interactions were highly significant for all of the highest LOD markers associated with these four traits (Table S2.10).

While native parental alleles conferred increased fitness for most QTLs, reflecting local adaptation, deviations from this pattern also occurred, where non-local alleles significantly increased fitness. Generally, foreign GFL alleles acted to increase reproductive output at the DMN and STL sites, while foreign DMN and STL alleles acted to increase vegetative area and survival traits at the GFL site (Table 2.1). This pattern was particularly apparent for regions of fitness QTL co-localization on linkage group 15. For both populations, we identified two non-overlapping regions of QTL co-localization on this linkage group where parental alleles exhibited trade-offs between vegetative growth and reproductive output. At these QTLs, northern genotypes (DD or SS) displayed increased vegetative area and survival, while southern genotypes (GG) increased reproductive output (Figure S2.6d, Figure S2.6e, Figure S2.7d, Figure S2.7e).

These results are consistent with energetic trade-offs between investment in vegetative growth and reproductive output. Moreover, they suggest that selection may favor different life history strategies in the northern sites compared to the subtropical GFL site. Whereas the DMN and STL sites appear to favor investment in vegetative growth and multi-year survival, fitness at the GFL site investment appears to be optimized by early investment in flowering and seed production at the expense of long-term persistence.

DISCUSSION

Identifying the most relevant traits and genetic variation underlying local adaptation is of major consequence for evolutionary, agricultural, conservation, and climate-change biology. Although intraspecific chemical defense polymorphisms are common in plant species and are known to evolve in response to heterogeneous environmental selection (Moore *et al.*, 2014), the relative importance of chemical defense variation for local adaptation has rarely been explicitly examined. Moreover, while it is well established that local adaptation is common in plants (Leimu and Fischer, 2008), the genetic mechanisms underlying this process have been studied in limited detail. Notably, outcrossing herbaceous species have been especially underrepresented in studies characterizing the genetic basis of local adaptation (Savolainen, Lascoux and Merilä, 2013).

Here, we have examined local adaptation in a perennial, obligately outcrossing herbaceous plant species, white clover, with a well-studied chemical defense polymorphism that varies across geographic clines, cyanogenesis. Using reciprocal

common garden experiments that span a U.S. latitudinal gradient, we demonstrate clear evidence of local adaptation (Figure 2.2), with an underlying genetic architecture characterized primarily by genetic trade-offs at fitness QTLs (i.e., antagonistic pleiotropy) (Figure 2.4, Figure S2.6, Table 2.1, Table S2.10). We find no evidence that cyanogenesis variation contributes to this local adaptation. Instead, divergent life-history strategies — specifically, early investment in flowering vs. delayed flowering and long-term persistence — appear to be the primary determinants of locally-adaptive fitness, as evidenced by trait correlations and allelic trade-offs within environments. Below we discuss these findings and their implications for understanding local adaptation in herbaceous plants.

Strong evidence for local adaptation

For both mapping populations in this study, genotypic fitness trade-offs across reciprocal common garden environments were evident for both vegetative growth and reproductive output fitness traits (Figure 2.2). This result indicates that multiple aspects of life history contribute to local adaptation in white clover and underscores the previously recognized need to consider more than just reproductive fitness in studies of local adaptation, especially for perennial species (Hereford, 2009; Friedman *et al.*, 2015; Wadgymar, Daws and Anderson, 2017). We believe that our findings are conservative and likely underestimate the magnitude of fitness trade-offs in white clover, given the necessarily limited two-year time frame of our experiment and other constraints of our experimental design (e.g., limits on vegetative area differences imposed by trimming; see Supplementary Results in Appendix II).

A chemical defense paradox?

More than 60 years of ecological genetic studies have provided evidence that selection acts on the cyanogenesis polymorphism in white clover (reviewed in Hughes, 1991; Kooyers *et al.*, 2018). While no study has assessed fitness variation among cyanotypes at different locations along a cyanogenesis cline to our knowledge, the fact that clines have evolved repeatedly along climatic gradients in North America and worldwide strongly suggests that one or more climate-associated selective factors act on this chemical defense polymorphism (Daday, 1958; de Araújo, 1976; Hughes, 1991; Kooyers and Olsen, 2012). Based upon locally abundant cyanotypes in wild populations near the three common garden sites in this study (Table S2.11), one would predict that in GFL, cyanogenic plants (AcLi), which are locally present at a frequency of >85%, would have highest relative fitness, while acyanogenic plants (acli, acLi, and Acli), which predominate in the two more northerly environments (>70% in STL, >80% in DMN), would have higher fitness in those locations (Kooyers and Olsen, 2012) (Wright & Olsen, *in prep*). However, we found no evidence that variation for any fitness trait we measured was attributable to cyanotype within or across the three common gardens (Figure 2.2, Tables S2.5-S2.8). Below we discuss potential factors that may explain why we do not see a contribution of the cyanogenesis polymorphism for fitness in these experiments.

One potential explanation for our seemingly paradoxical results is that cyanotype frequencies in a given location may not precisely predict which plants have differentially high fitness in that environment. Adaptive clines evolve through the interaction of divergent selection across populations and homogenizing gene flow between them

(Barton and Hewitt, 1985; Barton, 1999). Gene flow is expected to introduce maladaptive genotypes into populations, and this may be a substantial evolutionary force in an abundant, widely occurring, and obligately outcrossing species, such as white clover (Polechová and Barton, 2015). However, while this explanation could account for a lack of strong cyanotype-fitness associations in mid-cline populations, it cannot account for a complete lack of associations at the two cline ends (in our case, GFL and DMN), where the locally most-abundant cyanotype should still be expected to show differential fitness.

Another possible explanation is that the conditions plants experienced in the common gardens did not accurately capture all selective pressures in local wild populations — for example, natural levels of herbivore exposure. To assess whether our trimming and weeding practices within the common gardens may have altered herbivore abundances and community compositions, we compared rates of leaf herbivore damage within our common gardens and in natural plant communities near but outside of the common gardens (see Supplementary Methods in Appendix II). Measured rates of herbivory were low, both inside and outside of gardens; the lowest rates of herbivory were measured in the southernmost GFL environment, counter to expectations. Additionally, we found no differences in the rate of herbivory experienced by cyanogenic vs. acyanogenic plants within gardens (Table S2.13; Supplementary Results in Appendix II). These results suggest that leaf herbivore damage was likely not a major selective agent in any of the three environments during the years of our experiment. Previous herbivory studies in white clover, which have consistently revealed evidence for deterrent effects of cyanogenic plants in feeding chamber

experiments (e.g., Dirzo and Harper, 1982; Horrill and Richards, 1986; Burgess and Ennos, 1987), may therefore be reflecting levels of natural selection that occur primarily during episodes of intense herbivory that were not detected in our experiment. Given that selective pressures within clover populations are known to vary from year to year (e.g., Richards and Fletcher, 2002), it is possible that common garden experiments performed in different years would reveal greater evidence for cyanogenesis-related fitness variation.

Another possibility is that cyanogenesis variation may be most important for survival during the earliest life stages, when loss of vegetative tissue could have a greater negative effect on survival. To achieve genotypic replication, our common garden experiments necessarily utilized stolon cuttings of F_2 individuals planted from seed in a greenhouse, where germinant and seedling mortality is essentially 0%. Common garden fitness measurements therefore did not assess potentially important fitness variation that is known to be important for local adaptation during germination and seedling developmental stages (Postma and Ågren, 2016, 2018). Interestingly, a set of complementary germination experiments that we performed at the three common garden sites during Year 1 suggests that selection at the seedling or juvenile life stage likely does contribute to local cyanotype frequencies (Wright & Olsen, *in prep*). Consistent with that finding, a previous 24-year study of a single white clover population suggested that the germination environment determines the cyanotype frequencies for a given cohort, which persist through maturation, and that 2-3 year old plants may dominate wild populations (Richards and Fletcher, 2002). Thus, while our common garden findings suggest that traits unrelated to cyanogenesis are the major

determinants of fitness in reproductively mature plants, cyanotype variation could still be an important contributor to local adaptation at juvenile life stages.

Finally, cyanogenesis may be under weak but persistent selection that, while undetectable over the span of two years, can gradually lead to cline formation over many years. Our two-year experiment would not have captured these effects. Future studies that utilize wild population samples and population genomics methods may help to identify such an effect of cyanogenesis variation (e.g., F_{ST} outlier scans) (Wadgymar *et al.*, 2017; Price *et al.*, 2018).

Genetic trade-offs underlie local adaptation in an outcrossing plant

Simulation studies suggest that local adaptation occurs readily with QTLs of large effect but can also be achieved through the action of many small-effect loci (Whitlock, 2015; Yeaman, 2015). In this study, we identified a wide range of effect sizes among fitness QTLs in each of our common garden environments, suggesting that local adaptation in white clover occurs through the action of both small- and large-effect loci (Table 2.1). We detected loci with relatively small effects, despite known statistical limitations of QTL mapping approaches (Beavis, 1998). These findings are similar to empirical studies in other plant species (Ågren *et al.*, 2013; Savolainen, Lascoux and Merilä, 2013).

High levels of gene flow are predicted to lead to genomic clustering of small-effect loci that are locally adaptive; such clustering can empirically emerge as single large-effect QTLs in mapping studies (Yeaman and Whitlock, 2011). Thus, the large-

effect, locally adaptive loci we identified may represent single large-effect genes or many tightly linked small-effect genes acting together.

Despite these limitations, our study documented more evidence for allelic trade-offs at fitness QTLs (i.e., antagonistic pleiotropy) than has been seen in most previous studies in plants (Figure 2.4, Figures S2.6-S2.7, Table S2.10) (Savolainen, Lascoux and Merilä, 2013; but see Price *et al.*, 2018). Moreover, genetic trade-offs were more prevalent for the reciprocal comparison representing the greater difference in common garden environments (DG) than for the climatically less diverged comparison (SG). These results are predicted for a species with high levels of gene flow, although genetic trade-offs have rarely been empirically demonstrated to this extent in reciprocal common garden studies (Wadgymar *et al.*, 2017). Our results therefore contribute new evidence to the body of knowledge related to the genetic architecture of local adaptation.

We found that many of the fitness QTLs we identified were pleiotropic for multiple aspects of life history. That is, both growth/survival traits and reproductive output traits often co-localized to the same QTL regions (Figure 2.3, Figure 2.4); a similar result was found in a recent study in *Mimulus*, where QTLs in a bulk segregant analysis were pleiotropic for flowering time and stolon production (Friedman *et al.*, 2015). Here, we were able to show that within pleiotropic QTLs, allelic effects acted antagonistically for vegetative growth and reproduction— alleles from northern parents increased growth and survival in field experiments, while alleles from southern parents increased reproductive output (Figure S2.6a,d,e; Figure S2.7d,e). To our knowledge, this result is

novel; opposing allelic effects may reflect selection for divergent life history strategies in contrasting environments as discussed below.

Divergent life history strategies promote local adaptation in herbaceous species

Local adaptation via differential investment in sustained growth vs. early reproduction in contrasting environments has been documented in recent studies of several other well-studied herbaceous species; these include the model annual species *Arabidopsis thaliana* (Debieu *et al.*, 2013; Fournier-Level *et al.*, 2013); two related perennial species, *A. lyrata* (Leinonen *et al.*, 2009; Quilot-Turion *et al.*, 2013; Hämälä, Mattila and Savolainen, 2018) and *Boechera stricta* (Wadgymar, Daws and Anderson, 2017); and annual and perennial populations of *Mimulus*, (Friedman *et al.*, 2015; Peterson, 2016). These studies generally suggest that alternate life history strategies may be a common evolutionary strategy for local adaptation in herbaceous plant species.

In the case of white clover, the life history trade-offs we observed were most evident between investment in vegetative growth and reproductive output in the first year. Specifically, in the environments with low overall mortality (DMN and STL), greater investment in vegetative growth came at the cost of Year 1 reproductive output, but provided the benefit of high reproductive output in Year 2. In the northernmost DMN site, anti-correlation between growth and Year 1 reproduction was captured by PC2 of the within-site principal components analysis, which accounted for 27% of the total variation in measured fitness traits (Figure S2.2). In particular, we detected a significant negative correlation between vegetative growth in the first 120 days and Year 1 floral

count in DMN. While this correlation was not statistically significant in the STL common garden, PC2 for STL (explaining 27.9% of fitness variation) again suggested that growth and reproductive output traits were anti-correlated, albeit to a lesser extent than in DMN (Figure S2.3). The lack of a negative correlation between growth and reproduction in Year 1 may have been due to delayed planting of the STL garden (see Supplementary Results in Appendix II). For both populations grown in GFL, PC2 identified anti-correlation between early investment in both growth and reproduction vs. longevity (lifespan and later measures of vegetative area) (Figure S2.4, Figure S2.5). Unlike in the northern environments, plants with longer lifespans in GFL did not achieve substantial reproductive gains in Year 2 due to high Year 1 mortality. Thus, in a southern U.S. environment with harsh summer conditions, rapid growth and early reproduction appear to be selectively advantageous. Taken together, differences in trait correlations across sites strongly suggest that divergent optimal life history strategies are favored in different environments and contribute to local adaptation in white clover. At the genetic level, opposing allelic effects at fitness QTLs bolster this argument; northern alleles favor growth and survival, while southern alleles favor reproduction (Figure S2.6a,d,e; Figure S2.7d,e).

For white clover, we propose that these divergent optimal life history strategies across environments may be directly tied to the intensity of heat stress exposure in a given location. Heat stress can be a major limiting factor for vegetative growth and survival in plants (Moles *et al.*, 2014; Preite *et al.*, 2015). Hotter and drier conditions limit multi-year persistence and favor earlier investment in reproduction and more rapid life cycles (Kooyers, 2015). A previous white clover common garden experiment

conducted in STL with wild genotypes from 15 widespread North American locations showed that for the 2015 growing season, yearly temperature variability and average maximum summer temperature experienced by populations in their local environments were the best predictors of vegetative growth (Wright *et al.*, 2017). In line with this finding, patterns in the present study suggest that heat stress led to vegetative tissue loss and mortality. Plants in the DMN common garden did not appear to suffer from heat stress at any point, whereas plants in both STL and GFL displayed tissue loss and leaf senescence indicative of heat stress. The duration of heat stress lasted only 2-4 months each year in STL and was followed by a recovery period of several months before winter. In contrast, heat stress due to elevated temperatures was more intense and prolonged in GFL (5-6 months) and was associated with periods of massive tissue loss and high mortality (Figure 2.1, Table S2.12, Supplementary Results in Appendix II). As a result, hotter conditions in the southernmost common garden environment (GFL) favored genotypes that invested in reproduction earlier in the first growing season, prior to mortality during the summer months. In contrast, the two more northerly common garden environments (DMN and STL) favored genotypes with early and ongoing investment in vegetative growth over the two-year experiment; this investment came at the expense of floral production in the first year in DMN.

For widespread North American plant species, southern populations currently and increasingly experience natural selection due to climate change-associated heat stress (and potentially associated drought stress). Thus, life history variation is more likely to be a major contributor to local adaptation than chemical defense variation in white clover, as our results support, and also more broadly in herbaceous plant species.

Specifically, a drought/heat escape strategy involving rapid life cycles and early flowering, as opposed to the evolution of physiological tolerance, appears to be locally adaptive for herbaceous populations that experience prolonged periods of stress (Kooyers, 2015). Over time, one might predict natural selection to favor the evolution of annuality in populations of perennial herbaceous species that occur in hotter and more stressful environments.

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TABLE

Table 2.1. Locations and allelic effects for fitness trait QTLs (depicted in Figure 2.3) identified for each mapping population at each common garden site. Additional QTLs and results for square root transformed data are located in Table S2.9.

Pop-Site	Fitness trait	Measurement *(Total PVE*)*	Highest LOD Marker	Refined QTL Marker	Linkage group	Refined QTL Position	1-LOD Drop Interval	PVE*	LOD score	Allele of increased effect	Effect size per allele**	Mean Trait Estimate	% Effect
DG-DMN	Vegetative Area	Day 120 *(10.67%)*	13:13646557	13:13646557	4	141.203	136.60 - 142.82	3.94	5.63	GFL	-34.75	746.50	4.66
			10:18587313	15:33145691	15	249.485	211.53 - 319.00	3.56	4.57	DMN	30.08	746.50	4.03
			4:58955253	16:35058866	16	223.293	200.50 - 232.63	3.17	5.33	DMN	25.04	746.50	3.35
		Day 339 *(9.02%)*	9:23631957	9:23631957	10	178.702	153.50 - 239.59	4.92	6.80	DMN	43.33	390.35	11.10
			3:26735632	3:26735632	14	5.263	0.00 - 35.62	4.10	5.86	DMN	32.99	390.35	8.45
	Survival	Winter	4:32890845	4:32890845	14	59.920	48.86 - 121.46	4.33	4.83	DMN	0.05	1.07	4.52
	Flowering Duration	Year 1	4:59781115	4:59781115	15	213.296	207.63 - 260.75	9.68	11.10	GFL	-3.76	115.77	3.25
	Floral Count	Year 1 *(14.35%)*	5:3364109	5:3364109	5	147.083	23.20 - 156.31	2.75	4.53	DMN	8.27	173.15	4.77
			2:43914108	6:17008091	10	166.255	156.93 - 214.12	4.03	5.35	DMN	10.41	173.15	6.01
			2:24999810	13:40112209	13	276.003	258.18 - 301.29	3.39	4.93	DMN	9.03	173.15	5.21
			9:48242797	10:18367752	15	219.377	211.53 - 285.11	4.18	6.44	GFL	-9.29	173.15	5.37
		Year 2 *(7.81%)*	11:40901234	11:40901234	11	145.788	130.75 - 198.11	4.21	5.17	DMN	13.82	287.40	4.81
			8:52230082	8:52230082	14	6.847	5.26 - 22.18	3.60	4.48	DMN	13.06	287.40	4.54
DG-GFL	Vegetative Area	Day 235	12:25588091	12:25588091	15	20.359	0.00 - 37.45	12.66	14.79	DMN	36.99	201.59	18.35
	Flowering Duration	Year 1 *(20.98%)*	2:46629904	2:46629904	10	168.000	164.78 - 173.33	9.99	15.28	GFL	-10.36	101.13	10.25
			12:12548239	8:58495823	12	333.427	225.23 - 351.83	3.19	4.87	GFL	-5.82	101.13	5.75
			15:4065533	15:4065533	15	80.604	48.99 - 85.43	4.63	6.94	GFL	-4.16	101.13	4.12
			3:16346453	12:47751016	16	267.417	257.82 - 283.42	3.17	5.99	GFL	-5.73	101.13	5.67
	Floral Count	Year 1 *(23.87%)*	2:46629904	6:17008091	10	166.255	164.78 - 239.59	4.33	6.73	GFL	-19.36	181.55	10.66
			8:58495823	8:58495823	12	333.427	329.63 - 361.22	4.66	4.75	GFL	-19.22	181.55	10.59
			15:952339	15:952339	15	12.559	0.00 - 81.33	11.45	14.07	GFL	-26.94	181.55	14.84
			3:16346453	9:13212653	16	269.813	257.82 - 283.42	3.44	6.02	GFL	-16.20	181.55	8.92

Continued on next page

Table 2.1. (Continued)

Pop-Site	Fitness trait	Measurement (Total PVE*)	Highest LOD Marker	Refined QTL Marker	Linkage group	Refined QTL Position	1-LOD Drop Interval	PVE*	LOD score	Allele of increased effect	Effect size per allele**	Mean Trait Estimate	% Effect
SG-STL	Vegetative Area	Day 362	9:53788760	9:53788760	15	161.331	119.87 - 175.60	4.29	4.77	STL	52.60	1018.95	5.16
	Survival	Winter	4:59794978	4:59794978	15	119.214	111.55 - 126.64	3.96	4.38	STL	0.09	1.46	6.41
	Flowering Duration	Year 1 *(13.21%)*	13:13894869	13:13894869	13	121.105	113.82 - 134.38	4.01	5.82	GFL	-4.26	87.89	4.85
			1:95517659	1:95517659	15	126.639	124.09 - 135.94	9.19	11.93	GFL	-7.33	87.89	8.34
		Year 2	10:623367	10:623367	15	4.818	0.00 - 25.65	12.43	14.40	GFL	-9.13	173.15	5.27
	Floral Count	Year 1 *(12.77%)*	11:53202884	11:53202884	13	140.425	128.67 - 148.88	5.39	7.05	GFL	-8.43	48.03	17.55
			15:17467828	15:17467828	15	154.786	124.09 - 170.49	7.39	9.39	GFL	-10.27	48.03	21.38
		Year 2	2:32435469	2:32435469	15	117.433	98.72 - 159.63	6.47	7.25	GFL	-34.94	385.82	9.06
SG-GFL	Vegetative Area	Day 200	7:36845388	7:36845388	15	170.488	167.24 - 175.60	4.74	5.27	STL	42.89	574.38	7.47
		Day 295 *(12.57%)*	3:3834184	3:11692564	3	26.086	0.00 - 47.75	4.60	4.31	GFL	-7.09	29.84	23.76
			15:3511683	15:3511683	15	26.789	19.64 - 29.61	7.98	8.19	STL	10.90	29.84	36.52
	Survival	Lifespan	16:15693842	12:20254636	8	105.367	63.38 - 109.59	5.26	5.67	GFL	-0.54	12.86	4.23
		(9.90%)	15:3511683	15:3511683	15	26.789	19.64 - 41.04	4.64	4.94	STL	0.55	12.86	4.27
	Flowering Duration	Year 1 *(23.81%)*	1:27623384	8:22625723	7	67.297	61.71 - 79.00	3.54	4.26	GFL	-5.14	104.81	4.90
			15:4312433	1:73062464	8	140.951	71.00 - 216.26	2.88	4.80	GFL	-4.72	104.81	4.50
			6:22247148	10:28013669	10	154.732	121.58 - 176.64	3.22	4.71	GFL	-5.18	104.81	4.94
			10:725661	10:725661	15	8.430	0.00 - 17.42	14.17	15.82	GFL	-10.55	104.81	10.07
	Floral Count	Year 1 *(34.26%)*	12:45616154	12:45616154	1	246.700	239.22 - 257.09	2.45	4.53	GFL	-17.14	201.39	8.51
			6:18418652	6:18418652	6	275.968	190.04 - 284.44	1.88	4.66	GFL	-18.67	201.39	9.27
			6:22247148	10:31586103	10	173.517	121.58 - 177.62	5.21	8.56	GFL	-29.65	201.39	14.72
			10:623367	10:623367	15	4.818	2.56 - 10.32	24.73	32.29	GFL	-66.92	201.39	33.23

* PVE is the percent of the phenotypic variation explained by allelic variation at the refined QTL in the full QTL model for that trait at that common garden site. Total PVE is the sum of PVE across multiple QTLs.
** A positive value in Effect size represents an increased trait value from the DMN or STL parental allele, while a negative value represents an increased trait value from the GFL parental allele.

FIGURES

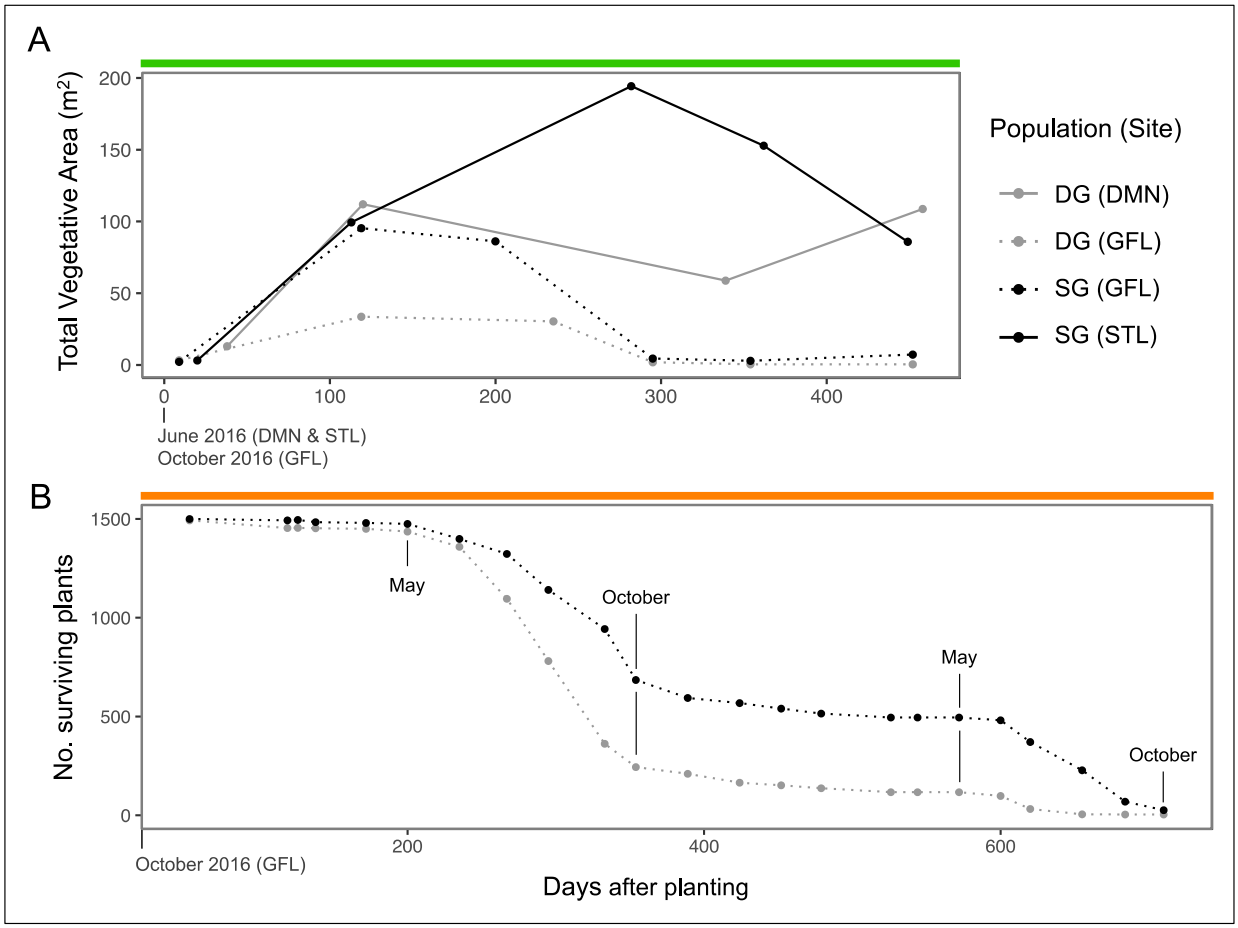

Figure 2.1. (A) Total vegetative area (m^2) at select time points in each of four common garden experiments, including reciprocal comparisons of both F_2 mapping populations (DG and SG). The x-axis corresponds to the number of days since common garden establishment at each site, which occurred in different months (June and October) of 2016. Each data point corresponds to the summed vegetative areas of individual plants, as determined by digital photo analysis using *Easy Leaf Area* software (Easlon and Bloom, 2014) (Table S2.1). **(B)** Survivorship over time in the two GFL common gardens. Plants were scored as living or dead from individual digital photos taken monthly throughout the experiment.

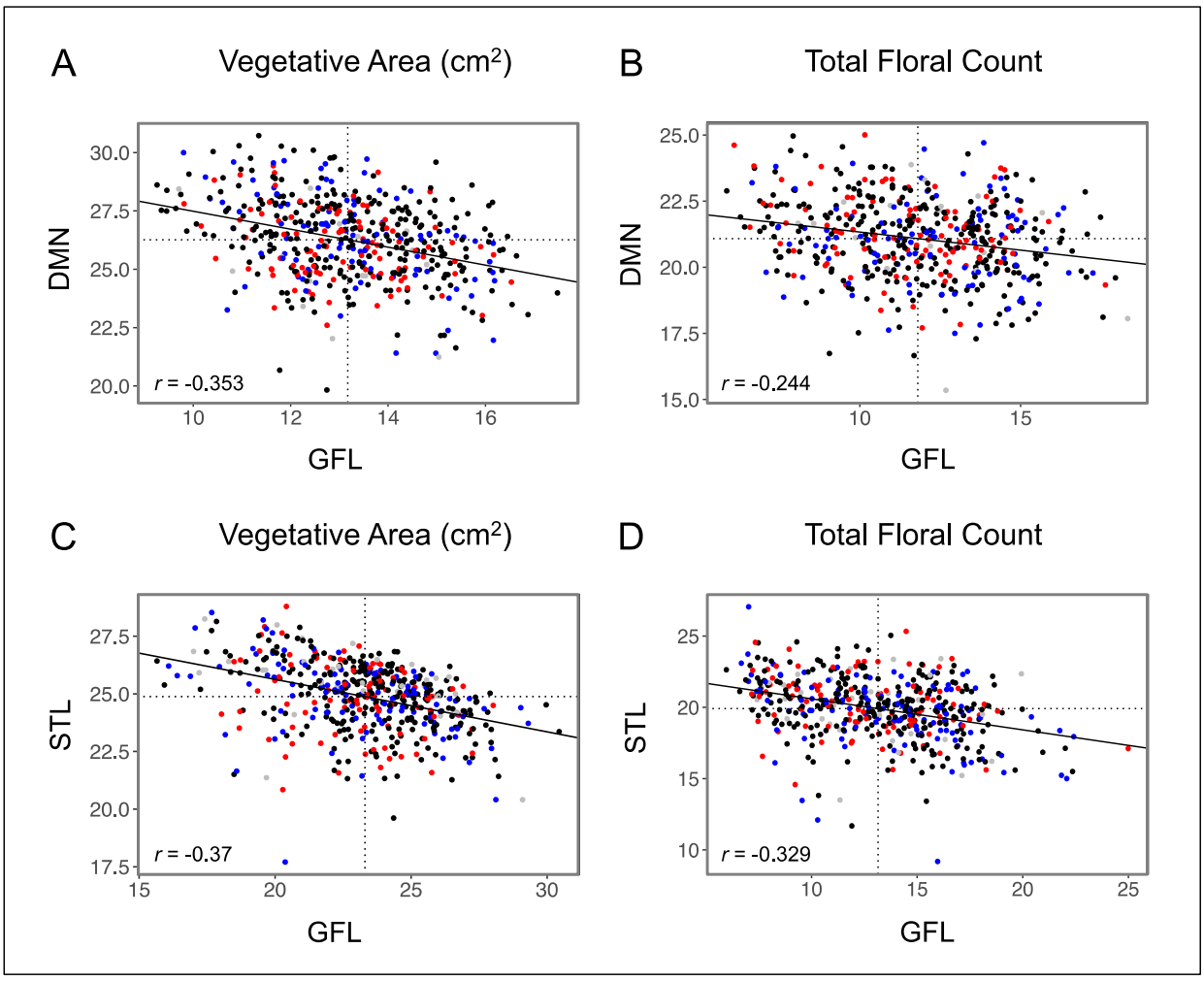

Figure 2.2. Genotype-environment correlations (r_{GE}) for both mapping populations (DG (**A**, **B**) and SG (**C**, **D**)), including comparisons for vegetative area during the initial growth period (~120 days) (**A**, **C**) and Total floral count over the duration of the two-year experiment (**B**, **D**). Data correspond to genotypic estimates from across-site trait models that were built using square root transformed data. Each data point corresponds to a single F_2 genotype colored by cyanotype (acli (gray), acLi (blue), Acli (red) and AcLi (black). Dotted axes denote the mean trait values at each site, and solid lines denote lines of best fit (Pearson correlation tests; $p < 0.0001$ in all cases; Table S2.4).

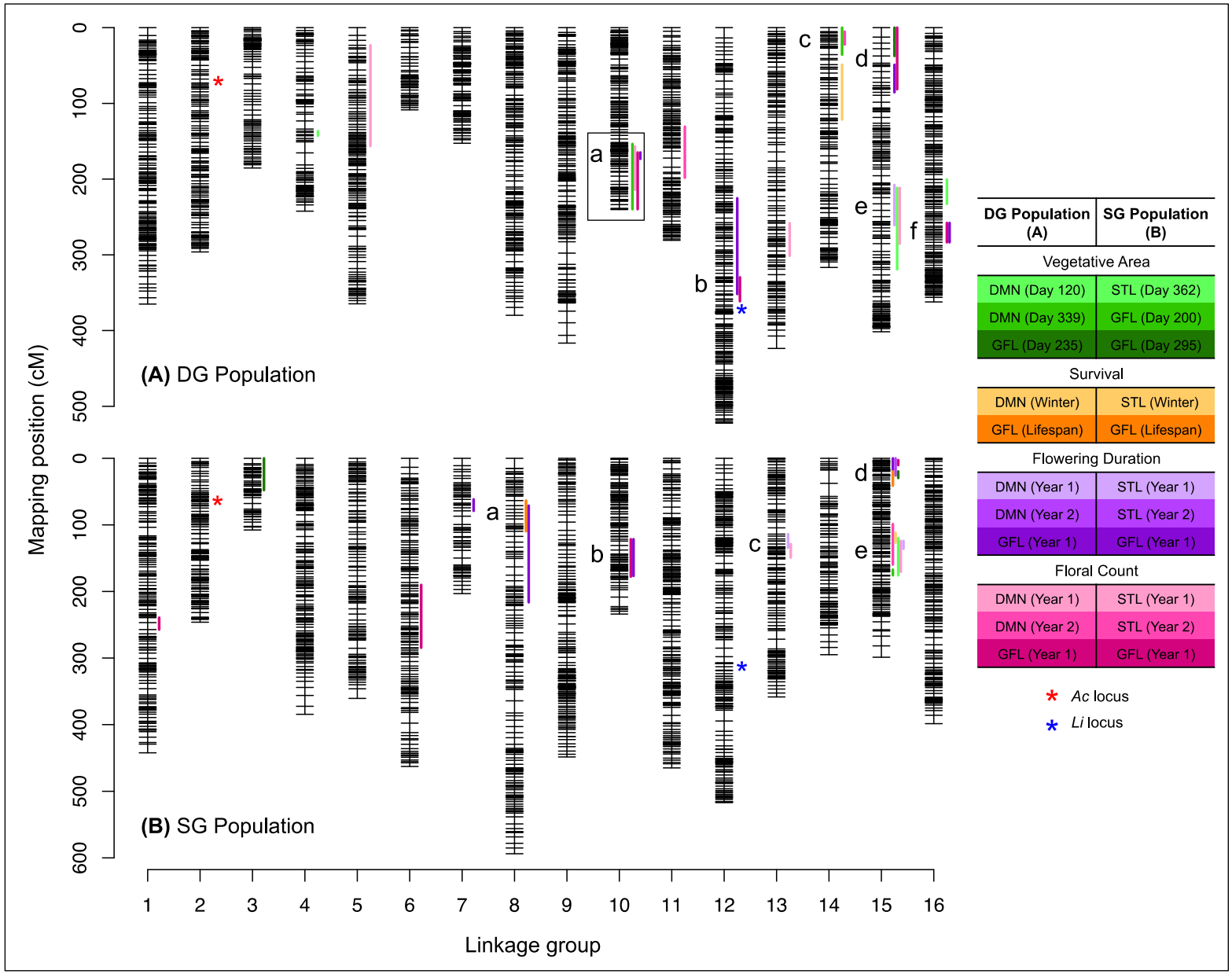

Figure 2.3. (Figure legend on next page)

Figure 2.3. Quantitative trait loci (QTLs) associated with fitness traits measured in each common garden for each mapping population (DG (**A**) and SG (**B**)). Colored bars indicate 1-LOD Drop intervals for refined QTLs (Table 2.1). Colored asterisks indicate the locations of the *Ac* and *Li* cyanogenesis loci. In each comparison, lower-case letters denote genetic regions where QTLs for multiple fitness traits co-localized, which are presented in greater detail in Figure 2.4, Figure S2.6 (a-f), and Figure S2.7 (a-e).

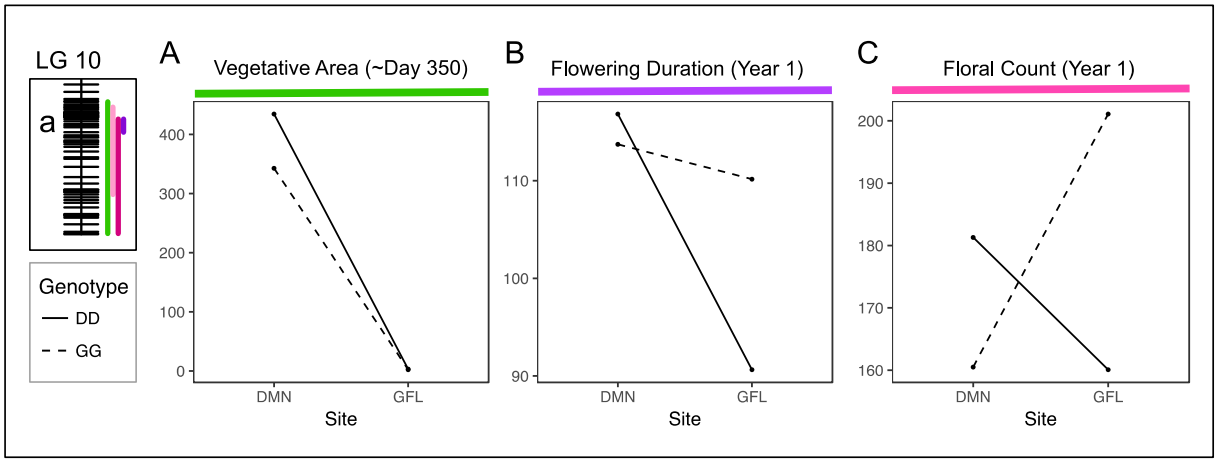

Figure 2.4. Multiple fitness traits (vegetative area (**A**), flowering duration (**B**), and floral count (**C**)) displaying antagonistic pleiotropy at a single major QTL on linkage group 10 (Figure S2.7a); homozygotes of native parental alleles (DD vs. GG) exhibited significant fitness trade-offs for markers located in this QTL region ($p < 0.0001$; see Table S2.10).

APPENDIX II

Chapter 2 Supplementary Material

SUPPLEMENTARY METHODS

Generation of F_2 mapping populations

Hand crosses were performed between parents in both directions to generate 50-100 F_1 genotypes per population. Within F_1 population, random cross-pollinations were performed by hand or using bee cages to generate ~500 F_2 genotypes per population. In the case of the DG population, commercially purchased solitary blue orchard mason bees (*Osmia lignaria*) were used to cross-pollinate F_1s in bee cages in the greenhouse (Crown Bees, Woodinville, WA). For the SG population, random F_1 crosses were performed by hand.

Reciprocal common gardens

The parental genotypes for the mapping populations used in this study were: DMN_010 (*ac/ac*, *li/li*, seed collected in Duluth, MN, 46.8185 N, -92.0850 W); STL_0701 (*ac/ac*, *li/li*, stolon cutting from St. Louis, MO, 38.6051 N, -90.2673 W) (Kooyers and Olsen, 2012); and GFL_007 (*Ac/Ac*, *Li/Li*, stolon cutting from Gainesville, FL, 29.6407 N, -82.3623 W). Studies of fitness variation in mapping populations commonly include the parental genotypes in the common garden experiment. In the present study, the parents were not included in field experiments because two of the parents (STL_0701 and GFL_007, collected as stolon cuttings from adult plants in natural populations) showed substantial declines in vigor (including growth deformities consistent with viral infection) during the two-year period between the initial crosses (2014) and the initiation of field experiments (2016).

Cuttings of F_2 genotypes for the STL garden were transported to the field site in greenhouse pots, while cuttings for the DMN and GFL gardens were placed into sealed plastic snack bags with damp paper towels and transported by van from WU to their respective sites, spending 3-5 days in bags before transplantation in the field.

F_2 genotypes from the DG mapping population were planted in Duluth, MN at the University of Minnesota-Duluth's Research and Field Studies Center (46.866, -92.048) and at the University of Florida-Gainesville's Plant Science Research and Educational Unit (PSREU) in Citra, FL (29.409, -82.171). The Research and Field Studies Center is a 114-acre site located along Amity Creek approximately four miles from the University of Minnesota-Duluth campus. The field site was located in one of the natural fields; it is regularly mowed and surrounded by a fence to protect experiments from deer grazing. The field was tilled prior to planting the common garden experiment. The PSREU is a 1,068-acre site, in which roughly 700 acres are cultivatable land. The remaining acreage comprises the "Hawthorne Prairie" and is wetland in nature. The field site of the common garden was newly tilled and comprised of sandy soils.

F_2 genotypes from the SG mapping population were planted at the PSREU in Florida in the same location as the DG population and in Eureka, MO at Washington University's Tyson Research Center (38.527, -90.562). The garden plot at Tyson Research Center consisted of local, native prairie soil and was surrounded by a fence to exclude deer and by an underground concrete barrier to exclude groundhogs.

Throughout the experiments, weeding was performed at all sites, primarily by hand; glyphosate herbicide was used once at GFL in between clover plants during a period of intense weed infestation. After several months of growth, small tillers and

lawn edgers were used to trim plants at the DMN and STL gardens as needed; a tractor with a cutter disc attachment was used at GFL. Excess vegetative tissue was removed from the garden plots when trimming was performed to ensure that stolons did not re-root.

Additional fitness measurements

Herbivory. Leaf herbivore damage was assessed multiple times for all plants at all common garden sites after plants were able to become established in common gardens. Plants in the DMN and STL common gardens were assessed during both growing seasons (August and September 2016, May and June 2017), while GFL plants were assessed only in the first growing season (March and May 2017), prior to massive vegetative tissue loss due to heat stress (see below).

For each date that measurements were recorded, leaf damage was quantified in an ordinal fashion (0=0%, 1=1-25%, 2=26-50%, 3=51-75% or 4= >75%) for 15 leaves on a randomly chosen stolon (Wright et al. 2017; see also Dirzo & Harper 1982a; Dirzo & Harper 1982b). For each plant in each environment and for each sampling date, we calculated three herbivory metrics: 1) Total Damage was calculated as the number of leaves with any herbivore damage, regardless of damage category, divided by the total number of leaves; 2) High Damage was calculated as the number of leaves with a damage classification of 2 or higher, divided by the total number of leaves; 3) Weighted Damage was calculated as the sum of leaf damage categories (0 = 0, 1 = 0.25, 2 = 0.5, 3 = 0.75, 4 = 1), each multiplied by the number of leaves in their respective category, and then divided by the total number of leaves.

To determine genotypic herbivory estimates for each of the herbivory metrics, we averaged across replicates of the same genotype within each environment and sampling date. Next, for each of the herbivory metrics, we calculated average herbivory measures for each cyanotype group with genotypic herbivory estimates; we did this separately for each sampling date in each environment.

Because leaf herbivore damage was low in the common garden experiments (i.e., the majority of leaves exhibited no damage, particularly in GFL), we also sampled approximately 100 white clover plants in natural plant communities near the STL and GFL common garden sites in Year 2 to compare herbivory rates inside and outside of the two southerly common garden plots, where herbivory might be expected to be higher overall.

Floret counts. Because we counted entire inflorescences as a measure of reproductive output in this experiment, any cyanotype-specific differences in seed production per inflorescence would not have been detected. Some experiments in white clover have suggested that reproductive effort within inflorescences may be associated with the cyanogenesis polymorphism. For example, petal size and floret number within inflorescences may vary according to cyanotype (Thompson and Johnson, 2016). To test this possibility, we counted florets within inflorescences produced by plants in Year 2 at STL; specifically, we counted florets for 21 cyanogenic and 18 acyanogenic SG genotypes. For each genotype, we counted florets for three inflorescences of each replicate (nine total inflorescences per genotype). We averaged floret counts across inflorescences from the same genotype, first averaging within the same block, then averaging across blocks, thereby creating one estimate per genotype.

We then compared average floret counts in cyanogenic vs. acyanogenic genotypes using a t-test.

SUPPLEMENTARY RESULTS

Common garden acclimation, maintenance, and notable weather events

At DMN, plants were watered only the day they were planted and received no fertilization. Only 12 plants out of 1,502 (representing 12 unique genotypes) died during the two-month acclimation period, and replacement cuttings were replanted on July 11, 2016. The four-month growing period occurred during a mild summer. The plants grew and flowered readily with little mortality. Weeds required constant attention, especially in block 3. Trimming began in August. The plants were healthy in the fall. A late, relatively mild winter (2016) led to prolonged flowering in the first year and low winter mortality, despite a reduction in vegetative tissue over winter (Figure 2.1A, Figure S2.1). In the second growing season (2017), there were no notable weather events. The plants generally initiated and ceased flowering as expected, relative to local plants. Plants were healthy in October 2017. The second winter was harsher than the first, with much higher mortality and substantially reduced vegetation in March 2018 (Table S2.2).

At STL, a delayed common garden establishment date (by approximately two months) and stressful summer weather contributed to higher mortality during the acclimation period. June and July 2016 were hot and dry. Plants were watered regularly until June 25, 2016, and spot watering continued until July 11. No fertilizer was provided. Sets of F_2 genotypes were replanted five times between late June and early August. In total, 215 out of 1,500 plants were re-planted, representing 174 unique

genotypes. The majority of the mortality occurred in block 1. Nonetheless, plants in all three blocks grew steadily over the summer, and trimming began in August. Due in part to prolonged watering during acclimation, weeds required much attention during the first year. The plants became large in the fall, continuing to grow well into December; a mild winter led to low winter mortality. After winter, record-setting rains and flooding in late April (2017) led to a burst of exceptionally high flowering in May and June (Figure S2.1). July and August were hotter and drier, which led to vegetative tissue loss and decreased flowering (Figure 2.1A); no trimming was necessary after July. Harsher conditions in the second winter reduced vegetation substantially and led to some winter mortality (Table S2.2).

At GFL, plants were watered regularly until November 25, 2016. Fertilizer (K and Mg) was provided on October 28, 2016, and *Rhizobium* inoculation was performed on November 21. Two sets of replacements were planted in GFL on November 18, 2016 and December 2, 2016. Across both mapping populations, 287 out of 3,002 plants were re-planted, representing 261 unique genotypes. Nitrogen fertilizer was applied once more on December 14, 2016, after which time no further care was provided. By late January 2017, after 3-4 months of growth, the plants looked healthy, and many had grown to fill their allotted individual plots; trimming occurred in February. As in other sites, weeds required ongoing attention. While plants flowered well in May and June, achieving similar floral counts to the DMN and STL gardens in their first year (Figure S2.1, Table S2.2), high summer temperatures slowed flowering and led to high mortality (Table S2.12). Visible leaf senescence was prevalent in June, and steady mortality occurred from June to October (Figure 2.1B); some plants survived the heat, likely due

in part to evening rains. Hurricane Irma passed over the plots in September 2017, near the end of the major summer mortality event; the remaining plants handled hurricane conditions remarkably well. Flooding cut some trenches in plot rows between plants. Due to high heat, new vegetation had not yet begun growing by late September. By January 2018, surviving plants were weak but growing, not flowering, and experienced some freezing temperatures. For the small number of remaining plants, flowering occurred in March, also with an increase in weeds. From April until the end of the experiment (October), mortality was again high, with steady declines in surviving plants.

Fitness measurements

Some experimental design considerations may have limited our ability to detect fitness trade-offs, or reduced the magnitude of the trade-offs we saw. These are discussed below.

Vegetative area. Plant vegetative area increased after common garden establishment periods for the first 4-5 months at all common garden sites. These initial increases were comparable for both mapping populations at all three locations except for the DG population in GFL, which experienced half the increase in vegetative growth seen in the other three populations. After the initial growth periods, vegetative area doubled in STL during the late fall months, owing in part to an unseasonably late frost; it decreased by half in DMN over winter; and it decreased almost to zero in GFL during the summer months. GFL gardens never regained vegetative area following the first summer season (2017). In contrast, the STL and DMN common gardens had, at the end of the second growing season and before the second winter season, maintained

roughly equivalent vegetative areas to what they achieved during the first growing season (Day ~120 vs. Day ~450) (Figure 2.1A).

Because it was necessary to keep genotypes separate by restricting their growth in our common gardens, trimming practices may have limited our ability to detect significant differences in vegetative area among genotypes, especially at the STL and DMN gardens, where most plants were large throughout the experiment and mostly filled their individually allotted 930 cm^2 plots (Figure 2.1A).

Survival. At both DMN and STL, mortality occurred primarily during winter months, particularly during the second winter, but it was low overall. At the GFL site, mortality was high for both populations. Summer heat in the first year led to steady declines in surviving plants beginning in May of the first summer and continuing to October at the end of the first year (Figure 2.1B). Mortality in the GFL gardens occurred during months where maximum temperatures exceeded 95°F at both 60 cm (above soil) and -10 cm (below soil) (Table S2.12). These results are consistent with previous findings that vegetative growth is associated with maximum summer temperatures in white clover (Wright *et al.*, 2017).

In GFL, Year 1 survivorship and plant lifespans were higher for the SG population than the DG population; 45.5% of the SG plants (84% of the genotypes) and 16.3% of DG plants (39% of the genotypes) survived the first year, and mean lifespans for the two populations were 12.9 months and 9.1 months, respectively. Ultimately, only 4 and 26 plants (representing 1% and 5% of the DG and SG genotypes) survived the 24-month experiment. (Table S2.2). These patterns are potentially consistent with a

greater selective disadvantage for alleles from the northernmost DMN parent at the subtropical GFL site.

Reproductive output. Plants in the DMN and STL common gardens began flowering immediately after the experiments were established, coinciding with their initial period of growth. In contrast, plants in the GFL common gardens first grew for 4-5 months (~120 days) and subsequently flowered (Figure S2.1), potentially due to short-day winter photoperiods that they experienced.

In the first year, plants in the DMN and STL gardens produced 260,761 and 72,039 inflorescences, respectively. The fact that the STL garden was planted approximately two months later than anticipated (June, instead of April) likely explains lower floral production in Year 1, compared to DMN (Figure S2.1); this may have affected our ability to detect Year 1 growth vs. reproduction trade-offs in STL (Figure S2.3), which we saw in DMN (Figure S2.2). In Year 2, these gardens produced 431,626 and 563,525, respectively (Table S2.2). Heavy spring rains and a longer growing season likely contributed to high inflorescence counts in STL.

In GFL, the DG population produced 278,375 inflorescences in Year 1—slightly more than the reciprocal comparison in the DMN garden, while the SG population produced 303,558 inflorescences. Following high Year 1 mortality, inflorescence counts were substantially reduced in Year 2, totaling 2,473 and 46,379 inflorescences for the DG and SG populations, respectively (Table S2.2).

Herbivory. Leaf herbivore damage was low for all sampling dates in all of the common garden environments. Averaging across all sampling dates, the proportion of leaves exhibiting any level of damage (Total Damage) was 0.20, 0.36, and 0.03 for the

DMN, STL, and GFL environments, respectively (Table S2.13). These values dropped by an order of magnitude at all sites when High Damage and Weighted Damage were considered, due to the fact that most leaves exhibiting damage were in the lowest damage category. Thus, while plants in STL exhibited higher herbivory than those in DMN, as expected, plants growing in the southernmost GFL environment experienced almost no herbivory, counter to expectations.

Across cyanotype groups and sampling dates, Total Damage measurements ranged from 0.16-0.23 (DMN), 0.21-0.44 (STL), and 0.02-0.04 (GFL). There was more variation between sampling dates in a given site than between cyanotype groups on the same sampling date, and cyanogenic (AcLi) plants were rarely the cyanotype that experienced the least amount of damage (Table S2.13). Thus, we saw little evidence that cyanogenic plants were differentially protected from herbivores, compared to acyanogenic plants, in our common garden experiments.

Approximately 100 wild plants sampled near the STL common gardens exhibited similar Total Damage to plants in the common garden plots at a comparable sampling date (wild=0.45, June (Year 2)=0.39). While wild cyanogenic plants did experience less herbivory than acyanogenic plants (0.41 vs. 0.45-0.47), the difference would not be expected to contribute greatly to significant differences in vegetative area or overall fitness.

Wild plants sampled near the GFL site also exhibited similar Total Damage to plants in the common gardens there (0.02 vs. 0.03). We did not measure cyanotypes for wild plants in GFL, so we cannot say whether cyanogenic plants were differentially protected from leaf herbivore damage (Table S2.13).

Overall, the results of our herbivory surveys do not support the hypothesis that a latitudinal gradient in herbivory drives cyanogenesis clines or contributes greatly to fitness trade-offs across contrasting environments in white clover. Other environmental factors, such as heat stress, likely play a greater role in local adaptation.

Floret counts. Cyanogenic genotypes averaged 46.5 florets per inflorescence, and acyanogenic genotypes averaged 49.1. These results suggest that acyanogenic plants that do not devote energy to cyanogenesis may experience a slight reproductive advantage. However, this result was not significant (t=1.04, df=33.8, p=0.3). We did not make floret count comparisons in the DMN or GFL common gardens, so we do not know whether this potential energetic trade-off changes across a latitudinal gradient. Because we did not detect significant differences in floret count in STL, we believe inflorescence count adequately captures important reproductive variation in these experiments. While insignificant differences in floret counts might be more important when considered together with the total number of inflorescences that a genotype produces, generating the data to perform such an analysis was beyond the scope of our study.

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SUPPLEMENTARY TABLES

Table S2.1. Summary of important dates, including vegetative area measurement dates, for common garden experiments.

Site-Population	Plant Date	End Est. Period	First Trim	Vegetative Area Measurements	End Date
DMN-DG	6/11/16	7/11/2016 (d30)	8/12/2016 (d62)	d38 (July 2016), d120 (Oct 2016), d339 (May 2017), d458 (Sept 2017)	5/26/2018 (d714)
GFL-DG	10/12/16	12/14/2016 (d63)	2/23/2017 (d134)	d9 (Oct 2016), d119 (Feb 2017), d235 (June 2017), d295 (Aug 2017), d354 (Oct 2017), d452 (Jan 2018)	10/15/2018 (d733)
STL-SG	6/14/16	8/4/2016 (d51)	8/9/2016 (d56)	d20 (July 2016), d113 (Oct 2016), d282 (Mar 2017), d362 (June 2017), d449 (Sept 2017)	3/30/2018 (d654)
GFL-SG	10/12/16	12/14/2016 (d63)	2/23/2017 (d134)	d9 (Oct 2016), d119 (Feb 2017), d200 (May 2017), d295 (Aug 2017), d354 (Oct 2017), d452 (Jan 2018)	10/15/2018 (d733)

Table S2.2. Summary of reproductive output and survival measurements in the common garden experiments.

Site-Population	Y1 Flowering Period			Y1 Duration (days)	Y1 Floral Count	Y1 Survival Count
DMN-DG	6/28/2016 (d14)	to	11/14/2016 (d153)	139	260,761	1,317
GFL-DG	2/8/2017 (d119)	to	11/28/2017 (d412)	293	278,375	245
STL-SG	6/28/2016 (d17)	to	11/15/2016 (d157)	140	72,039	1,457
GFL-SG	2/8/2017 (d119)	to	1/30/2018 (d475)	356	303,558	685

Site-Population	Y2 Flowering Period			Y2 Duration (days)	Y2 Floral Count	Total Floral Count
DMN-DG	6/1/2017 (d352)	to	10/23/2017 (d496)	144	431,626	692,387
GFL-DG	2/20/2018 (d496)	to	9/19/2018 (d707)	211	2,473	280,848
STL-SG	4/10/2017 (d303)	to	10/29/2017 (d505)	202	563,525	635,564
GFL-SG	2/20/2018 (d496)	to	9/10/2018 (d698)	202	46,379	349,937

Table S2.3. Trait means and quantitative genetic partitioning of fitness traits within each common garden site for each mapping population and in each year, with calculations of broad-sense heritability. Genotype (G) and replicate block (B) were included as random effects in within-site models. Vegetative area (cm^2), winter survival (0-4), lifespan (months), flowering duration (days), and floral count measurements are shown.

Population	Site	Trait	Mean	St.Error	V_G	St.Dev.	V_{Block}	V_E	V_P	$H^2=V_G/V_P$
DG	DMN	Vegetative Area (Day 38)	87.79	13.29	576.27	24.01	522.92	1907.08	3006.26	0.19
		Vegetative Area (Day 120)	745.72	97.32	52519.27	229.17	27940.55	77214.06	157673.88	0.33
		Vegetative Area (Day 339)	388.72	61.36	25179.56	158.68	11040.47	51373.72	87593.74	0.29
		Vegetative Area (Day 458)	721.89	44.49	34295.87	185.19	5573.09	78036.52	117905.48	0.29
		Winter Survival (Year 2)	1.07	0.10	0.08	0.29	0.03	0.55	0.66	0.12
		Flowering Duration (Year 1)	115.66	3.22	164.19	12.81	29.70	205.76	399.65	0.41
		Flowering Duration (Year 2)	122.19	2.19	43.59	6.60	12.98	538.07	594.64	0.07
		Flowering Duration	237.86	3.51	201.27	14.19	33.95	871.00	1106.22	0.18
		Floral Count (Year 1)	172.81	27.10	3591.96	59.93	2174.31	3573.18	9339.45	0.38
		Floral Count (Year 2)	287.40	13.30	3674.33	60.62	491.17	8655.97	12821.47	0.29
		Total Floral	460.25	21.47	10749.13	103.68	1290.09	13716.87	25756.09	0.42
	GFL	Vegetative Area (Day 9)	21.38	1.29	62.45	7.90	4.31	148.03	214.79	0.29
		Vegetative Area (Day 119)	224.09	46.34	10561.44	102.77	6330.85	23757.70	40650.00	0.26
		Vegetative Area (Day 235)	201.59	50.48	11249.76	106.06	7494.63	41817.06	60561.45	0.19
		Vegetative Area (Day 295)	12.46	3.15	116.78	10.81	27.03	983.07	1126.88	0.10
		Vegetative Area (Day 354)	3.00	1.43	14.06	3.75	5.54	271.82	291.42	0.05
		Vegetative Area (Day 452)	2.82	1.36	15.95	3.99	4.80	306.98	327.74	0.05
		Lifespan	9.12	0.24	1.67	1.29	0.13	11.67	13.47	0.12
		Flowering Duration (Year 1)	101.13	4.25	947.39	30.78	44.81	1892.17	2884.36	0.33
		Flowering Duration	103.12	4.54	1002.74	31.67	51.30	2244.55	3298.59	0.30
		Floral Count (Year 1)	181.55	30.23	9646.36	98.22	2648.46	18015.48	30310.30	0.32
		Total Floral	183.54	30.37	9734.33	98.66	2671.06	18719.44	31124.83	0.31
SG	STL	Vegetative Area (Day 20)	21.18	3.84	57.96	7.61	43.51	204.20	305.66	0.19
		Vegetative Area (Day 113)	677.03	66.24	13566.67	116.48	13004.25	37389.48	63960.39	0.21
		Vegetative Area (Day 282)	1326.06	56.36	121751.84	348.93	8617.64	85724.10	216093.59	0.56
		Vegetative Area (Day 362)	1046.97	27.96	45804.99	214.02	1946.14	59320.52	107071.66	0.43
		Vegetative Area (Day 449)	588.72	32.48	39342.54	198.35	2676.66	120978.78	162997.98	0.24
		Winter Survival (Year 2)	1.46	0.04	0.28	0.53	0.00	0.47	0.75	0.37

	Flowering Duration (Year 1)	90.21	5.58	1490.78	38.61	82.82	794.17	2367.77	0.63
	Flowering Duration (Year 2)	173.15	1.91	463.92	21.54	7.24	444.31	915.47	0.51
	Flowering Duration	263.27	6.95	2854.54	53.43	125.07	1223.26	4202.87	0.68
	Floral Count (Year 1)	49.16	8.15	1531.72	39.14	188.05	1021.56	2741.34	0.56
	Floral Count (Year 2)	385.82	6.40	12417.75	111.43	32.82	7448.08	19898.65	0.62
	Total Floral	435.08	7.39	19769.14	140.60	25.98	9071.29	28866.40	0.68
GFL	Vegetative Area (Day 9)	14.54	2.62	46.06	6.79	20.15	55.86	122.06	0.38
	Vegetative Area (Day 119)	635.49	113.06	75763.80	275.25	37674.41	108450.90	221889.11	0.34
	Vegetative Area (Day 200)	574.38	29.65	31862.30	178.50	2350.24	47980.93	82193.47	0.39
	Vegetative Area (Day 295)	29.84	6.60	500.65	22.38	121.90	2897.90	3520.45	0.14
	Vegetative Area (Day 354)	19.36	4.47	218.13	14.77	53.57	2539.16	2810.86	0.08
	Vegetative Area (Day 452)	48.17	10.74	2446.70	49.46	297.58	16926.15	19670.43	0.12
	Lifespan	12.86	0.82	7.38	2.72	1.93	22.73	32.04	0.23
	Flowering Duration (Year 1)	104.81	6.37	1709.59	41.35	106.89	2325.51	4142.00	0.41
	Flowering Duration	121.72	9.46	2728.91	52.24	242.93	4526.40	7498.25	0.36
	Floral Count (Year 1)	201.39	18.38	19007.24	137.87	871.89	13866.92	33746.06	0.56
	Total Floral	232.29	23.23	27659.86	166.31	1400.52	26427.05	55487.43	0.50

Table S2.4. Genotype-environment correlations for fitness traits that were comparable across sites. Pearson correlation coefficients (r_{GE}) are shown for each mapping population.

		r_{GE}	
Population	**Fitness Trait**	**Raw Data**	**Transformed Data**
DG	Vegetative Area (Day 38v9)	-0.281***	-0.296***
	Vegetative Area (Day 120v119)	-0.353***	-0.345***
	Vegetative Area (Day 339v354)	-0.111*	-0.155***
	Vegetative Area (Day 458v452)	-0.032^{NS}	-0.01^{NS}
	Flowering Duration (Year 1)	-0.107***	-0.092*
	Flowering Duration	-0.166***	-0.168***
	Floral Count (Year 1)	-0.219***	-0.178***
	TotalFloral	-0.244***	-0.229***
SG	Vegetative Area (Day 20v9)	-0.228***	-0.261***
	Vegetative Area (Day 113v119)	-0.37***	-0.388***
	Vegetative Area (Day 282v295)	-0.155***	-0.224***
	Vegetative Area (Day 362v354)	0.092*	-0.151***
	Vegetative Area (Day 449v452)	-0.181***	-0.223***
	Flowering Duration (Year 1)	-0.357***	-0.331***
	Flowering Duration	-0.377***	-0.391***
	Floral Count (Year 1)	-0.318***	-0.315***
	Total Floral	-0.329***	-0.341***

p < *0.01, **0.001, ***0.0001 (significance thresholds, following FDR correction)

NS = not significant at p=0.01 level

Table S2.5. Quantitative genetic partitioning of fitness trait variance across common garden sites and GxE interactions for each mapping population. Models with and without Year effects are shown for reproductive output traits. The significance of fixed and random effects in models was determined with ANOVAs and likelihood ratio tests (LRT), respectively.

DG Population											
Model	**Trait**	**Mean DMN**	**Mean GFL**	**Fixed effect**	**F value**	**p-value**	**Random effect**	**Variance**	**St.Dev.**	**LRT**	**p-value**
Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype)	Veg Area (Day ~120)	746.50	224.10	Site	1.26	1.00E+00	Genotype	13494.20	116.16	40.04	2.49E-10
							G x E	18203.62	134.92	121.92	2.40E-28
							Site(Block)	17274.03	131.43	562.99	1.88E-124
							Residual	50562.57	224.86	-	-
	Veg Area (Day ~350)	390.35	3.00	Site	1.65	1.99E-01	Genotype	234.61	15.32	0.06	8.07E-01
							G x E	12675.72	112.59	192.68	8.25E-44
							Site(Block)	5736.21	75.74	383.20	2.50E-85
							Residual	25863.04	160.82	-	-
	Veg Area (Day ~450)	723.01	2.88	Site	8.34	3.94E-03	Genotype	0.30	0.55	0.00	1.00E+00
							G x E	17157.96	130.99	157.95	3.17E-36
							Site(Block)	2967.96	54.48	128.89	7.18E-30
							Residual	39999.43	200.00	-	-
	Flowering Dur (Y1)	115.77	101.13	Site	1.09	2.98E-01	Genotype	64.50	8.03	2.55	1.10E-01
							G x E	490.68	22.15	180.65	3.49E-41
							Site(Block)	36.82	6.07	57.66	3.11E-14
							Residual	1048.03	32.37	-	-
	FloweringDuration	237.91	103.12	Site	1.73	1.00E+00	Genotype	153.19	12.38	9.46	2.10E-03
							G x E	448.08	21.17	87.26	9.53E-21
							Site(Block)	41.93	6.48	42.67	6.48E-11
							Residual	1556.26	39.45	-	-
	Floral Count (Y1)	173.15	181.55	Site	0.04	9.99E-01	Genotype	1900.42	43.59	17.57	2.76E-05
							G x E	4730.35	68.78	163.92	1.57E-37
							Site(Block)	2423.29	49.23	387.02	3.69E-86
							Residual	10810.17	103.97	-	-
	Total Floral	459.75	183.54	Site	1.35	2.45E-01	Genotype	3832.85	61.91	30.70	3.01E-08
							G x E	6501.73	80.63	144.68	2.52E-33
							Site(Block)	1932.55	43.96	209.90	1.45E-47
							Residual	16189.95	127.24	-	-
Year + Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype) + (1 | Year:Genotype) + (1 | Site:Year:Genotype)	Flowering Duration	120.83	88.54	Year	42.45	9.70E-11	Genotype	0.00	0.00	0.00	9.99E-01
				Site	0.16	6.87E-01	G x E	0.00	0.00	0.00	1.00E+00
							G x Y	0.00	0.00	0.00	1.00E+00
							G x E x Y	550.04	23.45	311.69	9.38E-70
							Site(Block)	24.26	4.93	53.63	2.42E-13
							Residual	899.81	30.00	-	-
	Total Floral	221.58	175.40	Year	119.71	1.49E-26	Genotype	1106.58	33.27	16.47	4.94E-05
				Site	0.47	1.00E+00	G x E	0.00	0.00	0.00	9.97E-01
							G x Y	0.00	0.01	0.00	1.00E+00
							G x E x Y	5753.67	75.85	184.50	5.05E-42
							Site(Block)	1334.25	36.53	254.45	2.78E-57
							Residual	10923.17	104.51	-	-

Table S2.5. (Continued)

SG Population											
Model	**Trait**	**Mean STL**	**Mean GFL**	**Fixed effect**	**F value**	**p value**	**Random effect**	**Variance**	**St.Dev.**	**LRT**	**p value**
Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype)	Veg Area (Day ~120)	662.55	635.49	Site	0.04	8.53E-01	Genotype	21567.18	146.86	48.08	4.08E-12
							G x E	24656.36	157.02	107.08	4.27E-25
							Site(Block)	27452.64	165.69	604.38	1.86E-133
							Residual	74837.89	273.57	-	-
	Veg Area (Day ~350)	1018.95	19.35	Site	16.32	5.54E-05	Genotype	0.02	0.14	0.00	9.98E-01
							G x E	26807.35	163.73	271.21	6.17E-61
							Site(Block)	113.88	10.67	1.84	1.75E-01
							Residual	41088.16	202.70	-	-
	Veg Area (Day ~450)	572.45	48.17	Site	0.77	1.00E+00	Genotype	4732.58	68.79	5.26	2.19E-02
							G x E	18205.76	134.93	74.02	7.73E-18
							Site(Block)	544.73	23.34	9.22	2.39E-03
							Residual	69997.22	264.57	-	-
	Flowering Dur (Y1)	87.89	104.81	Site	0.14	7.13E-01	Genotype	742.32	27.25	65.78	5.04E-16
							G x E	821.73	28.67	228.51	1.26E-51
							Site(Block)	125.69	11.21	132.53	1.15E-30
							Residual	1645.46	40.56	-	-
	FloweringDuration	256.43	121.72	Site	1.86	1.00E+00	Genotype	1507.99	38.83	76.11	2.68E-18
							G x E	1299.63	36.05	122.82	1.53E-28
							Site(Block)	317.96	17.83	154.43	1.87E-35
							Residual	3604.18	60.03	-	-
	Floral Count (Y1)	48.03	201.39	Site	1.74	1.87E-01	Genotype	2070.37	45.50	13.41	2.50E-04
							G x E	8151.49	90.29	531.64	1.24E-117
							Site(Block)	549.26	23.44	127.35	1.55E-29
							Residual	7472.10	86.44	-	-
	Total Floral	423.71	232.29	Site	1.73	1.88E-01	Genotype	9708.14	98.53	54.52	1.54E-13
							G x E	13866.83	117.76	310.07	2.11E-69
							Site(Block)	943.69	30.72	80.12	3.53E-19
							Residual	19821.26	140.79	-	-
Year + Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype) + (1 | Year:Genotype) + (1 | Site:Year:Genotype)	Flowering Duration	127.58	85.60	Year	110.83	5.69E-25	Genotype	167.09	12.93	8.20	4.19E-03
				Site	0.86	1.00E+00	G x E	0.00	0.00	0.00	1.00E+00
							G x Y	0.00	0.00	0.00	9.99E-01
							G x E x Y	2107.17	45.90	1309.52	9.63E-287
							Site(Block)	49.20	7.01	103.60	2.48E-24
							Residual	1297.48	36.02	-	-
	Total Floral	201.51	161.90	Year	344.86	1.36E-70	Genotype	0.00	0.01	0.00	1.00E+00
				Site	0.52	1.00E+00	G x E	0.00	0.00	0.00	9.93E-01
							G x Y	0.00	0.00	4.00	4.55E-02
							G x E x Y	23012.52	151.70	2028.97	0.00E+00
							Site(Block)	203.73	14.27	47.14	6.62E-12
							Residual	8419.06	91.76	-	-

Table S2.6. Means and quantitative genetic partitioning of fitness traits within each common garden site for each mapping population with calculations of broad-sense heritability. Included are models that use transformed data, as well as those that replace genotype with cyanotype.

			Raw Data								Square root Transformed Data							
Pop-Site	**Model**	**Trait**	**Mean**	**St. Err**	**V_G**	**St. Dev**	**V_B**	**V_E**	**V_P**	**$H^2=V_G/V_P$**	**Mean**	**St. Err**	**V_G**	**St. Dev**	**V_B**	**V_E**	**V_P**	**$H^2=V_G/V_P$**
DG-DMN	(1 | Genotype) + (1 | Block)	Veg Area (Day 38)	87.79	13.29	576.27	24.01	522.92	1907.08	3006.26	0.19	8.96	0.69	1.71	1.31	1.42	4.95	8.08	0.21
		Veg Area (Day 120)	745.72	97.32	52519.27	229.17	27940.55	77214.06	157673.88	0.33	26.26	1.99	20.57	4.54	11.71	28.06	60.34	0.34
		Veg Area (Day 339)	388.72	61.36	25179.56	158.68	11040.47	51373.72	87593.74	0.29	17.89	1.86	20.37	4.51	10.21	41.51	72.08	0.28
		Veg Area (Day 458)	721.89	44.49	34295.87	185.19	5573.09	78036.52	117905.48	0.29	25.74	0.82	15.15	3.89	1.84	42.86	59.85	0.25
		Winter Survival (Y2)	1.07	0.10	0.08	0.29	0.03	0.55	0.66	0.12	0.90	0.06	0.03	0.17	0.01	0.22	0.26	0.12
		Flowering Dur (Y1)	115.66	3.22	164.19	12.81	29.70	205.76	399.65	0.41	10.70	0.16	0.49	0.70	0.07	0.66	1.22	0.40
		Flowering Dur (Y2)	122.19	2.19	43.59	6.60	12.98	538.07	594.64	0.07	10.89	0.17	0.20	0.45	0.08	3.35	3.63	0.06
		Flowering Duration	237.86	3.51	201.27	14.19	33.95	871.00	1106.22	0.18	15.37	0.12	0.24	0.49	0.04	1.32	1.59	0.15
		Floral Count (Y1)	172.81	27.10	3591.96	59.93	2174.31	3573.18	9339.45	0.38	12.59	1.10	6.56	2.56	3.60	5.37	15.53	0.42
		Floral Count (Y2)	287.40	13.30	3674.33	60.62	491.17	8655.97	12821.47	0.29	16.46	0.51	3.48	1.87	0.73	12.42	16.62	0.21
		Total Floral	460.25	21.47	10749.13	103.68	1290.09	13716.87	25756.09	0.42	21.10	0.51	6.09	2.47	0.73	8.55	15.37	0.40
	(1 | Cyanotype) + (1 | Block)	Veg Area (Day 38)	87.01	13.31	5.02	2.24	521.27	2477.68	3003.97	0.00	8.89	0.70	0.03	0.18	1.41	6.64	8.09	0.00
		Veg Area (Day 120)	709.57	103.46	5530.34	74.37	27436.13	127937.45	160903.92	0.03	25.56	2.11	2.09	1.45	11.53	48.03	61.65	0.03
		Veg Area (Day 339)	388.32	61.09	0.00	0.00	11040.53	76548.85	87589.39	0.00	17.90	1.86	0.06	0.24	10.21	61.84	72.11	0.00
		Veg Area (Day 458)	705.87	48.13	1370.25	37.02	5532.80	111655.15	118558.20	0.01	25.39	0.93	0.75	0.86	1.83	57.76	60.33	0.01
		Winter Survival (Y2)	1.08	0.10	0.00	0.06	0.03	0.63	0.66	0.01	0.90	0.07	0.00	0.05	0.01	0.25	0.26	0.01
		Flowering Dur (Y1)	115.66	3.18	0.00	0.00	29.54	370.55	400.08	0.00	10.70	0.16	0.00	0.00	0.07	1.15	1.22	0.00
		Flowering Dur (Y2)	122.19	2.18	0.15	0.39	12.90	581.65	594.69	0.00	10.88	0.18	0.01	0.11	0.08	3.55	3.64	0.00
		Flowering Duration	237.84	3.46	0.00	0.00	33.79	1072.68	1106.47	0.00	15.37	0.12	0.00	0.00	0.04	1.56	1.60	0.00
		Floral Count (Y1)	174.91	27.29	61.16	7.82	2165.07	7129.28	9355.51	0.01	12.68	1.11	0.13	0.36	3.57	11.86	15.56	0.01
		Floral Count (Y2)	287.30	13.07	0.00	0.00	487.42	12340.68	12828.10	0.00	16.46	0.50	0.00	0.00	0.72	15.91	16.63	0.00
		Total Floral	461.15	21.31	24.19	4.92	1286.63	24461.63	25772.46	0.00	21.11	0.51	0.01	0.10	0.73	14.64	15.38	0.00
DG-GFL	(1 | Genotype) + (1 | Block)	Veg Area (Day 9)	21.38	1.29	62.45	7.90	4.31	148.03	214.79	0.29	4.31	0.20	0.74	0.86	0.12	1.98	2.84	0.26
		Veg Area (Day 119)	224.09	46.34	10561.44	102.77	6330.85	23757.70	40650.00	0.26	13.17	1.74	14.13	3.76	8.98	30.43	53.54	0.26
		Veg Area (Day 235)	201.59	50.48	11249.76	106.06	7494.63	41817.06	60561.45	0.19	11.54	2.04	13.38	3.66	12.26	46.98	72.62	0.18
		Veg Area (Day 295)	12.46	3.15	116.78	10.81	27.03	983.07	1126.88	0.10	1.97	0.21	1.21	1.10	0.11	7.29	8.60	0.14
		Veg Area (Day 354)	3.00	1.43	14.06	3.75	5.54	271.82	291.42	0.05	0.51	0.17	0.17	0.41	0.09	2.52	2.77	0.06

(1 | Genotype) + (1 | Block)	Veg Area (Day 452)	2.82	1.36	15.95	3.99	4.80	306.98	327.74	0.05	0.38	0.17	0.08	0.29	0.08	2.57	2.74	0.03
	Lifespan	9.12	0.24	1.67	1.29	0.13	11.67	13.47	0.12	2.95	0.04	0.05	0.22	0.00	0.36	0.41	0.12
	Flowering Dur (Y1)	101.13	4.25	947.39	30.78	44.81	1892.17	2884.36	0.33	9.40	0.33	4.11	2.03	0.29	8.56	12.95	0.32
	Flowering Duration	103.12	4.54	1002.74	31.67	51.30	2244.55	3298.59	0.30	9.47	0.33	4.19	2.05	0.29	9.11	13.59	0.31
	Floral Count (Y1)	181.55	30.23	9646.36	98.22	2648.46	18015.48	30310.30	0.32	11.74	1.21	17.63	4.20	4.23	23.34	45.19	0.39
	Total Floral	183.54	30.37	9734.33	98.66	2671.06	18719.44	31124.83	0.31	11.80	1.20	17.57	4.19	4.20	23.93	45.69	0.38
(1 | Cyanotype) + (1 | Block)	Veg Area (Day 9)	21.38	1.24	0.00	0.00	4.19	210.48	214.67	0.00	4.31	0.20	0.00	0.00	0.11	2.72	2.84	0.00
	Veg Area (Day 119)	211.69	47.97	611.13	24.72	6321.00	34022.19	40954.31	0.01	12.88	1.78	0.46	0.68	8.97	44.30	53.73	0.01
	Veg Area (Day 235)	205.41	50.96	220.25	14.84	7474.94	52911.09	60606.28	0.00	11.69	2.05	0.30	0.55	12.24	60.14	72.68	0.00
	Veg Area (Day 295)	12.97	3.34	4.54	2.13	26.83	1096.90	1128.27	0.00	2.00	0.23	0.03	0.17	0.11	8.48	8.61	0.00
	Veg Area (Day 354)	3.17	1.52	0.82	0.90	5.51	285.34	291.67	0.00	0.53	0.18	0.01	0.11	0.08	2.68	2.77	0.00
	Veg Area (Day 452)	2.98	1.46	0.88	0.94	4.87	322.19	327.94	0.00	0.38	0.17	0.00	0.05	0.08	2.65	2.74	0.00
	Lifespan	9.12	0.23	0.00	0.00	0.13	13.34	13.47	0.00	2.95	0.04	0.00	0.00	0.00	0.41	0.41	0.00
	Flowering Dur (Y1)	101.64	4.27	6.07	2.46	42.94	2836.25	2885.26	0.00	9.40	0.32	0.00	0.00	0.28	12.67	12.94	0.00
	Flowering Duration	103.55	4.51	5.02	2.24	49.29	3244.42	3298.72	0.00	9.47	0.32	0.00	0.00	0.28	13.30	13.58	0.00
	Floral Count (Y1)	181.71	30.78	171.49	13.10	2629.46	27570.53	30371.47	0.01	11.77	1.22	0.17	0.42	4.19	40.87	45.24	0.00
	Total Floral	183.65	30.89	167.32	12.94	2651.07	28364.65	31183.03	0.01	11.83	1.21	0.15	0.39	4.17	41.41	45.73	0.00
SG-STL	Veg Area (Day 20)	21.18	3.84	57.96	7.61	43.51	204.20	305.66	0.19	4.25	0.41	0.70	0.84	0.49	2.09	3.28	0.21
(1 | Genotype) + (1 | Block)	Veg Area (Day 113)	677.03	66.24	13566.67	116.48	13004.25	37389.48	63960.39	0.21	25.47	1.42	5.77	2.40	5.98	18.61	30.36	0.19
	Veg Area (Day 282)	1326.06	56.36	121751.84	348.93	8617.64	85724.10	216093.59	0.56	35.55	0.86	40.00	6.32	1.96	19.83	61.79	0.65
	Veg Area (Day 362)	1046.97	27.96	45804.99	214.02	1946.14	59320.52	107071.66	0.43	31.83	0.41	17.93	4.23	0.37	14.33	32.63	0.55
	Veg Area (Day 449)	588.72	32.48	39342.54	198.35	2676.66	120978.78	162997.98	0.24	22.48	0.74	27.03	5.20	1.37	54.53	82.94	0.33
	Winter Survival (Y2)	1.46	0.04	0.28	0.53	0.00	0.47	0.75	0.37	1.12	0.02	0.08	0.29	0.00	0.12	0.21	0.40
	Flowering Dur (Y1)	90.21	5.58	1490.78	38.61	82.82	794.17	2367.77	0.63	8.73	0.42	8.78	2.96	0.47	4.91	14.15	0.62
	Flowering Dur (Y2)	173.15	1.91	463.92	21.54	7.24	444.31	915.47	0.51	13.07	0.09	1.23	1.11	0.02	1.05	2.29	0.54
	Flowering Duration	263.27	6.95	2854.54	53.43	125.07	1223.26	4202.87	0.68	16.07	0.23	3.35	1.83	0.13	1.44	4.92	0.68
	Floral Count (Y1)	49.16	8.15	1531.72	39.14	188.05	1021.56	2741.34	0.56	5.94	0.61	8.90	2.98	1.06	4.35	14.31	0.62
	Floral Count (Y2)	385.82	6.40	12417.75	111.43	32.82	7448.08	19898.65	0.62	19.25	0.18	10.10	3.18	0.03	4.74	14.86	0.68
	Total Floral	435.08	7.39	19769.14	140.60	25.98	9071.29	28866.40	0.68	20.43	0.18	12.65	3.56	0.01	4.93	17.59	0.72
Next page	Veg Area (Day 20)	21.91	4.02	6.31	2.51	42.90	260.51	309.71	0.02	4.30	0.41	0.03	0.17	0.48	2.79	3.29	0.01
	Veg Area (Day 113)	683.90	66.71	628.16	25.06	12725.81	50634.11	63988.07	0.01	25.59	1.43	0.24	0.49	5.87	24.25	30.36	0.01
	Veg Area (Day 282)	1332.35	60.65	3180.92	56.40	8023.08	202716.17	213920.17	0.01	35.70	0.90	0.62	0.79	1.79	57.41	59.82	0.01

	(1 | Cyanotype) + (1 | Block)	Veg Area (Day 362)	1051.67	28.64	240.19	15.50	2018.76	103991.12	106250.08	0.00	31.94	0.40	0.03	0.18	0.38	31.16	31.58	0.00
		Veg Area (Day 449)	590.04	33.15	219.00	14.80	2750.94	160088.28	163058.22	0.00	22.52	0.75	0.12	0.34	1.43	81.15	82.69	0.00
		Winter Survival (Y2)	1.46	0.03	0.00	0.00	0.00	0.75	0.75	0.00	1.12	0.01	0.00	0.00	0.00	0.21	0.21	0.00
		Flowering Dur (Y1)	88.74	6.35	39.39	6.28	84.31	2277.40	2401.10	0.02	8.64	0.48	0.23	0.48	0.48	13.64	14.35	0.02
		Flowering Dur (Y2)	173.48	1.73	0.00	0.00	7.16	882.14	889.30	0.00	13.09	0.09	0.00	0.00	0.02	2.16	2.17	0.00
		Flowering Duration	261.87	7.66	50.09	7.08	126.39	4027.23	4203.71	0.01	16.03	0.25	0.05	0.23	0.13	4.72	4.91	0.01
		Floral Count (Y1)	48.30	8.67	36.22	6.02	190.67	2533.74	2760.63	0.01	5.87	0.66	0.24	0.48	1.08	13.14	14.46	0.02
		Floral Count (Y2)	387.28	6.07	73.37	8.57	3.34	19641.04	19717.75	0.00	19.32	0.15	0.04	0.20	0.00	14.27	14.32	0.00
		Total Floral	436.24	9.26	232.95	15.26	0.00	28488.00	28720.95	0.01	20.47	0.22	0.12	0.35	0.00	17.02	17.15	0.01
SG-GFL	(1 | Genotype) + (1 | Block)	Veg Area (Day 9)	14.54	2.62	46.06	6.79	20.15	55.86	122.06	0.38	3.54	0.37	0.85	0.92	0.41	0.87	2.13	0.40
		Veg Area (Day 119)	635.49	113.06	75763.80	275.25	37674.41	108450.90	221889.11	0.34	23.30	2.29	38.95	6.24	15.39	43.41	97.75	0.40
		Veg Area (Day 200)	574.38	29.65	31862.30	178.50	2350.24	47980.93	82193.47	0.39	22.86	0.67	22.44	4.74	1.16	28.81	52.40	0.43
		Veg Area (Day 295)	29.84	6.60	500.65	22.38	121.90	2897.90	3520.45	0.14	3.89	0.49	3.30	1.82	0.69	10.96	14.96	0.22
		Veg Area (Day 354)	19.36	4.47	218.13	14.77	53.57	2539.16	2810.86	0.08	2.26	0.48	2.18	1.48	0.65	11.66	14.48	0.15
		Veg Area (Day 452)	48.17	10.74	2446.70	49.46	297.58	16926.15	19670.43	0.12	3.23	0.63	6.83	2.61	1.07	30.20	38.11	0.18
		Lifespan	12.86	0.82	7.38	2.72	1.93	22.73	32.04	0.23	3.50	0.11	0.15	0.39	0.04	0.43	0.62	0.24
		Flowering Dur (Y1)	104.81	6.37	1709.59	41.35	106.89	2325.51	4142.00	0.41	9.49	0.38	6.95	2.64	0.38	7.66	14.98	0.46
		Flowering Duration	121.72	9.46	2728.91	52.24	242.93	4526.40	7498.25	0.36	10.11	0.47	8.60	2.93	0.60	10.44	19.65	0.44
		Floral Count (Y1)	201.39	18.38	19007.24	137.87	871.89	13866.92	33746.06	0.56	12.37	0.70	31.25	5.59	1.24	16.42	48.91	0.64
		Total Floral	232.29	23.23	27659.86	166.31	1400.52	26427.05	55487.43	0.50	13.15	0.80	35.93	5.99	1.65	22.50	60.08	0.60
	(1 | Cyanotype) + (1 | Block)	Veg Area (Day 9)	14.40	2.62	0.27	0.52	20.10	101.72	122.09	0.00	3.53	0.37	0.00	0.06	0.41	1.72	2.13	0.00
		Veg Area (Day 119)	620.92	114.21	1487.16	38.56	37501.46	183027.54	222016.17	0.01	22.92	2.32	0.74	0.86	15.32	81.77	97.82	0.01
		Veg Area (Day 200)	565.09	30.87	472.08	21.73	2288.67	79586.86	82347.61	0.01	22.60	0.72	0.38	0.62	1.12	51.07	52.57	0.01
		Veg Area (Day 295)	31.27	6.89	16.70	4.09	120.84	3390.34	3527.88	0.00	3.89	0.49	0.00	0.00	0.68	14.27	14.95	0.00
		Veg Area (Day 354)	19.36	4.42	0.00	0.00	53.08	2757.31	2810.39	0.00	2.26	0.47	0.00	0.00	0.64	13.84	14.48	0.00
		Veg Area (Day 452)	48.17	10.51	0.00	0.00	292.55	19372.83	19665.38	0.00	3.23	0.61	0.00	0.00	1.06	37.03	38.10	0.00
		Lifespan	12.86	0.81	0.00	0.00	1.92	30.11	32.03	0.00	3.50	0.11	0.00	0.00	0.03	0.58	0.62	0.00
		Flowering Dur (Y1)	104.81	6.10	0.00	0.00	103.46	4035.10	4138.56	0.00	9.49	0.36	0.00	0.00	0.36	14.61	14.97	0.00
		Flowering Duration	121.72	9.16	0.00	0.00	237.47	7255.35	7492.81	0.00	10.11	0.46	0.00	0.00	0.59	19.05	19.63	0.00
		Floral Count (Y1)	197.40	20.33	414.42	20.36	833.87	32655.88	33904.16	0.01	12.21	0.74	0.45	0.67	1.17	47.44	49.06	0.01
		Total Floral	229.38	24.33	374.57	19.35	1345.70	53883.33	55603.61	0.01	13.03	0.82	0.35	0.59	1.58	58.24	60.17	0.01

Table S2.7. Quantitative genetic partitioning of fitness trait variance across common garden sites and GxE interactions for the DG mapping population. Models with and without year effects are shown for reproductive output traits. Also included are models that use both raw and transformed data, as well as those that replace genotype with cyanotype.

		Raw Data								Square root Transformed Data							
	Trait (DG Pop)	Mean DMN	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value	Mean DMN	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value
Model: Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype)	VegArea d38v9	87.7	21.4	5.51	1.0E+00	Genotype	103.3	10.2	12.56	3.9E-04	8.95	4.31	10.24	1.0E+00	Genotype	0.64	0.80	38.14	6.6E-10
						G x E	212.9	14.6	50.44	1.2E-12					G x E	0.57	0.76	34.39	4.5E-09
						Site(Block)	266.8	16.3	445.32	7.5E-99					Site(Block)	0.78	0.88	386.24	5.4E-86
						Residual	1017.1	31.9	-	-					Residual	3.45	1.86	-	-
	VegArea d120v119	746.5	224.1	1.26	1.0E+00	Genotype	13494.2	116.2	40.04	2.5E-10	26.27	13.17	3.00	1.0E+00	Genotype	9.36	3.06	62.85	2.2E-15
						G x E	18203.6	134.9	121.92	2.4E-28					G x E	8.10	2.85	81.84	1.5E-19
						Site(Block)	17274.0	131.4	562.99	1.9E-124					Site(Block)	10.39	3.22	586.15	1.7E-129
						Residual	50562.6	224.9	-	-					Residual	29.21	5.41	-	-
	VegArea d339v354	390.4	3.0	1.65	2.0E-01	Genotype	234.6	15.3	0.06	8.1E-01	17.92	0.51	7.14	1.0E+00	Genotype	0.91	0.96	1.32	2.5E-01
						G x E	12675.7	112.6	192.68	8.3E-44					G x E	9.64	3.10	165.43	7.4E-38
						Site(Block)	5736.2	75.7	383.20	2.5E-85					Site(Block)	5.23	2.29	411.02	2.2E-91
						Residual	25863.0	160.8	-	-					Residual	21.89	4.68	-	-
	VegArea d458v452	723.0	2.9	8.34	3.9E-03	Genotype	0.3	0.6	0.00	1.0E+00	25.75	0.38	8.55	3.5E-03	Genotype	0.05	0.23	0.01	9.4E-01
						G x E	17158.0	131.0	157.95	3.2E-36					G x E	7.67	2.77	107.24	3.9E-25
						Site(Block)	2968.0	54.5	128.89	7.2E-30					Site(Block)	1.02	1.01	74.23	6.9E-18
						Residual	39999.4	200.0	-	-					Residual	23.04	4.80	-	-
	Y1 FloweringDur	115.8	101.1	1.09	3.0E-01	Genotype	64.5	8.0	2.55	1.1E-01	10.70	9.40	0.10	1.0E+00	Genotype	0.20	0.45	1.42	2.3E-01
						G x E	490.7	22.2	180.65	3.5E-41					G x E	2.09	1.45	173.19	1.5E-39
						Site(Block)	36.8	6.1	57.66	3.1E-14					Site(Block)	0.18	0.42	64.33	1.1E-15
						Residual	1048.0	32.4	-	-					Residual	4.60	2.15	-	-
	FloweringDur	237.9	103.1	1.73	1.0E+00	Genotype	153.2	12.4	9.46	2.1E-03	15.37	9.47	2.88	9.0E-02	Genotype	0.37	0.61	4.44	3.5E-02
						G x E	448.1	21.2	87.26	9.5E-21					G x E	1.84	1.36	119.84	6.9E-28
						Site(Block)	41.9	6.5	42.67	6.5E-11					Site(Block)	0.16	0.40	50.86	9.9E-13
						Residual	1556.3	39.4	-	-					Residual	5.21	2.28	-	-
	Y1 FloralCount	173.1	181.5	0.04	1.0E+00	Genotype	1900.4	43.6	17.57	2.8E-05	12.60	11.74	0.04	8.4E-01	Genotype	2.60	1.61	12.01	5.3E-04
						G x E	4730.3	68.8	163.92	1.6E-37					G x E	9.50	3.08	289.44	6.6E-65
						Site(Block)	2423.3	49.2	387.02	3.7E-86					Site(Block)	3.92	1.98	464.71	4.5E-103
						Residual	10810.2	104.0	-	-					Residual	14.36	3.79	-	-
	TotalFloral	459.7	183.5	1.35	2.4E-01	Genotype	3832.9	61.9	30.70	3.0E-08	21.09	11.80	0.88	3.5E-01	Genotype	3.48	1.87	20.76	5.2E-06
						G x E	6501.7	80.6	144.68	2.5E-33					G x E	8.40	2.90	208.15	3.5E-47
						Site(Block)	1932.5	44.0	209.90	1.4E-47					Site(Block)	2.43	1.56	263.28	3.3E-59
						Residual	16189.9	127.2	-	-					Residual	16.22	4.03	-	-

Table S2.7. (Continued)

Model: Site + (1 | Cyanotype) + (1 | Site/Block) + (1 | Site:Cyanotype)	Raw Data									Square root Transformed Data								
Trait (DG Pop)	Mean DMN	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value	Mean DMN	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value
VegArea d38v9	86.9	21.3	1.49	2.2E-01	Cyanotype	0.3	0.5	0.00	9.5E-01	8.91	4.30	0.89	1.0E+00	Cyanotype	0.00	0.04	0.01	9.3E-01
					Cyano x E	2.0	1.4	0.28	6.0E-01					Cyano x E	0.01	0.08	0.21	6.5E-01
					Site(Block)	267.2	16.3	358.32	6.5E-80					Site(Block)	0.78	0.88	298.39	7.4E-67
					Residual	1330.3	36.5	-	-					Residual	4.66	2.16	-	-
VegArea d120v119	713.4	201.1	2.10	1.5E-01	Cyanotype	2824.4	53.1	2.20	1.4E-01	25.66	12.58	2.62	1.0E+00	Cyanotype	1.44	1.20	5.58	1.8E-02
					Cyano x E	352.3	18.8	0.83	3.6E-01					Cyano x E	0.00	0.00	0.00	1.0E+00
					Site(Block)	17162.5	131.0	375.15	1.4E-83					Site(Block)	10.35	3.22	397.96	1.5E-88
					Residual	81140.0	284.9	-	-					Residual	46.18	6.80	-	-
VegArea d339v354	390.4	3.0	1.14	1.0E+00	Cyanotype	0.0	0.0	0.00	1.0E+00	17.93	0.51	6.28	1.2E-02	Cyanotype	0.04	0.20	0.38	5.4E-01
					Cyano x E	0.0	0.0	0.00	1.0E+00					Cyano x E	0.00	0.00	0.00	1.0E+00
					Site(Block)	5706.4	75.5	264.71	1.6E-59					Site(Block)	5.21	2.28	288.84	8.9E-65
					Residual	38773.6	196.9	-	-					Residual	32.43	5.69	-	-
VegArea d458v452	705.0	2.9	12.07	1.0E+00	Cyanotype	0.0	0.1	0.00	1.0E+00	25.36	0.37	30.94	3.2E-08	Cyanotype	0.03	0.17	0.00	9.5E-01
					Cyano x E	831.0	28.8	6.18	1.3E-02					Cyano x E	0.41	0.64	3.91	4.8E-02
					Site(Block)	2931.4	54.1	88.64	4.7E-21					Site(Block)	1.01	1.00	53.88	2.1E-13
					Residual	56828.1	238.4	-	-					Residual	30.61	5.53	-	-
Y1 FloweringDur	115.8	101.6	2.21	1.0E+00	Cyanotype	0.0	0.0	0.00	1.0E+00	10.70	9.40	0.16	1.0E+00	Cyanotype	0.00	0.00	0.00	1.0E+00
					Cyano x E	2.7	1.7	0.45	5.0E-01					Cyano x E	0.00	0.00	0.00	9.8E-01
					Site(Block)	35.7	6.0	34.46	4.4E-09					Site(Block)	0.17	0.42	39.74	2.9E-10
					Residual	1601.7	40.0	-	-					Residual	6.90	2.63	-	-
FloweringDur	237.9	103.3	5.78	1.6E-02	Cyanotype	0.0	0.0	0.00	1.0E+00	15.37	9.47	296.55	5.5E-05	Cyanotype	0.00	0.00	0.00	1.0E+00
					Cyano x E	1.2	1.1	0.08	7.8E-01					Cyano x E	0.00	0.00	0.00	1.0E+00
					Site(Block)	40.7	6.4	28.35	1.0E-07					Site(Block)	0.16	0.40	32.93	9.6E-09
					Residual	2156.8	46.4	-	-					Residual	7.42	2.72	-	-
Y1 FloralCount	174.8	181.7	0.03	1.0E+00	Cyanotype	0.0	0.0	0.00	1.0E+00	12.66	11.78	0.23	1.0E+00	Cyanotype	0.00	0.00	0.00	1.0E+00
					Cyano x E	111.1	10.5	3.94	4.7E-02					Cyano x E	0.14	0.38	2.75	9.7E-02
					Site(Block)	2408.8	49.1	249.09	4.1E-56					Site(Block)	3.88	1.97	265.34	1.2E-59
					Residual	17383.5	131.8	-	-					Residual	26.38	5.14	-	-
TotalFloral	461.7	183.6	1.73	1.0E+00	Cyanotype	0.0	0.0	0.00	1.0E+00	21.12	11.83	1.31	2.5E-01	Cyanotype	0.00	0.00	0.00	9.9E-01
					Cyano x E	84.0	9.2	1.45	2.3E-01					Cyano x E	0.07	0.27	0.91	3.4E-01
					Site(Block)	1911.0	43.7	127.06	1.8E-29					Site(Block)	2.41	1.55	152.45	5.0E-35
					Residual	26480.5	162.7	-	-					Residual	28.07	5.30	-	-

Table S2.7. (Continued)

		Raw Data								Square root Transformed Data							
Model	Trait (DG Pop)	Mean DMN	Mean GFL	F (Site) (Year)	p-value	Random effect	Var	St. Dev	LRT	p-value	Mean DMN	Mean GFL	F (Site) (Year)	p-value	Random effect	Var	St. Dev	LRT	p-value
Year + Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype) + (1 | Year:Genotype) + (1 | Site:Year:Genotype)	Flowering Dur	120.8	88.5	42.45	9.7E-11	Genotype	0.0	0.0	0.00	1.0E+00	11.00	8.28	100.01	7.9E-23	Genotype	0.00	0.00	0.00	1.0E+00
						G x E	0.0	0.0	0.00	1.0E+00					G x E	0.00	0.00	0.00	1.0E+00
				0.16	6.9E-01	G x Y	0.0	0.0	0.00	1.0E+00			0.71	4.0E-01	G x Y	0.00	0.00	0.00	1.0E+00
						G x E x Y	550.0	23.5	311.69	9.4E-70					G x E x Y	2.88	1.70	335.83	5.2E-75
						Site(Block)	24.3	4.9	53.63	2.4E-13					Site(Block)	0.15	0.38	64.45	9.9E-16
						Residual	899.8	30.0	-	-					Residual	4.34	2.08	-	-
	TotalFloral	221.6	175.4	119.71	1.5E-26	Genotype	1106.6	33.3	16.47	4.9E-05	14.40	10.67	19.44	1.1E-05	Genotype	0.66	0.81	2.15	1.4E-01
						G x E	0.0	0.0	0.00	1.0E+00					G x E	0.00	0.00	0.00	1.0E+00
				0.47	1.0E+00	G x Y	0.0	0.0	0.00	1.0E+00			1.10	3.0E-01	G x Y	0.00	0.00	0.00	1.0E+00
						G x E x Y	5753.7	75.9	184.50	5.1E-42					G x E x Y	12.70	3.56	366.81	9.2E-82
						Site(Block)	1334.2	36.5	254.45	2.8E-57					Site(Block)	2.21	1.49	306.08	1.6E-68
						Residual	10923.2	104.5	-	-					Residual	14.94	3.87	-	-
Year + Site + (1 | Cyanotype) + (1 | Site/Block) + (1 | Site:Cyanotype) + (1 | Year:Cyanotype) + (1 | Site:Year:Cyanotype)	Flowering Dur	124.6	67.6	9.41	9.4E-03	Cyanotype	0.0	0.1	0.00	1.0E+00	11.29	6.52	11.22	5.7E-03	Cyanotype	0.00	0.00	0.01	9.3E-01
						Cyano x E	0.0	0.0	0.01	9.3E-01					Cyano x E	0.00	0.00	0.00	9.6E-01
				18.24	1.0E+00	Cyano x Y	0.0	0.0	0.00	1.0E+00			1.73	1.9E-01	Cyano x Y	0.00	0.00	0.00	9.8E-01
						Cyano xExY	614.7	24.8	554.16	1.6E-122					Cyano xExY	4.17	2.04	725.57	8.2E-160
						Site(Block)	23.9	4.9	37.16	1.1E-09					Site(Block)	0.14	0.37	44.29	2.8E-11
						Residual	1242.7	35.3	-	-					Residual	5.86	2.42	-	-
	TotalFloral	236.7	106.1	0.38	5.5E-01	Cyanotype	0.5	0.7	0.00	1.0E+00	15.06	7.41	2.53	1.4E-01	Cyanotype	0.00	0.00	0.00	1.0E+00
						Cyano x E	0.0	0.2	0.00	1.0E+00					Cyano x E	0.00	0.00	0.00	1.0E+00
				2.51	1.0E+00	Cyano x Y	0.0	0.0	0.00	1.0E+00			3.12	1.0E+00	Cyano x Y	0.00	0.00	0.00	1.0E+00
						Cyano xExY	5871.1	76.6	454.75	6.7E-101					Cyano xExY	14.43	3.80	723.17	2.7E-159
						Site(Block)	1265.6	35.6	173.50	1.3E-39					Site(Block)	2.05	1.43	192.79	7.8E-44
						Residual	15725.4	125.4	-	-					Residual	23.01	4.80	-	-

Table S2.8. Quantitative genetic partitioning of fitness trait variance across common garden sites and GxE interactions for the SG mapping population. Models with and without year effects are shown for reproductive output traits. Also included are models that use both raw and transformed data, as well as those that replace genotype with cyanotype.

		Raw Data									Square root Transformed Data								
	Trait (SG Pop)	Mean STL	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value	Mean STL	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value
Model: Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype)	VegArea d20v9	21.0	14.5	0.31	1.0E+00	Genotype	20.5	4.5	23.81	1.1E-06	4.22	3.54	0.15	1.0E+00	Genotype	0.33	0.58	34.92	3.4E-09
						G x E	31.6	5.6	67.81	1.8E-16					G x E	0.45	0.67	92.06	8.4E-22
						Site(Block)	32.4	5.7	430.12	1.5E-95					Site(Block)	0.46	0.68	522.07	1E-115
						Residual	128.4	11.3	-	-					Residual	1.49	1.22	-	-
	VegArea d113v119	662.5	635.5	0.04	8.5E-01	Genotype	21567.2	146.9	48.08	4.1E-12	24.88	23.30	0.03	1.0E+00	Genotype	12.16	3.49	60.85	6.1E-15
						G x E	24656.4	157.0	107.08	4.3E-25					G x E	11.87	3.45	109.21	1.5E-25
						Site(Block)	27452.6	165.7	604.38	1.9E-133					Site(Block)	12.83	3.58	595.22	1E-131
						Residual	74837.9	273.6	-	-					Residual	35.57	5.96	-	-
	VegArea d282v295	1295.5	29.8	13.97	2.0E-04	Genotype	2428.8	49.3	0.42	5.2E-01	34.72	3.89	13.32	2.7E-04	Genotype	4.53	2.13	9.55	2.0E-03
						G x E	62507.2	250.0	530.91	1E-117					G x E	19.68	4.44	343.25	1.2E-76
						Site(Block)	8969.1	94.7	272.95	2.6E-61					Site(Block)	3.54	1.88	238.48	8.4E-54
						Residual	57363.9	239.5	-	-					Residual	25.98	5.10	-	-
	VegArea d362v354	1018.9	19.4	16.32	5.5E-05	Genotype	0.0	0.1	0.00	1.0E+00	30.99	2.26	36.71	1.0E+00	Genotype	2.21	1.49	5.72	1.7E-02
						G x E	26807.4	163.7	271.21	6.2E-61					G x E	10.78	3.28	178.13	1.2E-40
						Site(Block)	113.9	10.7	1.84	1.7E-01					Site(Block)	0.48	0.69	31.19	2.3E-08
						Residual	41088.2	202.7	-	-					Residual	23.19	4.82	-	-
	VegArea d449v452	572.5	48.2	0.77	1.0E+00	Genotype	4732.6	68.8	5.26	2.2E-02	21.88	3.23	72.84	3.0E-16	Genotype	6.64	2.58	18.63	1.6E-05
						G x E	18205.8	134.9	74.02	7.7E-18					G x E	12.73	3.57	81.40	1.8E-19
						Site(Block)	544.7	23.3	9.22	2.4E-03					Site(Block)	0.57	0.75	16.54	4.8E-05
						Residual	69997.2	264.6	-	-					Residual	46.07	6.79	-	-
	Y1 FloweringDur	87.9	104.8	0.14	7.1E-01	Genotype	742.3	27.2	65.78	5.0E-16	8.53	9.49	0.09	7.7E-01	Genotype	3.31	1.82	57.92	2.7E-14
						G x E	821.7	28.7	228.51	1.3E-51					G x E	4.37	2.09	269.98	1.1E-60
						Site(Block)	125.7	11.2	132.53	1.1E-30					Site(Block)	0.63	0.80	159.59	1.4E-36
						Residual	1645.5	40.6	-	-					Residual	6.94	2.64	-	-
	FloweringDur	256.4	121.7	1.86	1.0E+00	Genotype	1508.0	38.8	76.11	2.7E-18	15.68	10.11	1.54	1.0E+00	Genotype	3.30	1.82	72.19	2.0E-17
						G x E	1299.6	36.1	122.82	1.5E-28					G x E	2.89	1.70	114.72	9.1E-27
						Site(Block)	318.0	17.8	154.43	1.9E-35					Site(Block)	0.68	0.83	141.58	1.2E-32
						Residual	3604.2	60.0	-	-					Residual	8.40	2.90	-	-
	Y1 FloralCount	48.0	201.4	1.74	1.9E-01	Genotype	2070.4	45.5	13.41	2.5E-04	5.81	12.37	3.75	1.0E+00	Genotype	6.05	2.46	34.63	4.0E-09
						G x E	8151.5	90.3	531.64	1E-117					G x E	13.75	3.71	636.86	1E-140
						Site(Block)	549.3	23.4	127.35	1.6E-29					Site(Block)	1.33	1.15	217.72	2.8E-49
						Residual	7472.1	86.4	-	-					Residual	10.71	3.27	-	-
	TotalFloral	423.7	232.3	1.73	1.9E-01	Genotype	9708.1	98.5	54.52	1.5E-13	19.91	13.15	0.49	4.8E-01	Genotype	9.45	3.07	50.13	1.4E-12
						G x E	13866.8	117.8	310.07	2.1E-69					G x E	15.12	3.89	390.12	7.8E-87
						Site(Block)	943.7	30.7	80.12	3.5E-19					Site(Block)	1.17	1.08	111.94	3.7E-26
						Residual	19821.3	140.8	-	-					Residual	18.01	4.24	-	-

Table S2.8. (Continued)

		Raw Data									Square root Transformed Data								
	Trait (SG Pop)	Mean STL	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value	Mean STL	Mean GFL	F (Site)	p-value	Random effect	Var	St. Dev	LRT	p-value
Model: Site + (1 | Cyanotype) + (1 | Site/Block) + (1 | Site:Cyanotype)	VegArea d20v9	21.5	14.4	0.98	3.3E-01	Cyanotype	0.8	0.9	0.16	6.9E-01	4.23	3.55	0.79	3.9E-01	Cyanotype	0.01	0.12	1.09	3.0E-01
						Cyano x E	1.7	1.3	0.82	3.6E-01					Cyano x E	0.00	0.00	0.00	1.0E+00
						Site(Block)	32.7	5.7	321.11	8.3E-72					Site(Block)	0.46	0.68	365.08	2.2E-81
						Residual	179.6	13.4	-	-					Residual	2.26	1.50	-	-
	VegArea d113v119	663.9	622.2	0.09	1.0E+00	Cyanotype	387.5	19.7	0.23	6.3E-01	24.89	22.94	0.37	1.0E+00	Cyanotype	0.16	0.39	0.13	7.2E-01
						Cyano x E	610.1	24.7	2.15	1.4E-01					Cyano x E	0.36	0.60	2.08	1.5E-01
						Site(Block)	27409.4	165.6	404.48	5.8E-90					Site(Block)	12.79	3.58	384.45	1.3E-85
						Residual	120362.3	346.9	-	-					Residual	59.23	7.70	-	-
	VegArea d282v295	1292.6	32.4	1.50	2.2E-01	Cyanotype	245.3	15.7	0.03	8.7E-01	34.65	3.99	9.40	2.2E-03	Cyanotype	0.14	0.38	0.12	7.3E-01
						Cyano x E	1708.7	41.3	8.17	4.3E-03					Cyano x E	0.35	0.59	4.36	3.7E-02
						Site(Block)	8840.5	94.0	127.88	1.2E-29					Site(Block)	3.50	1.87	122.39	1.9E-28
						Residual	121221.9	348.2	-	-					Residual	49.91	7.06	-	-
	VegArea d362v354	1018.7	19.4	7.12	1.0E+00	Cyanotype	0.0	0.0	0.34	5.6E-01	30.99	2.26	25.11	5.8E-07	Cyanotype	0.00	0.00	3.86	5.0E-02
						Cyano x E	19.0	4.4	0.03	8.7E-01					Cyano x E	0.00	0.00	0.00	1.0E+00
						Site(Block)	60.2	7.8	0.31	5.8E-01					Site(Block)	0.45	0.67	17.00	3.7E-05
						Residual	67887.4	260.6	-	-					Residual	36.18	6.02	-	-
	VegArea d449v452	571.1	48.9	1.52	2.2E-01	Cyanotype	37.8	6.1	0.02	8.8E-01	21.82	3.27	498.2	5.8E-09	Cyanotype	0.00	0.05	0.00	9.9E-01
						Cyano x E	110.9	10.5	0.30	5.8E-01					Cyano x E	0.09	0.30	0.41	5.2E-01
						Site(Block)	501.4	22.4	5.56	1.8E-02					Site(Block)	0.53	0.73	10.27	1.4E-03
						Residual	92843.1	304.7	-	-					Residual	65.39	8.09	-	-
	Y1 FloweringDur	86.8	103.6	1.17	1.0E+00	Cyanotype	8.5	2.9	0.32	5.7E-01	8.48	9.42	1.89	2.3E-01	Cyanotype	0.00	0.02	0.00	1.0E+00
						Cyano x E	4.7	2.2	0.20	6.6E-01					Cyano x E	0.07	0.27	1.62	2.0E-01
						Site(Block)	122.5	11.1	63.56	1.6E-15					Site(Block)	0.62	0.79	71.40	2.9E-17
						Residual	3202.7	56.6	-	-					Residual	14.58	3.82	-	-
	FloweringDur	254.8	120.1	0.46	5.0E-01	Cyanotype	17.6	4.2	1.13	2.9E-01	15.63	10.06	0.44	1.0E+00	Cyanotype	0.02	0.14	0.83	3.6E-01
						Cyano x E	0.0	0.0	0.00	1.0E+00					Cyano x E	0.00	0.00	0.00	1.0E+00
						Site(Block)	313.3	17.7	83.42	6.6E-20					Site(Block)	0.67	0.82	77.97	1.0E-18
						Residual	6402.0	80.0	-	-					Residual	14.58	3.82	-	-
	Y1 FloralCount	47.3	197.4	0.34	5.6E-01	Cyanotype	0.0	0.0	0.00	1.0E+00	5.76	12.21	10.96	1.0E+00	Cyanotype	0.00	0.00	0.00	1.0E+00
						Cyano x E	222.1	14.9	8.74	3.1E-03					Cyano x E	0.32	0.56	8.17	4.2E-03
						Site(Block)	529.0	23.0	48.50	3.3E-12					Site(Block)	1.29	1.14	71.55	2.7E-17
						Residual	17577.0	132.6	-	-					Residual	30.36	5.51	-	-
	TotalFloral	422.7	229.8	0.37	1.0E+00	Cyanotype	0.0	0.0	0.00	1.0E+00	19.87	13.05	50.23	1.0E+00	Cyanotype	0.00	0.00	0.00	1.0E+00
						Cyano x E	233.1	15.3	3.45	6.3E-02					Cyano x E	0.17	0.41	2.06	1.5E-01
						Site(Block)	895.9	29.9	31.42	2.1E-08					Site(Block)	1.12	1.06	41.74	1.0E-10
						Residual	43266.1	208.0	-	-					Residual	42.49	6.52	-	-

Table S2.8. (Continued)

		Raw Data									Square root Transformed Data								
Model	Trait (SG Pop)	Mean STL	Mean GFL	F (Site) (Year)	p-value	Random effect	Var	St. Dev	LRT	p-value	Mean STL	Mean GFL	F (Site) (Year)	p-value	Random effect	Var	St. Dev	LRT	p-value
Year + Site + (1 | Genotype) + (1 | Site/Block) + (1 | Site:Genotype) + (1 | Year:Genotype) + (1 | Site:Year:Genotype)	Flowering Dur	127.6	85.6	110.83	5.7E-25	Genotype	167.1	12.9	8.20	4.2E-03	10.67	8.14	39.85	3.7E-10	Genotype	1.03	1.01	15.85	6.9E-05
						G x E	0.0	0.0	0.00	1.0E+00					G x E	0.00	0.00	0.00	1.0E+00
				0.86	1.0E+00	G x Y	0.0	0.0	0.00	1.0E+00			0.49	1.0E+00	G x Y	0.00	0.00	0.00	1.0E+00
						G x E x Y	2107.2	45.9	1309.5	9E-287					G x E x Y	9.00	3.00	1228.6	3E-269
						Site(Block)	49.2	7.0	103.60	2.5E-24					Site(Block)	0.23	0.47	117.66	2.1E-27
						Residual	1297.5	36.0	-	-					Residual	5.57	2.36	-	-
	TotalFloral	201.5	161.9	344.86	1.4E-70	Genotype	0.0	0.0	0.00	1.0E+00	11.98	10.24	260.58	7.6E-55	Genotype	0.00	0.00	0.00	1.0E+00
						G x E	0.0	0.0	0.00	9.9E-01					G x E	0.00	0.00	0.00	1.0E+00
				0.52	1.0E+00	G x Y	0.0	0.0	4.00	4.5E-02			0.26	1.0E+00	G x Y	0.00	0.00	0.00	1.0E+00
						G x E x Y	23012.5	151.7	2028.9	0.0E+00					G x E x Y	39.82	6.31	2552.7	0.0E+00
						Site(Block)	203.7	14.3	47.14	6.6E-12					Site(Block)	0.45	0.67	101.33	7.8E-24
						Residual	8419.1	91.8	-	-					Residual	10.68	3.27	-	-
Year + Site + (1 | Cyanotype) + (1 | Site/Block) + (1 | Site:Cyanotype) + (1 | Year:Cyanotype) + (1 | Site:Year:Cyanotype)	Flowering Dur	127.8	72.1	0.49	5.0E-01	Cyanotype	0.0	0.0	0.00	1.0E+00	10.71	7.33	0.06	8.2E-01	Cyanotype	0.00	0.00	0.00	1.0E+00
						Cyano x E	0.0	0.0	0.00	1.0E+00					Cyano x E	0.00	0.00	0.00	1.0E+00
				0.29	1.0E+00	Cyano x Y	0.0	0.0	0.05	8.1E-01			3.65	6.2E-02	Cyano x Y	0.00	0.00	0.00	1.0E+00
						Cyano xExY	1632.2	40.4	1697.0	0.0E+00					Cyano xExY	5.93	2.43	1315.21	5E-288
						Site(Block)	53.4	7.3	57.94	2.7E-14					Site(Block)	0.24	0.49	57.98	2.6E-14
						Residual	2412.7	49.1	-	-					Residual	11.23	3.35	-	-
	TotalFloral	205.2	127.9	3.32	9.2E-02	Cyanotype	0.1	0.4	0.00	1.0E+00	12.11	8.82	2.08	1.7E01	Cyanotype	0.00	0.00	0.00	1.0E+00
						Cyano x E	0.0	0.0	0.00	1.0E+00					Cyano x E	0.00	0.00	0.02	8.9E-01
				1.45	2.5E-01	Cyano x Y	0.0	0.2	0.01	9.1E-01			1.42	6.5E-01	Cyano x Y	0.00	0.00	0.01	9.0E-01
						Cyano xExY	15796.2	125.7	2090.8	0.0E+00					Cyano xExY	29.10	5.39	2520.08	0.0E+00
						Site(Block)	201.8	14.2	19.15	1.2E-05					Site(Block)	0.44	0.66	36.70	1.4E-09
						Residual	19072.1	138.1	-	-					Residual	27.42	5.24	-	-

Table S2.9. QTL locations for additional fitness traits not depicted in Figure 2.3, as well as transformed data QTLs.

Site-Population	Fitness trait	Linkage group	Refined QTL Position‡	1-LOD Drop Interval
DMN-DG	sqrt_VegArea_d120	4	141.203	111.86 - 185.91
		16	228.148	200.50 - 232.63
	sqrt_VegArea_d339	10	176.377	153.50 - 210.94
		14	5.263	0.00 - 35.62
	sqrt_Y2_WinterSurvival	14	59.920	48.86 - 80.55
	FloweringDuration	10	176.377	164.78 - 215.93
		15	256.441	207.63 - 273.98
	sqrt_Y1_FloweringDur	15	256.686	207.63 - 260.75
	sqrt_Y2_FloweringDur	7	84.135	75.67 - 87.50
	sqrt_FloweringDuration	10	176.377	164.78 - 215.93
		15	258.551	207.63 - 273.98
	TotalFloral	10	206.973	164.78 - 215.93
		15	249.485	216.80 - 295.51
	sqrt_Y1_FloralCount	5	147.083	26.60 - 156.31
		10	176.377	146.94 - 215.93
		13	280.535	258.18 - 384.24
		15	256.686	214.41 - 273.98
	sqrt_Y2_FloralCount	8	290.733	241.68 - 379.79
		11	151.686	130.75 - 198.11
		14	6.847	5.26 - 24.43
	sqrt_TotalFloral	10	166.255	157.53 - 210.94
		13	391.818	263.50 - 423.62
		15	277.716	216.80 - 295.51
GFL-DG	sqrt_VegArea_d235	15	27.884	0.00 - 37.45
	sqrt_Lifespan	15	89.352	20.36 - 150.94
	FloweringDuration	10	168.000	164.78 - 173.33
		12	333.427	225.23 - 351.83
		15	80.604	66.34 - 85.43
		16	267.417	257.82 - 362.16
	sqrt_Y1_FloweringDur	10	169.077	164.78 - 173.33
		12	236.823	225.23 - 351.83
		15	80.604	0.00 - 110.28
		16	267.417	257.82 - 283.42
	sqrt_FloweringDuration	10	169.077	164.78 - 173.33
		12	236.823	225.23 - 347.69
		15	80.604	0.00 - 85.43
		16	267.417	257.82 - 283.42
	TotalFloral	10	166.255	164.78 - 176.79
		12	333.427	330.32 - 351.83
		15	79.665	0.00 - 85.43
		16	269.813	245.24 - 283.42
	sqrt_Y1_FloralCount	10	168.000	164.78 - 176.79
		12	333.427	329.63 - 351.83
		15	49.841	0.00 - 58.08
		16	262.156	257.82 - 272.47
	sqrt_TotalFloral	10	168.000	164.78 - 176.38
		12	333.427	329.63 - 351.83
		15	49.841	46.88 - 80.39
		16	262.156	257.82 - 273.06

STL-SG	VegArea_d282	8	515.505	512.76	-	519.44
	sqrt_VegArea_d282	8	515.505	491.01	-	519.44
	sqrt_VegArea_d362	15	161.331	119.87	-	175.60
	FloweringDuration	13	121.105	78.16	-	134.38
		15	117.433	23.86	-	170.49
	sqrt_Y1_FloweringDur	13	121.105	113.82	-	134.38
		15	126.639	119.87	-	175.60
	sqrt_Y2_FloweringDur	15	0.000	0.00	-	25.65
	sqrt_FloweringDuration	13	121.105	116.96	-	134.38
	TotalFloral	15	154.786	124.09	-	168.77
	sqrt_Y1_FloralCount	13	134.377	87.92	-	159.62
		15	169.022	124.09	-	170.49
	sqrt_Y2_FloralCount	15	117.433	98.72	-	159.63
	sqrt_TotalFloral	15	38.029	0.00	-	44.32
GFL-SG	VegArea_d354	15	26.789	19.64	-	58.70
	VegArea_d452	15	26.789	13.47	-	38.03
	sqrt_VegArea_d200	8	91.120	86.40	-	106.07
		15	170.488	159.63	-	176.59
	sqrt_VegArea_d295	15	26.789	10.32	-	29.61
	sqrt_VegArea_d354	15	26.789	19.64	-	29.61
	sqrt_VegArea_d452	15	26.789	19.64	-	32.31
	sqrt_Lifespan	8	105.367	63.38	-	109.59
		15	26.789	19.64	-	83.97
	FloweringDuration	8	101.553	63.38	-	163.10
		10	154.732	121.58	-	162.00
		15	16.166	0.00	-	45.61
	sqrt_Y1_FloweringDur	8	143.939	71.00	-	216.26
		10	169.512	151.11	-	176.64
		15	8.430	6.13	-	11.49
	sqrt_FloweringDuration	8	76.500	56.91	-	216.26
		10	154.732	148.89	-	176.64
		15	8.430	6.13	-	17.42
	TotalFloral	1	246.700	239.22	-	287.85
		10	148.892	130.82	-	162.00
		15	5.330	2.56	-	18.39
	sqrt_Y1_FloralCount	6	195.487	179.37	-	285.19
		10	173.517	121.58	-	177.62
		15	4.818	2.56	-	10.32
	sqrt_TotalFloral	1	246.700	239.22	-	287.85
		6	195.487	94.31	-	338.22
		10	154.732	121.58	-	180.38
		15	7.530	2.56	-	10.32

Table S2.10. For each population, results of post-hoc QTL x E analysis for highest LOD markers in Table 2.1, only for traits that were comparably measured across reciprocal sites.

Pop	Trait	Highest LOD Marker	Linkage Group	Mean GFL	Mean DMN	Effect	Test Statistic		p-value	
DG	Vegetative Area (Day 120v119)	13:13646557	4	227.95	752.23	Genotype (DD vs. GG)	F	222.36	4.26E-02	*
						Site	F	1.03	4.96E-01	
						QTLxE	LRT	13.83	**2.00E-04**	***
		10:18587313	15	225.34	752.76	Genotype (DD vs. GG)	F	255.58	3.98E-02	*
						Site	F	0.84	5.28E-01	
						QTLxE	LRT	12.15	**4.92E-04**	***
	Vegetative Area (Day 339v354)	9:23631957	10	2.69	388.75	Genotype (DD vs. GG)	F	67.92	7.68E-02	
						Site	F	0.98	5.03E-01	
						QTLxE	LRT	25.75	**3.88E-07**	****
		3:26735632	14	2.76	405.82	Genotype (DD vs. GG)	F	136.42	5.43E-02	
						Site	F	1.01	4.98E-01	
						QTLxE	LRT	13.93	**1.90E-04**	***
	Flowering Duration (Year 1)	2:46629904	10	100.40	115.28	Genotype (DD vs. GG)	F	1.67	4.19E-01	
						Site	F	0.51	6.06E-01	
						QTLxE	LRT	49.54	**1.94E-12**	****
		12:12548239	12	102.88	115.49	Genotype (DD vs. GG)	F	4.54	2.79E-01	
						Site	F	1.30	4.59E-01	
						QTLxE	LRT	11.94	**5.49E-04**	***
		15:4065533	15	96.26	116.16	Genotype (DD vs. GG)	F	19.73	1.41E-01	
						Site	F	1.65	4.22E-01	
						QTLxE	LRT	4.91	**2.67E-02**	*
		4:59781115	15	99.91	115.43	Genotype (DD vs. GG)	F	12.15	1.78E-01	
						Site	F	0.35	6.61E-01	
						QTLxE	LRT	3.76	5.24E-02	(NS)
		3:16346453	16	100.27	115.53	Genotype (DD vs. GG)	F	4.30	2.86E-01	
						Site	F	0.93	5.11E-01	
						QTLxE	LRT	19.29	**1.12E-05**	****
	Floral Count (Year 1)	2:43914108	10	181.92	171.45	Genotype (DD vs. GG)	F	0.15	7.64E-01	
						Site	F	0.06	8.41E-01	
						QTLxE	LRT	31.33	**2.18E-08**	****
		2:46629904	10	180.59	170.90	Genotype (DD vs. GG)	F	0.09	8.10E-01	
						Site	F	0.10	8.02E-01	
						QTLxE	LRT	45.61	**1.44E-11**	****

		8:58495823	12	182.72	173.96	Genotype (DD vs. GG)	F	0.16	7.57E-01	
						Site	F	0.32	6.73E-01	
						QTLxE	LRT	23.40	**1.31E-06**	****
		2:24999810	13	179.18	173.73	Genotype (DD vs. GG)	F	0.07	8.33E-01	
						Site	F	0.00	9.90E-01	
						QTLxE	LRT	5.75	**1.65E-02**	*
		15:952339	15	172.15	173.01	Genotype (DD vs. GG)	F	0.00	9.77E-01	
						Site	F	1.52	4.34E-01	
						QTLxE	LRT	30.29	**3.72E-08**	****
		9:48242797	15	176.79	171.39	Genotype (DD vs. GG)	F	0.35	6.61E-01	
						Site	F	1.71	4.15E-01	
						QTLxE	LRT	2.08	1.50E-01	(NS)
		3:16346453	16	178.25	172.23	Genotype (DD vs. GG)	F	0.09	8.17E-01	
						Site	F	0.74	5.49E-01	
						QTLxE	LRT	19.82	**8.52E-06**	****
SG	Vegetative Area (Day 282v295)	3:3834184	3	30.26	1291.71	Genotype (SS vs. GG)	F	2083.19	1.60E-02	*
						Site	F	2.05	3.88E-01	
						QTLxE	LRT	0.51	4.77E-01	(NS)
		15:3511683	15	26.94	1297.45	Genotype (SS vs. GG)	F	1401.19	2.01E-02	*
						Site	F	0.30	6.82E-01	
						QTLxE	LRT	0.61	4.33E-01	(NS)
	Vegetative Area (Day 362v354)	9:53788760	15	18.42	1023.13	Genotype (SS vs. GG)	F	608.17	2.64E-02	*
						Site	F	1.65	4.21E-01	
						QTLxE	LRT	6.81	**9.08E-03**	**
	Flowering Duration (Year 1)	1:27623384	7	106.43	87.76	Genotype (SS vs. GG)	F	18.94	1.44E-01	
						Site	F	0.49	6.12E-01	
						QTLxE	LRT	3.67	5.55E-02	(NS)
		15:4312433	8	104.79	87.29	Genotype (SS vs. GG)	F	3.78	3.03E-01	
						Site	F	0.06	8.52E-01	
						QTLxE	LRT	25.97	**3.47E-07**	****
		6:22247148	10	105.89	86.46	Genotype (SS vs. GG)	F	15.48	1.59E-01	
						Site	F	2.48	3.60E-01	
						QTLxE	LRT	2.38	1.23E-01	(NS)
		13:13894869	13	105.81	87.70	Genotype (SS vs. GG)	F	13.89	1.67E-01	
						Site	F	0.72	5.53E-01	
						QTLxE	LRT	6.73	**9.46E-03**	**

	10:725661	15	104.98	87.68	Genotype (SS vs. GG)	F	5.41	2.59E-01	
					Site	F	1.27	4.62E-01	
					QTLxE	LRT	10.10	**1.49E-03**	**
	1:95517659	15	105.18	87.55	Genotype (SS vs. GG)	F	7.51	2.23E-01	
					Site	F	2.03	3.89E-01	
					QTLxE	LRT	10.52	**1.18E-03**	**
Floral Count (Year 1)	12:45616154	1	206.23	48.28	Genotype (SS vs. GG)	F	49.62	8.98E-02	
					Site	F	0.54	5.96E-01	
					QTLxE	LRT	10.22	**1.39E-03**	**
	6:18418652	6	212.11	47.60	Genotype (SS vs. GG)	F	20.07	1.40E-01	
					Site	F	0.48	6.15E-01	
					QTLxE	LRT	18.91	**1.37E-05**	****
	6:22247148	10	209.92	45.31	Genotype (SS vs. GG)	F	17.84	1.48E-01	
					Site	F	0.89	5.18E-01	
					QTLxE	LRT	14.99	**1.08E-04**	***
	11:53202884	13	201.67	48.82	Genotype (SS vs. GG)	F	123.81	5.71E-02	
					Site	F	0.03	8.89E-01	
					QTLxE	LRT	2.79	9.51E-02	(NS)
	10:623367	15	195.99	50.78	Genotype (SS vs. GG)	F	4.39	2.84E-01	
					Site	F	0.72	5.51E-01	
					QTLxE	LRT	138.07	**7.03E-32**	****
	15:17467828 *(used marker 7:34111585)*	15	210.76	47.19	Genotype (SS vs. GG)	F	96.42	6.52E-02	
					Site	F	3.24	3.23E-01	
					QTLxE	LRT	1.49	2.23E-01	(NS)

p< *0.05, **0.01, ***0.001, ****0.0001 (significance thresholds)
NS = not significant at p=0.05 level

Table S2.11. Cyanotype frequencies in wild populations near the common garden experiments in this study.

Nearest City	Latitude	N	acli	acLi	Acli	AcLi
Duluth, MN (DMN)	46.8	82*	0.33	0.10	0.38	0.20
St. Louis, MO (STL)	38.6	57*	0.12	0.14	0.47	0.26
Gainesville, FL (GFL)	29.6	62*	0.00	0.05	0.05	0.90
Wausau, WI (WI)	44.9	136**	0.36	0.13	0.40	0.11
St. Louis, MO (STL)	38.6	299**	0.15	0.28	0.42	0.15
New Orleans, LA (LA)	30.2	141**	0.01	0.07	0.06	0.86

* Wright & Olsen *(in prep)*

** (Kooyers and Olsen, 2012)

Table S2.12. Summary of FAWN[§] monthly above- and below-ground temperatures and rainfall at the GFL common gardens in Citra, FL. Temperatures (T) are reported in degrees Fahrenheit.

Month-Year*	N (# obs)	T min(avg) 60cm	T max(avg) 60cm	T avg 60cm	Tsoil min(avg) -10cm	Tsoil max(avg) -10cm	Tsoil avg -10cm	2m Rain tot (in)	2m Rain max over 15min(in)
Oct-16	2976	40.97	92.37	72.3	66.52	95.07	79.75	1.43	0.08
Nov-16	2880	33.06	86.43	63.93	58.73	82.81	71.89	0.03	0.03
Dec-16	2976	28.97	86.49	62.96	51.01	77.81	68.12	0.74	0.17
Jan-17	2976	29.03	85.03	60.2	46.85	75.56	65.3	1.32	0.08
Feb-17	2688	33.01	87.91	63.36	56.16	81.68	67.93	1.48	0.24
Mar-17	2976	33.48	90.81	64.15	52.29	85.89	71	1.33	0.17
Apr-17	2880	42.29	97.02	71.63	63.7	91.47	78.8	3.8	0.89
May-17	2976	49.42	101.89	76.79	68.92	97.72	83.85	3.2	0.32
Jun-17	2880	64.94	95.94	78.5	72	100.56	84.18	12.69	0.88
Jul-17	2976	70.16	97.63	80.74	78.19	103.87	87.74	6.58	0.64
Aug-17	2976	70.43	96.78	81.22	78.13	103.06	87.47	7.74	0.59
Sep-17	2880	63.43	96.06	78.6	71.73	97.14	85.24	10.64	0.47
Oct-17	2976	37.61	94.5	72.75	61.11	93.13	79.02	2.18	0.7
Nov-17	2874	41.76	85.3	64.5	58.28	82.47	70.71	3.09	0.18
Dec-17	2976	31.04	83.44	58.74	45.36	77.02	64.21	1.6	0.24
Jan-18	2976	23.88	81.45	51.52	38.21	71.28	55.88	5.23	0.39
Feb-18	2688	39.67	87.96	67.73	50.7	84.88	70.4	2.51	0.4
Mar-18	2976	29.15	85.59	60.98	55.72	82.2	68.92	3.16	0.35
Apr-18	2880	44.33	88.02	68.63	59.45	90.86	75.06	6.72	0.55
May-18	2976	52.07	96.37	75.25	71.02	95.81	81.25	8.09	0.7
Jun-18	2880	67.6	96.53	80.02	75.11	104.29	86.68	3.37	0.55
Jul-18	2976	69.93	96.12	79.84	74.03	102.76	85.35	6.51	0.51
Aug-18	2976	70.59	96.15	80.41	77.43	101.95	87.41	6.28	0.84
Sep-18	2880	69.62	96.28	80.81	77.86	97.92	86.84	4.08	0.39
Oct-18	2976	48.94	95.7	74.3	63.66	92.7	81	0.86	0.23

[§]Florida Automated Weather Network (https://fawn.ifas.ufl.edu/data/); the University of Florida-Gainesville's Plant Science Research and Educational Unit (Citra FAWN station)

* Periods of high plant mortality in common gardens are highlighted.

Table S2.13. Herbivory measurements (three metrics) for cyanotype groups in the common garden environments.

Site-Pop	Sampling Date	Cyano-type	N	Avg Total Damage	St. Dev	Avg High Damage	St. Dev	Avg Weighted Damage	St. Dev
DMN-DG	August (Year 1)	acli	20	0.203	0.060	0.031	0.026	0.063	0.022
		acLi	98	0.155	0.056	0.018	0.020	0.045	0.018
		Acli	102	0.170	0.068	0.019	0.025	0.049	0.022
		AcLi	282	0.164	0.065	0.021	0.024	0.048	0.021
	September (Year 1)	acli	20	0.206	0.084	0.026	0.026	0.061	0.027
		acLi	98	0.217	0.077	0.035	0.032	0.067	0.026
		Acli	102	0.195	0.078	0.029	0.028	0.060	0.027
		AcLi	282	0.203	0.078	0.029	0.030	0.061	0.026
	May (Year 2)	acli	20	0.155	0.059	0.034	0.032	0.053	0.026
		acLi	98	0.200	0.081	0.043	0.040	0.066	0.030
		Acli	102	0.189	0.073	0.039	0.035	0.062	0.028
		AcLi	281	0.197	0.074	0.048	0.047	0.067	0.031
	June (Year 2)	acli	20	0.234	0.098	0.034	0.039	0.070	0.033
		acLi	98	0.229	0.089	0.036	0.031	0.070	0.029
		Acli	102	0.210	0.085	0.030	0.031	0.062	0.027
		AcLi	282	0.207	0.080	0.037	0.036	0.065	0.029
	Mean DMN (gardens)			**0.196**		**0.032**		**0.060**	
STL-SG	August (Year 1)	acli	33	0.213	0.070	0.071	0.052	0.080	0.035
		acLi	116	0.229	0.087	0.085	0.054	0.087	0.038
		Acli	91	0.209	0.081	0.078	0.056	0.081	0.039
		AcLi	259	0.210	0.084	0.072	0.049	0.079	0.036
	September (Year 1)	acli	33	0.388	0.101	0.173	0.074	0.159	0.051
		acLi	116	0.399	0.091	0.173	0.066	0.159	0.044
		Acli	91	0.405	0.100	0.171	0.068	0.160	0.045
		AcLi	259	0.415	0.089	0.183	0.065	0.167	0.042
	May (Year 2)	acli	33	0.440	0.125	0.040	0.052	0.121	0.039
		acLi	115	0.443	0.120	0.026	0.027	0.119	0.035
		Acli	91	0.417	0.132	0.032	0.046	0.114	0.041
		AcLi	258	0.436	0.133	0.030	0.045	0.118	0.040
	June (Year 2)	acli	33	0.384	0.087	0.025	0.032	0.103	0.026
		acLi	115	0.379	0.117	0.028	0.034	0.103	0.034
		Acli	91	0.386	0.131	0.026	0.028	0.104	0.037
		AcLi	259	0.406	0.132	0.028	0.034	0.110	0.038
	Mean STL (gardens)			**0.289**		**0.081**		**0.100**	
STL-Wild	Year 2	acli	23	0.473	0.178	0.161	0.102	0.176	0.083
		acLi	14	0.468	0.134	0.105	0.081	0.149	0.054
		Acli	33	0.451	0.164	0.140	0.070	0.163	0.064
		AcLi	34	0.413	0.147	0.110	0.083	0.143	0.063
	Mean STL (wild)			**0.451**		**0.129**		**0.158**	

GFL on next page

GFL-DG	March (Year 1)	acli	20	0.026	0.026	0.003	0.008	0.008	0.008
		acLi	98	0.044	0.042	0.007	0.014	0.013	0.014
		Acli	101	0.039	0.038	0.008	0.017	0.013	0.015
		AcLi	282	0.047	0.045	0.008	0.016	0.015	0.016
	May (Year 1)	acli	20	0.027	0.035	0.012	0.022	0.012	0.017
		acLi	98	0.024	0.033	0.008	0.015	0.009	0.013
		Acli	101	0.018	0.024	0.005	0.012	0.007	0.010
		AcLi	282	0.020	0.028	0.006	0.016	0.007	0.013
GFL-SG	March (Year 1)	acli	33	0.031	0.036	0.009	0.018	0.012	0.015
		acLi	117	0.032	0.042	0.011	0.021	0.012	0.017
		Acli	91	0.022	0.032	0.008	0.021	0.008	0.015
		AcLi	258	0.027	0.044	0.009	0.025	0.010	0.021
	May (Year 1)	acli	33	0.012	0.020	0.003	0.008	0.004	0.009
		acLi	117	0.022	0.035	0.006	0.015	0.007	0.012
		Acli	91	0.017	0.024	0.005	0.011	0.006	0.009
		AcLi	258	0.017	0.026	0.004	0.011	0.006	0.009
Mean GFL (gardens)				**0.027**		**0.007**		**0.009**	
GFL-Wild	Year 2	*unknown*		0.024	0.007	0.003	0.015	0.033	0.011
Mean GFL (wild)				**0.024**		**0.003**		**0.033**	

SUPPLEMENTARY FIGURES

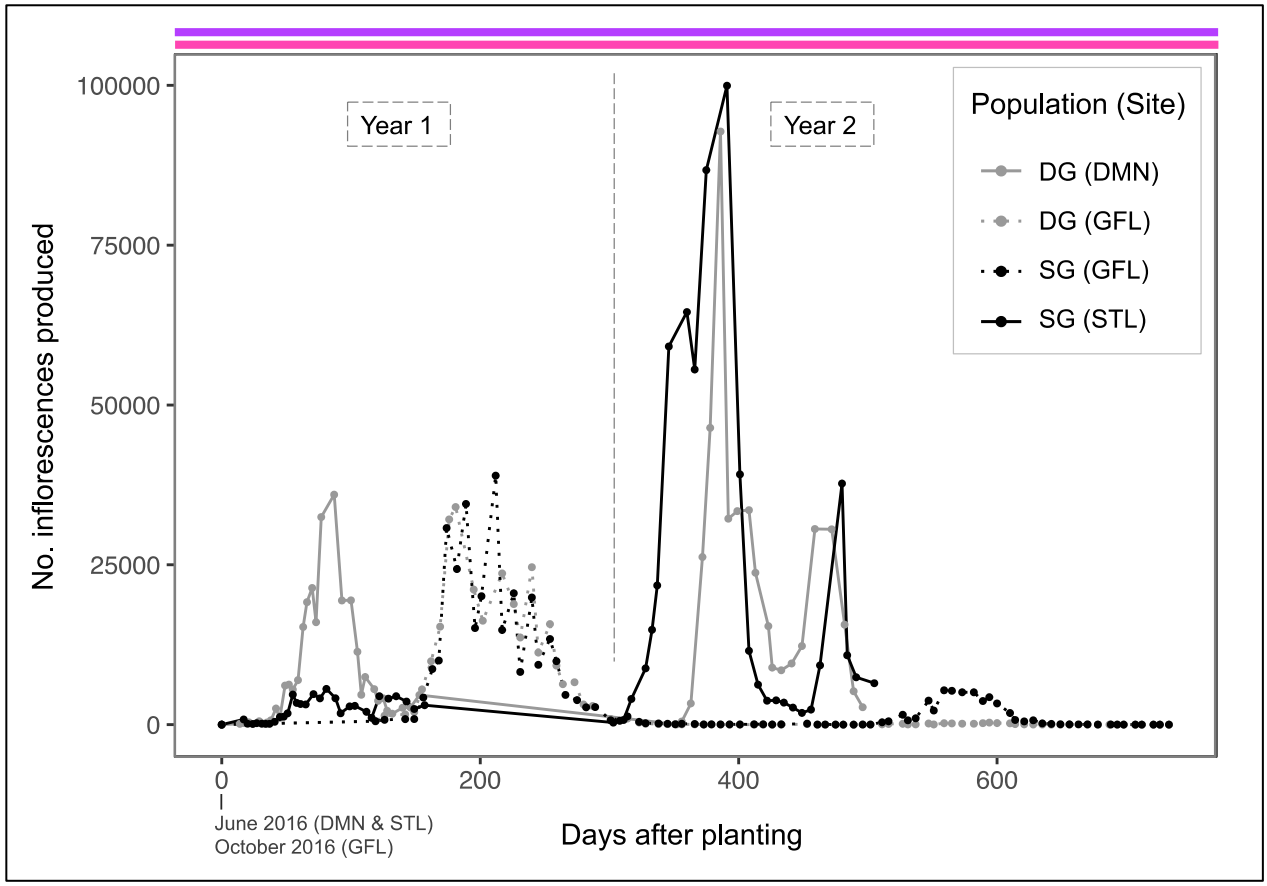


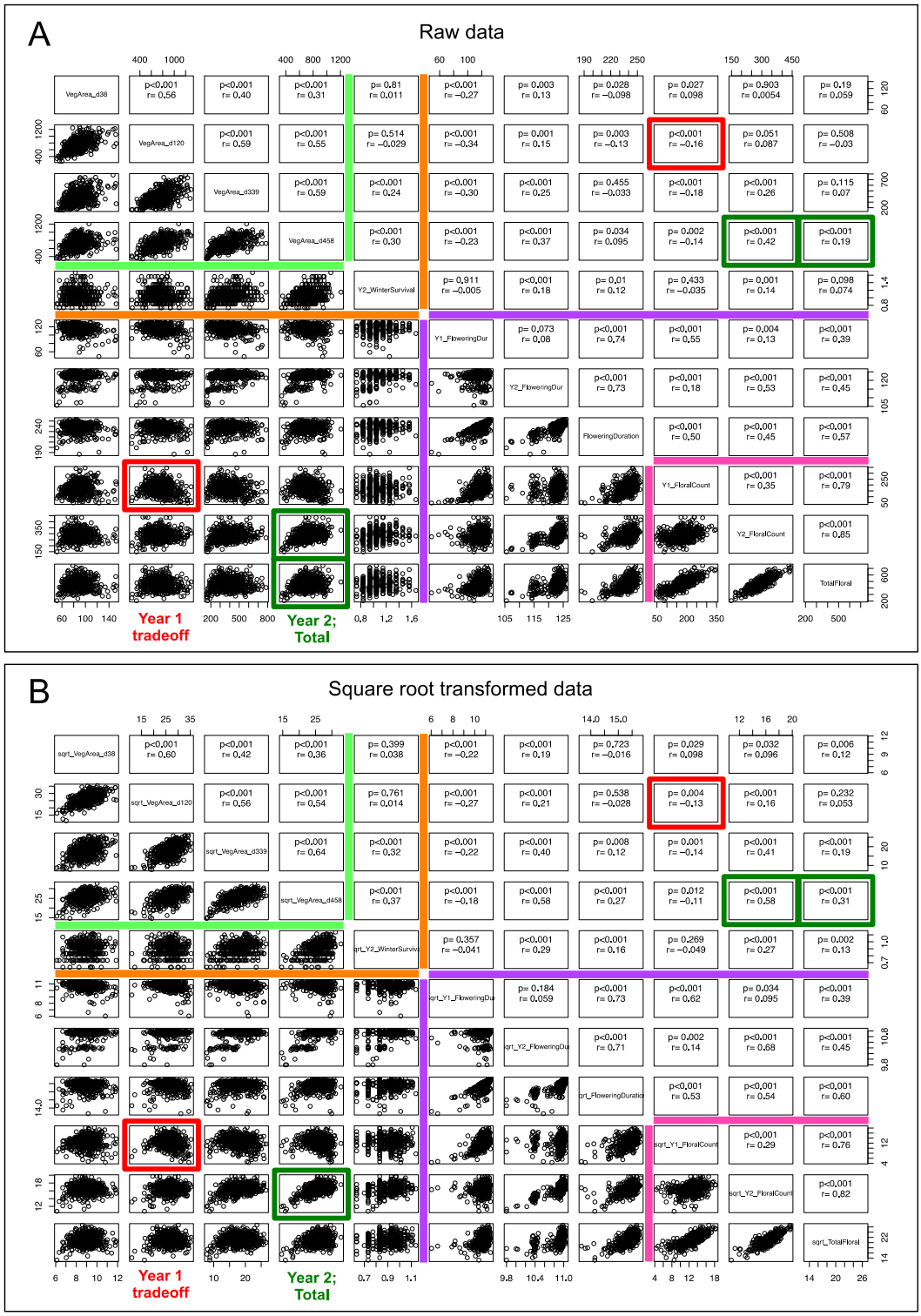
Figure S2.1. The total number of inflorescences produced over time in each of the four common garden experiments, including reciprocal comparisons for both F_2 mapping populations (DG and SG). The x-axis corresponds to the number of days since common garden establishment at each site, which occurred in different months (June and October) of 2016. (Table S2.1, Table S2.2)**.**

SUPPLEMENTARY FIGURE LEGENDS

Large Composite Figures

Figures S2.2-S2.5. Within-site, pairwise trait correlations for all life history traits measured in the DMN (Figure S2.2), STL (Figure S2.3), GFL-DG (Figure S2.4), and GFL-SG (Figure S2.5) common gardens (Pearson correlation tests (*r*), FDR corrected p-values). Analyses were performed with genotypic estimates from within-site trait models that were built using both raw (non-transformed) data (**A**) and square root transformed data (**B**); each data point corresponds to one F_2 genotype. Colored lines between plots draw distinctions between vegetative area (green), survival (orange), flowering duration (purple), and floral count (pink) traits. Colored boxes around plots indicate examples of negative (red), non-significant (blue), and positive (green) correlations between different aspects of life history (growth vs. reproductive output). Also shown are the results of complementary principal components analyses (PCA) that identify major axes of correlated fitness trait variation (PC1, PC2 and PC3) within each common garden site. Within each PC, green vs. red shading denotes anti-correlation between traits, with the degree of shading indicating the magnitude of a given trait's association in the PC.

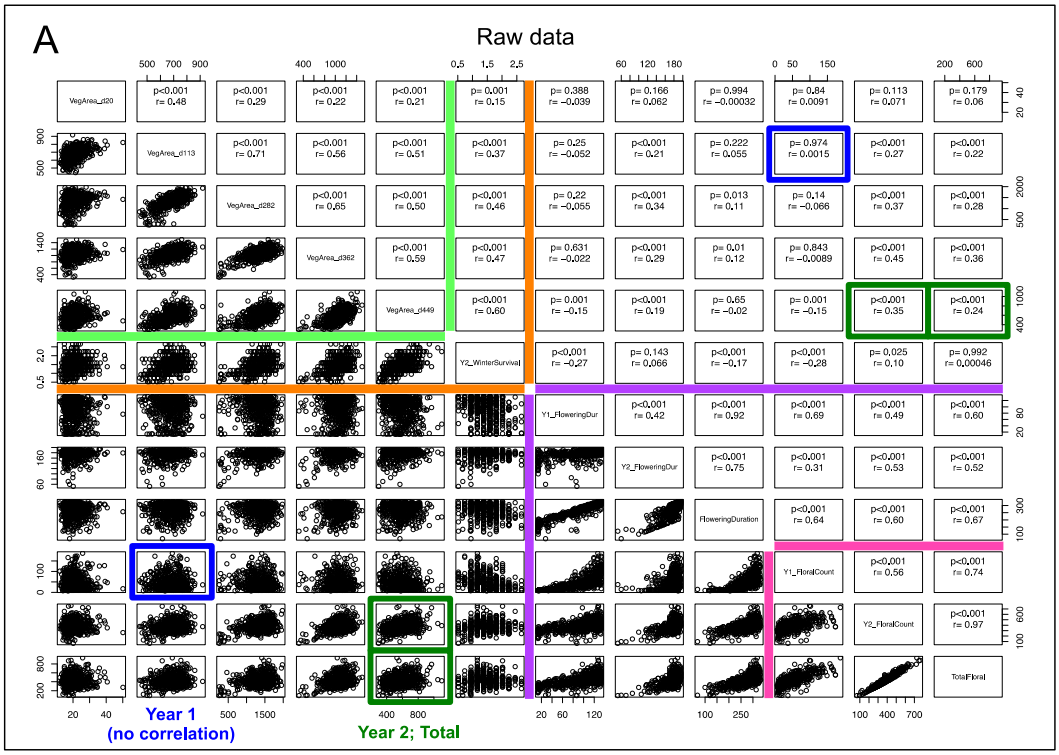
Figures S2.6-S2.7. Regions of genomic co-localization among fitness QTLs in the DG and SG populations (Figure S2.6 (**a-f**) and Figure S2.7 (**a-e**), respectively). For each QTL within each genomic region, phenotypic distributions are shown for homozygotes and heterozygotes, with respect to the native parental alleles, within the common garden environment where the QTL was significant. Means and standard error bars are indicated for each genotype. Additionally, for traits that were comparably measured in each reciprocal environment, interaction plots for post-hoc QTL×E analyses at the highest LOD markers are shown; significant QTL×E interactions, indicating antagonistic pleiotropy, are denoted (p < *0.05, **0.01, ***0.001, ****0.0001; NS = not significant at p=0.05 level) (Table S2.10).



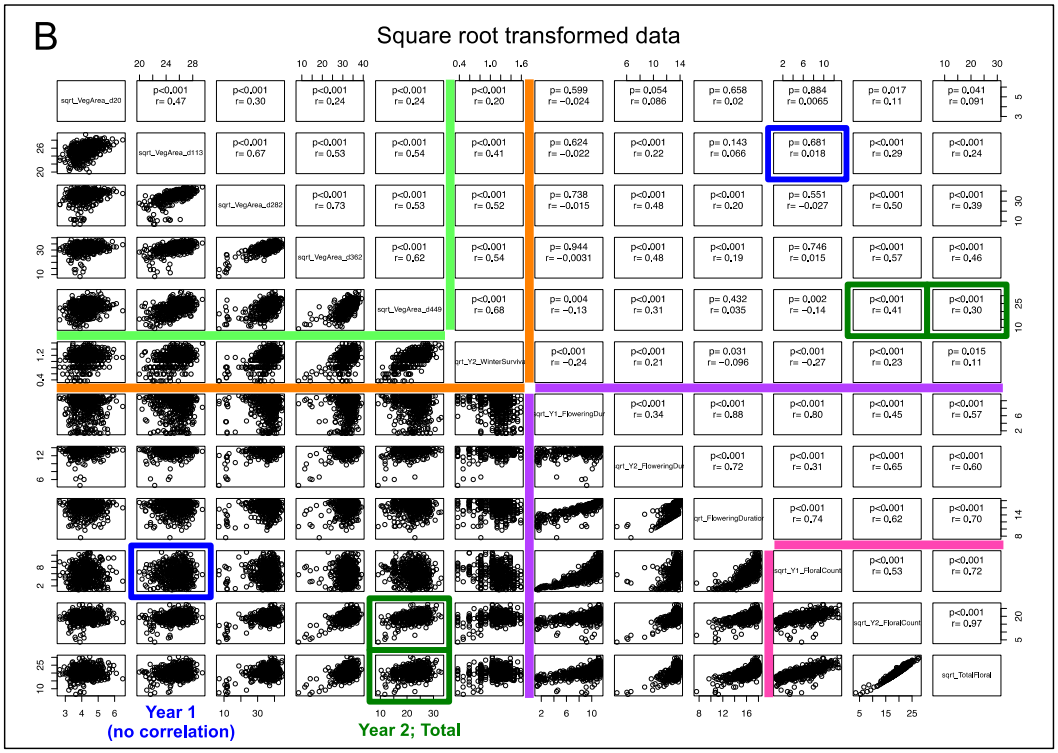
Trait	PC1	PC2	PC3
VegArea_d38	0.03	-0.35	-0.49
VegArea_d120	0.01	-0.46	-0.29
VegArea_d339	0.08	-0.47	0.06
VegArea_d458	0.17	-0.44	0.18
Y2_WinterSurvival	0.10	-0.14	0.60
Y1_FloweringDur	0.28	0.35	-0.01
Y2_FloweringDur	0.37	-0.16	0.22
FloweringDuration	0.45	0.14	0.14
Y1_FloralCount	0.36	0.21	-0.41
Y2_FloralCount	0.42	-0.12	0.06
TotalFloral	0.48	0.04	-0.19
Proportion of Variation Explained	32.40%	27.02%	11.13%

Trait	PC1	PC2	PC3
sqrt_VegArea_d38	0.17	-0.29	-0.55
sqrt_VegArea_d120	0.18	-0.39	-0.40
sqrt_VegArea_d339	0.28	-0.36	0.00
sqrt_VegArea_d458	0.35	-0.31	0.13
sqrt_Y2_WinterSurvival	0.18	-0.11	0.52
sqrt_Y1_FloweringDur	0.13	0.47	-0.10
sqrt_Y2_FloweringDur	0.40	-0.04	0.23
sqrt_FloweringDuration	0.37	0.30	0.07
sqrt_Y1_FloralCount	0.22	0.40	-0.38
sqrt_Y2_FloralCount	0.43	0.02	0.15
sqrt_TotalFloral	0.39	0.24	-0.15
Proportion of Variation Explained	36.56%	25.94%	11.61%

Figure S2.2. DMN

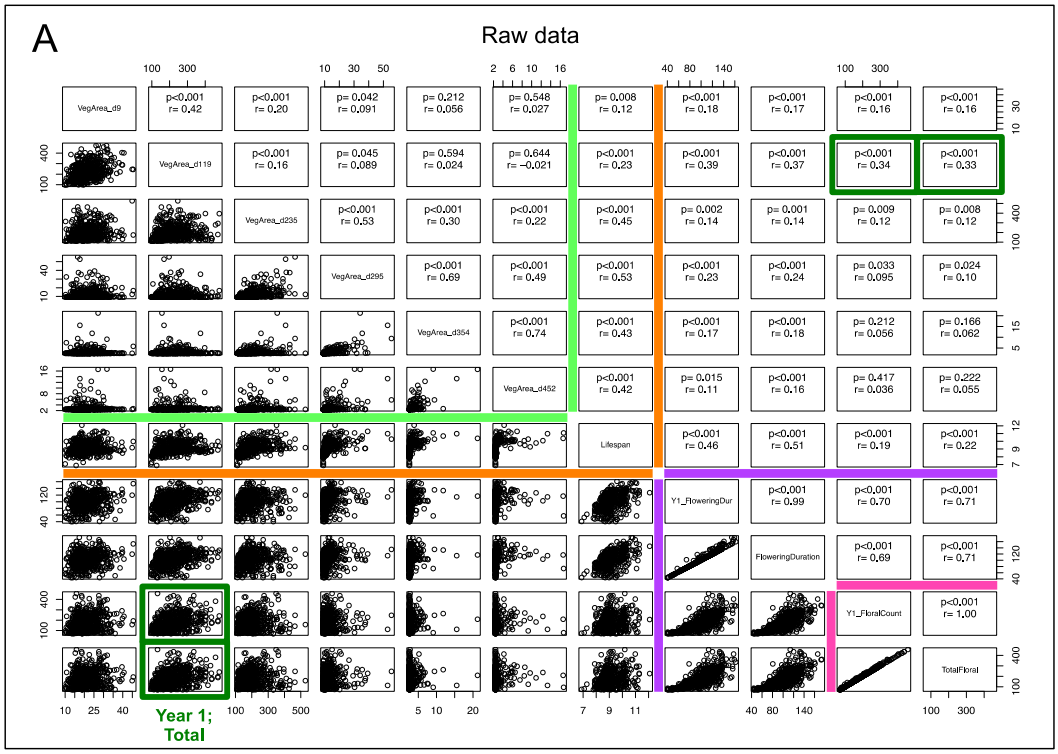


Trait	PC1	PC2	PC3
VegArea_d20	0.10	-0.20	-0.79
VegArea_d113	0.22	-0.35	-0.36
VegArea_d282	0.25	-0.35	-0.07
VegArea_d362	0.26	-0.33	0.13
VegArea_d449	0.19	-0.37	0.25
Y2_WinterSurvival	0.08	-0.39	0.31
Y1_FloweringDur	0.30	0.34	-0.09
Y2_FloweringDur	0.33	0.06	0.09
FloweringDuration	0.36	0.27	-0.02
Y1_FloralCount	0.29	0.31	-0.14
Y2_FloralCount	0.42	0.04	0.17
TotalFloral	0.42	0.12	0.10
Proportion of Variation Explained	37.75%	27.90%	8.75%

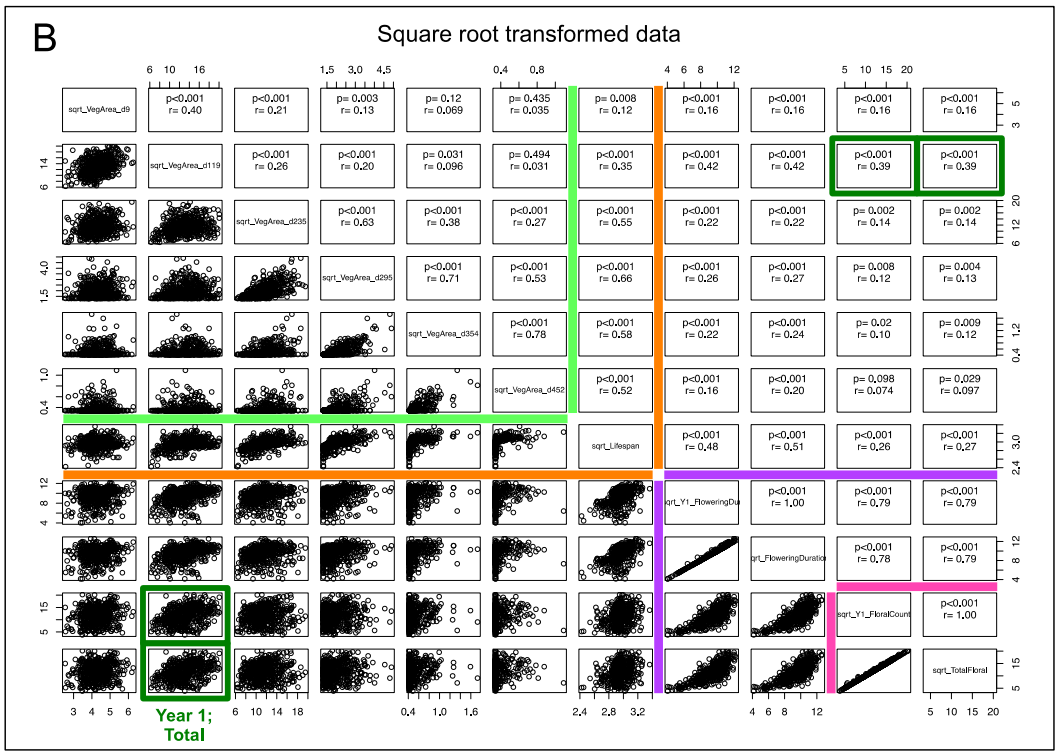


Trait	PC1	PC2	PC3
sqrt_VegArea_d20	0.12	-0.18	-0.77
sqrt_VegArea_d113	0.24	-0.29	-0.42
sqrt_VegArea_d282	0.31	-0.28	-0.02
sqrt_VegArea_d362	0.32	-0.27	0.14
sqrt_VegArea_d449	0.24	-0.34	0.14
sqrt_Y2_WinterSurvival	0.17	-0.38	0.21
sqrt_Y1_FloweringDur	0.23	0.40	-0.16
sqrt_Y2_FloweringDur	0.34	0.05	0.23
sqrt_FloweringDuration	0.32	0.33	-0.01
sqrt_Y1_FloralCount	0.24	0.40	-0.19
sqrt_Y2_FloralCount	0.40	0.08	0.16
sqrt_TotalFloral	0.39	0.17	0.08
Proportion of Variation Explained	41.34%	27.25%	8.89%

Figure S2.3. STL

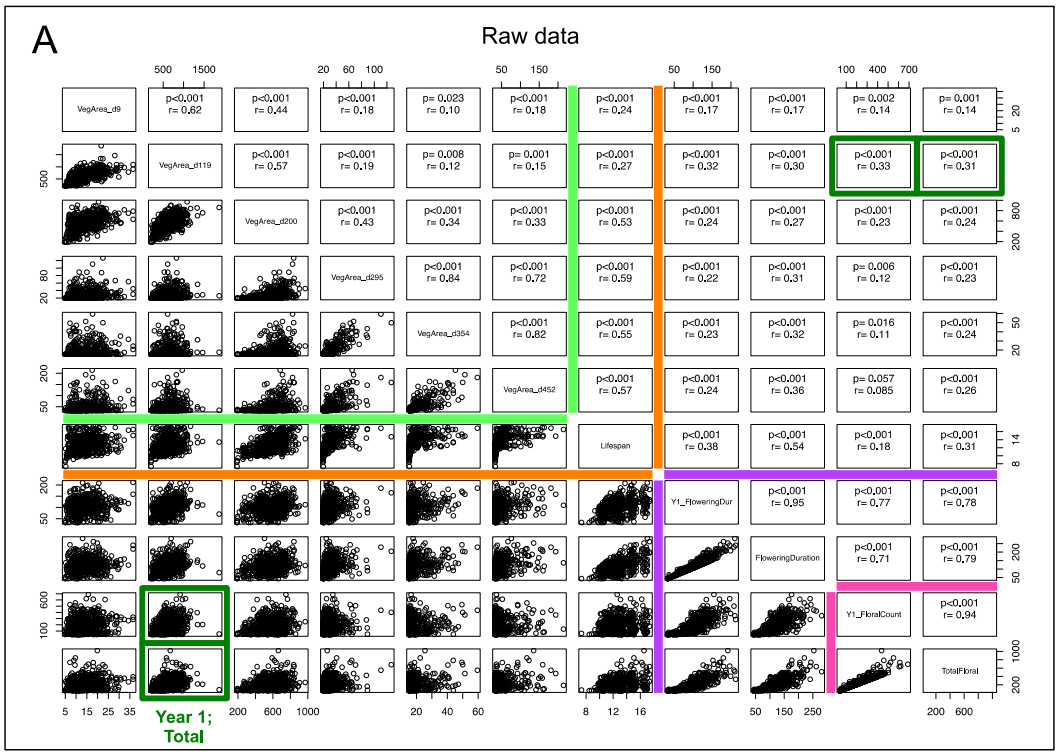


Trait	PC1	PC2	PC3
VegArea_d9	0.15	0.04	-0.68
VegArea_d119	0.23	0.15	-0.52
VegArea_d235	0.20	-0.27	-0.33
VegArea_d295	0.26	-0.43	-0.02
VegArea_d354	0.22	-0.46	0.15
VegArea_d452	0.19	-0.43	0.20
Lifespan	0.32	-0.25	-0.04
Y1_FloweringDur	0.42	0.20	0.12
FloweringDuration	0.42	0.18	0.14
Y1_FloralCount	0.37	0.31	0.16
TotalFloral	0.37	0.30	0.17
Proportion of Variation Explained	38.50%	22.69%	11.43%

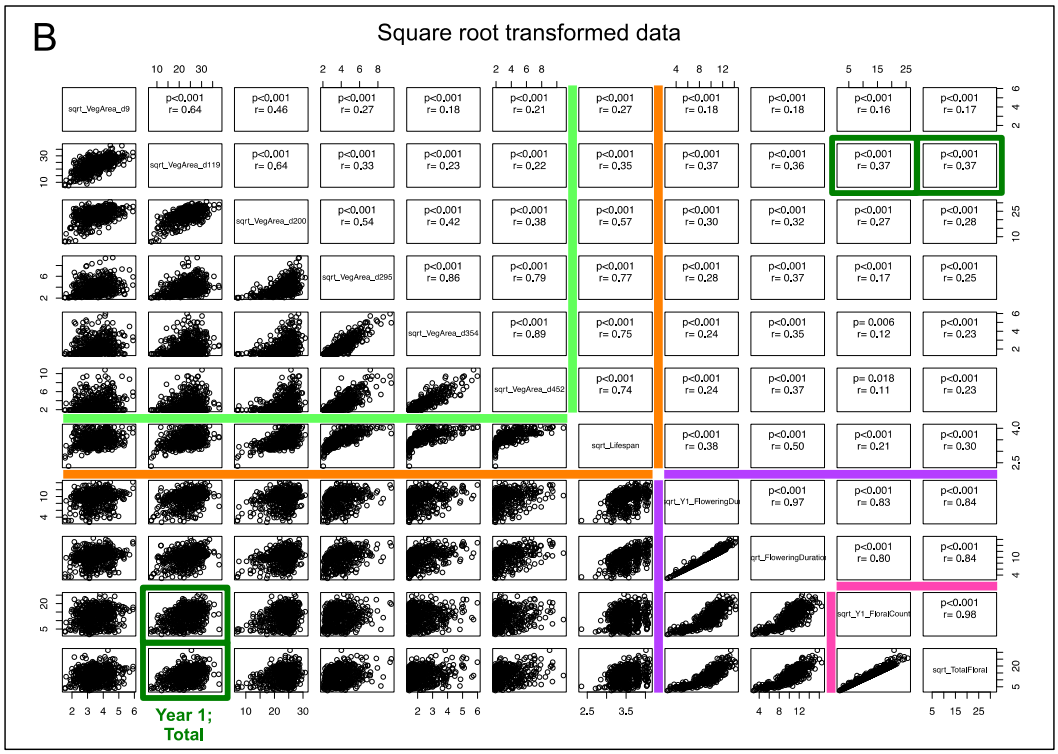


Trait	PC1	PC2	PC3
sqrt_VegArea_d9	0.13	0.03	-0.68
sqrt_VegArea_d119	0.25	0.12	-0.51
sqrt_VegArea_d235	0.24	-0.28	-0.31
sqrt_VegArea_d295	0.28	-0.40	-0.05
sqrt_VegArea_d354	0.26	-0.42	0.19
sqrt_VegArea_d452	0.22	-0.39	0.27
sqrt_Lifespan	0.34	-0.26	-0.01
sqrt_Y1_FloweringDur	0.39	0.25	0.13
sqrt_FloweringDuration	0.40	0.23	0.14
sqrt_Y1_FloralCount	0.35	0.35	0.13
sqrt_TotalFloral	0.35	0.34	0.14
Proportion of Variation Explained	42.74%	23.12%	11.31%

Figure S2.4. GFL-DG



Trait	PC1	PC2	PC3
VegArea_d9	0.18	-0.06	-0.58
VegArea_d119	0.24	0.04	-0.57
VegArea_d200	0.27	-0.17	-0.39
VegArea_d295	0.30	-0.39	0.14
VegArea_d354	0.29	-0.39	0.24
VegArea_d452	0.29	-0.37	0.19
Lifespan	0.32	-0.23	0.02
Y1_FloweringDur	0.35	0.33	0.12
FloweringDuration	0.38	0.25	0.16
Y1_FloralCount	0.30	0.42	0.06
TotalFloral	0.35	0.35	0.13
Proportion of Variation Explained	44.26%	21.29%	14.61%



Trait	PC1	PC2	PC3
sqrt_VegArea_d9	0.19	-0.07	-0.60
sqrt_VegArea_d119	0.26	0.02	-0.56
sqrt_VegArea_d200	0.28	-0.15	-0.37
sqrt_VegArea_d295	0.32	-0.34	0.12
sqrt_VegArea_d354	0.30	-0.36	0.24
sqrt_VegArea_d452	0.30	-0.35	0.24
sqrt_Lifespan	0.34	-0.27	0.10
sqrt_Y1_FloweringDur	0.33	0.35	0.12
sqrt_FloweringDuration	0.35	0.29	0.17
sqrt_Y1_FloralCount	0.29	0.43	0.05
sqrt_TotalFloral	0.32	0.38	0.11
Proportion of Variation Explained	48.63%	22.99%	13.65%

Figure S2.5. GFL-SG

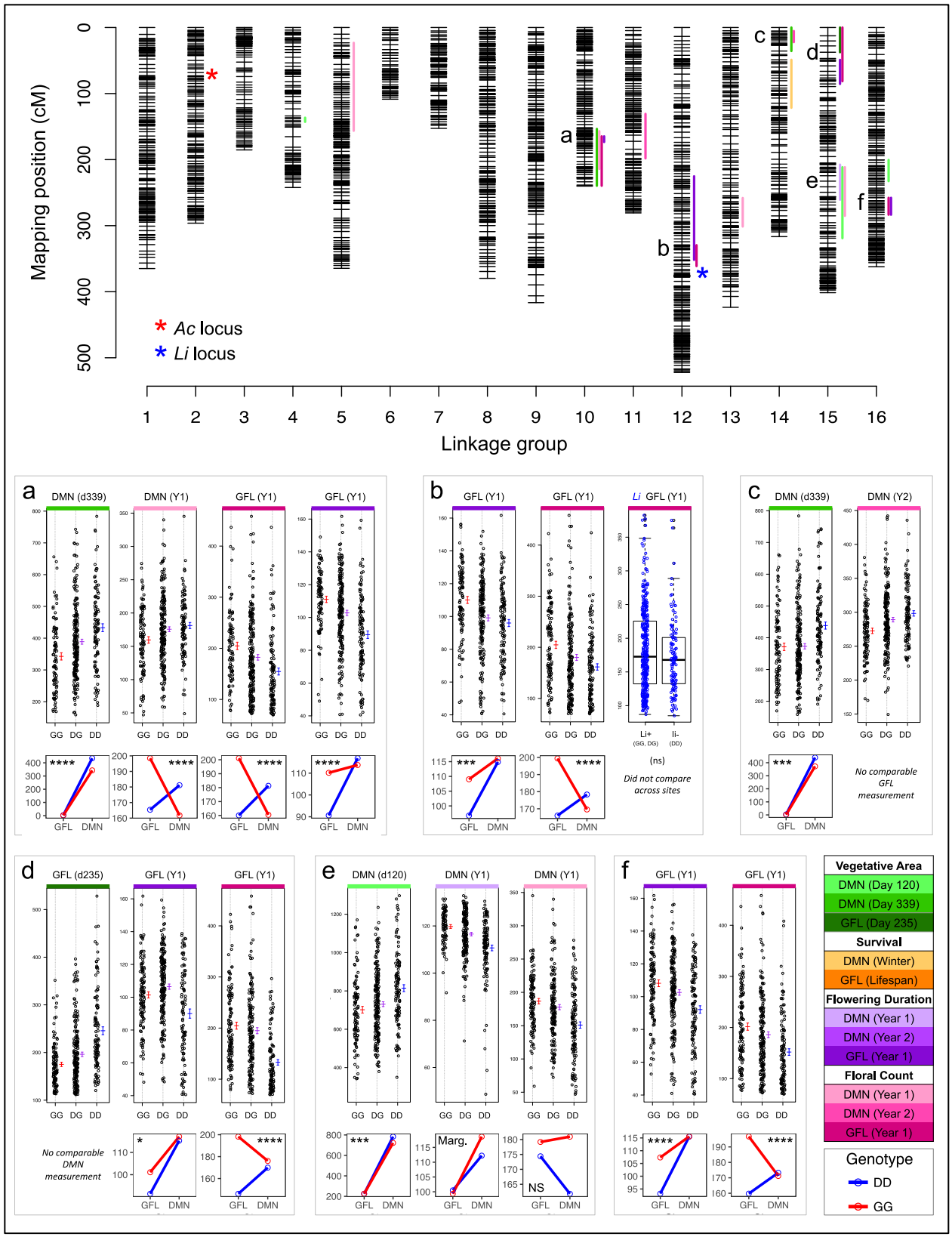


Figure S2.6. DG Population

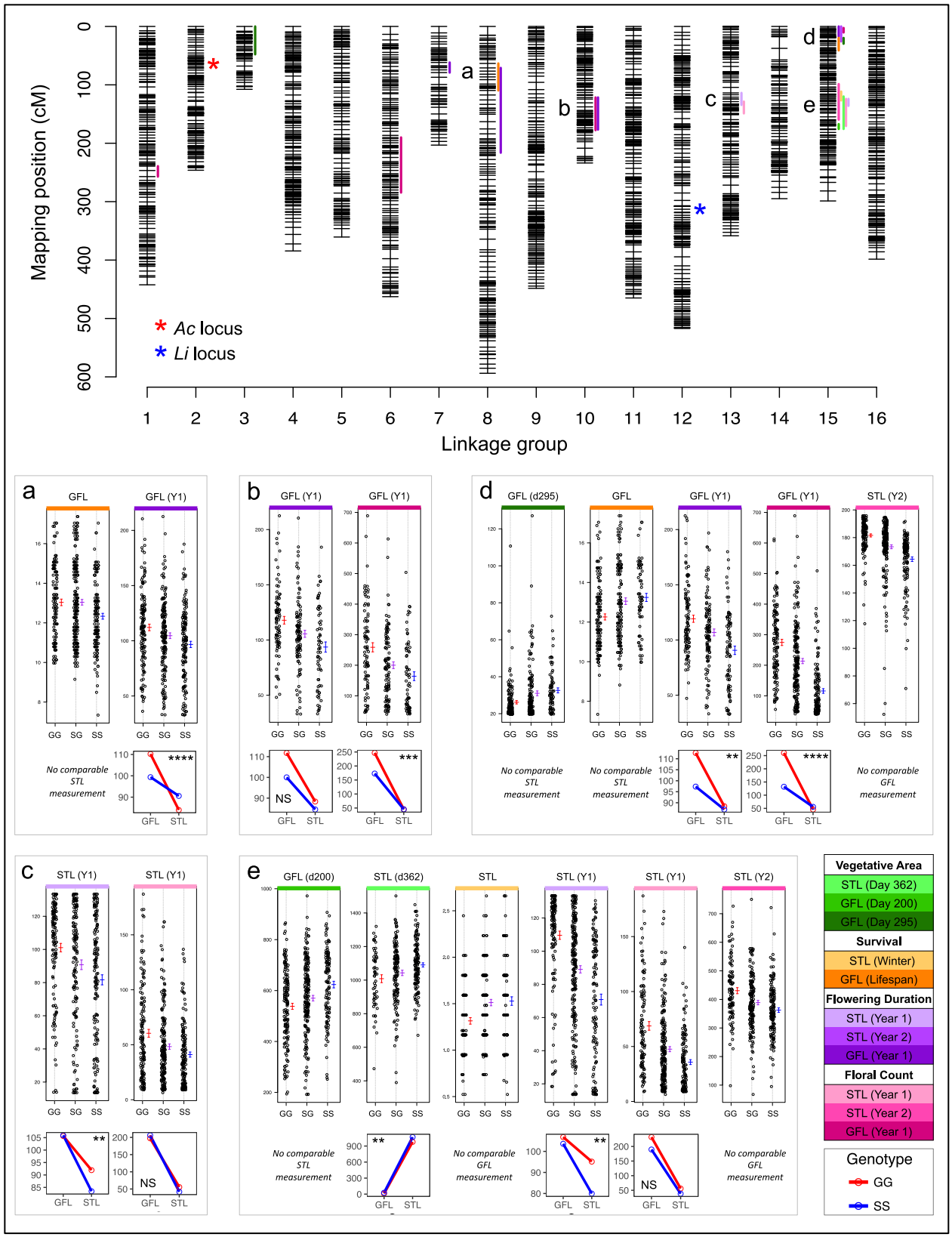


Figure S2.7. SG Population

CHAPTER 3

Selection at the seedling life stage contributes to cyanogenesis cline evolution in white clover (*Trifolium repens* L.)

ABSTRACT

Widespread plant species often evolve adaptive clines across heterogeneous environments, and clines in chemical defense polymorphisms are among the best-documented cases. However, the role of selection at the seedling life stage for the evolution of chemical defense clines is largely unknown. We examined this question in white clover, a species that is polymorphic for cyanogenesis and has repeatedly evolved climate-associated cyanogenesis clines throughout its range. We assessed whether selection at the seedling life stage favors locally-abundant cyanogenesis variants in three environments that span a U.S. latitudinal cyanogenesis cline. We first compared cyanogenesis frequencies between wild adult populations that have experienced natural selection in each environment and wild-collected seed grown in a benign greenhouse environment. We then performed field seedling survival experiments in each environment to test for frequency shifts in cyanogenesis variants among survivors. For two of the three environments, there were significant differences in cyanogenesis variant frequencies between greenhouse-grown seedlings and wild adults. Field survival experiments further revealed significant shifts towards the locally most abundant variants, consistent with selection at the juvenile life stage. Our results indicate that selection at the seedling life stage contributes to the evolution of cyanogenesis clines, and that cyanogenesis clines can potentially evolve within a few generations in white clover. They further suggest that the costs and benefits of producing secondary metabolites are substantial in juvenile herbaceous plants and that this life stage may play a critical role in the evolution of other chemical defense clines.

INTRODUCTION

Chemical defenses against herbivores are common across the plant kingdom. The prevalence and diversity of secondary metabolites produced by plants, as well as evidence of long-standing coevolution between plants and herbivores, demonstrate that chemical defenses are both effective on an ecological time scale and evolutionarily advantageous (Mithöfer and Boland, 2012). Nevertheless, herbivores and other environmental challenges vary across space and time, and energetic costs associated with producing chemical defenses can reduce their benefits in some contexts, such as conditions of low herbivore abundance or nutrient limitation (Strauss *et al.*, 2002; Fine *et al.*, 2006; Kooyers, Blackman and Holeski, 2017; Züst and Agrawal, 2017). Natural selection may therefore favor or disfavor chemical defenses in different environments or at different stages of a plant's life cycle (Lankau and Kliebenstein, 2009; Sampedro, Moreira and Zas, 2011). Intraspecific chemical defense polymorphisms provide variation upon which this heterogeneous selection can act (Moore *et al.*, 2014).

Species with widespread geographical distributions often evolve clines in adaptive polymorphisms, such that phenotypic and/or genotypic frequencies change gradually across latitudinal, altitudinal, or other environmental gradients (Savolainen, Pyhäjärvi and Knürr, 2007; Samis *et al.*, 2012a; Woods *et al.*, 2012). In plants, chemical defense polymorphisms are among the best documented examples of traits that have evolved adaptive clinal variation (e.g., Daday, 1958; Levin, 1976; Martz *et al.*, 2009; Pratt *et al.*, 2014). Clinal patterns typically emerge from ongoing divergent selection for alternate variants in different environments (i.e., locally adaptive variants), with some degree of homogenizing gene flow between populations (Haldane, 1948; Su,

Lam and Bürger, 2019). However, while chemical defense clines have been readily described for multiple species, little is known about which life stages are most important for the evolution and maintenance of these clines.

Recent empirical studies have highlighted the importance of early life stages for lifetime fitness and local adaptation in natural settings (Debieu *et al.*, 2013; Burghardt, Metcalf and Donohue, 2016; Postma and Ågren, 2016, 2018; Zettlemoyer, Prendeville and Galloway, 2017). Because juveniles are likely to be differentially affected by herbivory and other environmental stressors (Cook, 1979; Züst and Agrawal, 2017), relative to established adult plants, it is reasonable to hypothesize that early life stages should play a determining role the evolution of chemical defense clines. On the other hand, the juvenile life stage represents only a brief window in the total life span of a plant, particularly for perennial species, and fitness variation associated with a chemical defense polymorphism could have cumulative effects over multiple years. Here, we take advantage of a well-studied chemical defense polymorphism that has repeatedly evolved climate-associated adaptive clines. For three populations that span a broad environmental gradient corresponding to a North American latitudinal cline in the defense, we assess whether selection at the seedling life stage favors locally abundant chemical defense variants.

Study system

White clover (*Trifolium repens* L., Fabaceae) is a widespread herbaceous perennial that is native to Europe and has been introduced to mesic temperate regions worldwide as an important forage crop; it exhibits large population sizes across its

global range (USDA, 2002; Kjærgaard, 2003). Evolutionary and ecological genetic studies have been performed in white clover for over 60 years, in large part because it possesses an adaptive chemical defense polymorphism for cyanogenesis (the ability to produce hydrogen cyanide following tissue damage) (Daday, 1954a, 1954b). Clines in the frequency of cyanogenesis have repeatedly evolved across the native and introduced range, such that higher frequencies of cyanogenic plants are found in warmer environments, and acyanogenic plants are dominant in cooler environments (Daday, 1954a, 1954b; Till-Bottraud, Kakes and Dommée, 1988; Caradus *et al.*, 1990; Kooyers and Olsen, 2013; Kooyers *et al.*, 2014; Thompson, Renaudin and Johnson, 2016). In the United States, for example, the proportion of cyanogenic plants ranges from >85% in the Gulf States (e.g., Louisiana, Florida) to <20% in states that share a border with Canada (e.g., Wisconsin, Minnesota) (Kooyers and Olsen, 2012). Similar patterns have been documented across latitudinal and elevation gradients worldwide (Daday, 1958; de Araújo, 1976), providing strong evidence that natural selection acts on this polymorphism and that alternate cyanotypes are locally adaptive in contrasting environments.

Two biochemical components, cyanogenic glucosides (CNglcs) and their hydrolyzing enzyme linamarase, must both be present for a white clover plant to produce the cyanogenic response; these components are spatially separated in plant tissue, so plants produce the defense only when tissue damage occurs (Gleadow and Møller, 2014). Two unlinked Mendelian polymorphisms control the inheritance of these cyanogenesis components, *Ac/ac* and *Li/li* for CNglcs and linamarase, respectively (Olsen, Sutherland and Small, 2007; Olsen, Hsu and Small, 2008). For both loci,

recessive alleles correspond to gene deletions, and homozygous recessive genotypes lack the cyanogenic component (Olsen, Kooyers and Small, 2013; Olsen and Small, 2018). These presence/absence polymorphisms thus create four cyanogenesis variants or 'cyanotypes' in white clover, all of which occur in wild populations: cyanogenic plants (AcLi cyanotypes) which produce both components, and acyanogenic plants (Acli, acLi, and acli cyanotypes), which lack one or both components.

Because the *Ac/ac* and *Li/li* polymorphisms are independently segregating, and because white clover is an obligately outcrossing species, recombination during sexual reproduction is expected to generate cyanotype frequencies for seedlings in a population that are determined by Hardy-Weinberg predictions from parental allele frequencies in that location (Ennos, 1982). These seedling cyanotype frequencies may or may not be the same as the optimal cyanotype frequencies favored by selection in that environment. Thus, to the extent that selection acts on cyanotype variation at the seedling and juvenile life stages, cyanotype frequencies would be expected to change between seedlings and reproductively mature plants in a given location, and any cyanotype frequency shifts between seedling and adult cohorts can be attributed to the action of selection at the juvenile life stage.

Experiments using herbivore feeding chambers and other controlled conditions have repeatedly demonstrated that cyanotypes exhibit fitness variation when subjected to different herbivory, temperature and soil moisture treatments (reviewed in Hughes, 1991). However, field experiments have been mixed in their ability to attribute significant fitness trade-offs across contrasting environments to the cyanogenesis

polymorphism (Wright et al., in prep). Moreover, field experiments have rarely focused on the seedling life stage, as most have involved transplanting mature plants from greenhouse environments into the field. Nonetheless, the few studies that have assessed seedling fitness in natural environments have detected cyanotype frequency shifts that suggest selection may be occurring at juvenile life stages (R. Ennos, 1981; Pederson and Brink, 1998; Richards and Fletcher, 2002). While these field studies highlight the potential for selection at the seedling life stage related to the cyanogenesis polymorphism, shifts in cyanotype frequencies have not been assessed for multiple populations across cyanogenesis clines.

Here, we assess the role of fitness variation at the seedling life stage in contributing to white clover local adaptation and the evolution of cyanogenesis clines. Using population genetic surveys and germination experiments in three environments spanning a latitudinal cyanogenesis cline in North America, we address the following specific questions: 1) Are cyanotype frequencies of wild-collected seeds grown in a benign greenhouse environment different from frequencies found in adult plants in local wild populations? 2) If so, do differences between the seedling and adult cohorts suggest that selection favors a shift toward frequencies of the locally most abundant cyanotypes? And 3) When seedlings are germinated and grown in contrasting field environments, do cyanotype frequencies for surviving plants display an increased frequency of the locally most abundant cyanotype, consistent with selection favoring that cyanotype at the juvenile life stage?

MATERIALS AND METHODS

Germplasm

White clover seeds and stolon cuttings were collected on separate occasions from mature adult plants in three environments between 2014 and 2016. The environments span a broad latitudinal gradient in the United States: Duluth, Minnesota (46.8°N, -92.1°W; hereafter DMN), St. Louis, Missouri (38.6°N, -90.3°W; hereafter STL), and Gainesville, Florida (29.6°N, -82.3°W, hereafter GFL). Duluth (USDA climate zone 4b) is located on Lake Superior near the U.S.- Canadian border, while Gainesville (USDA zone 9a) is located near the transition from temperate to subtropical climate, essentially the southern limit of naturalized North American white clover populations. St. Louis (USDA zone 6b) is centrally located in the United States.

Seed samples. In each environment, mature seed heads were collected from approximately 10 maternal plants in 10 distinct localities (e.g., empty lots, parks, schoolyards). In total, samples from 287 maternal plants were collected across the three environments (DMN: 83 samples from 10 localities; STL: 115 samples from 13 localities; GFL: 89 samples from 10 localities) (Table S3.1). Following collection, samples were stored in individual coin envelopes and dried with silica gel; mature seeds were harvested by hand and stored at 4°C.

Seeds from different maternal plants were pooled within localities to create seed mixes for germination experiments. Specifically, three seeds per maternal plant were pooled in 1.5 mL microcentrifuge tubes for each distinct locality (10-13 tubes per environment, approximately 30 seeds per tube). This pooling procedure was repeated three additional times to create four sets of pooled seeds, each containing 861 seeds

from across the 33 localities. Three of the four sets were stored in the dark at 4°C for 5-9 months prior to their use in field experiments.

The fourth set of pooled seeds was scarified using fine-grain sandpaper and planted on mist benches in the Washington University greenhouses in January 2016. Individual tubes of pooled seeds, corresponding to 33 localities across the three environments (Table S3.1), were planted in separate 4.5” square pots with Metro-Mix 360 potting soil (Hummert International, Earth City, Missouri). Following germination, seedlings were removed from mist benches and grown under standard greenhouse conditions. Germinants and seedlings were counted by hand daily for each locality for 45 days after planting; 99.3% of the 399 germinants survived to 45 days, indicating that the greenhouse provided a benign growth environment.

After 45 days, a subset of the greenhouse seedlings (designated GH) were transferred to individual 3.5” square pots (N=52, 59, and 48 from DMN, STL, and GFL, respectively, with proportional representation based on the number of seedlings that emerged in each locality). These GH seedling populations represent plants originating from each of the three environments grown under benign conditions with relaxed selection. Cyanotypes were determined as described below, and baseline (pre-selection) cyanotype frequencies were calculated for GH seedlings from each environment of origin. These baseline frequencies were used in comparisons to wild adult and field-grown seedling populations (described below).

Stolon cuttings. Reproductively mature wild plants were sampled as stolon cuttings in each environment. In total, we collected stolon cuttings from 82, 57, and 62 adult white clover plants in DMN, STL, and GFL, respectively (Table S3.2). In these

sampling efforts, stolons were collected broadly across each environment, without defining distinct localities, with a minimum distance of 5 m between samples to avoid collecting multiple ramets of the same genotype. Stolon cuttings were transplanted into 3.5" square pots and grown under standard greenhouse conditions. Cyanotypes were determined as described below.

Cyanotype assignments

Cyanotypes were first determined using PCR genotyping of the cyanogenesis genes. DNA extractions were performed with 100 mg of pulverized fresh young leaf tissue and *IBI Scientific* Mini Genomic DNA extraction kits (IBI Scientific, Dubuque, Iowa). Standard PCR reaction conditions and primers for the *Ac* and *Li* genes were used as described previously (Olsen, Sutherland and Small, 2007; Olsen, Hsu and Small, 2008). Negative PCR results (indicating ac- and li- phenotypes) were repeated twice to confirm the negative result. Half of all cyanotypes inferred by PCR-genotyping were further confirmed with cyanotype phenotyping assays using Feigl-Anger HCN test paper, as previously described (Olsen, Sutherland and Small, 2007).

Statistical analysis (GH seedlings vs. adults)

To assess whether white clover populations display overall shifts in cyanotype frequencies from the seedling to adult life stage, we compared observed cyanotype counts from wild adult samples to the baseline cyanotype counts of GH seedling populations for each environment of origin using χ^2-tests with contingency tables. We also tested specifically for an increase in locally abundant cyanotypes by performing two

proportion *z*-tests between seedling and adult population frequencies for the most abundant cyanotype in each environment.

Field experiments

The remaining three sets of pooled seeds were planted in each environment (DMN, STL, GFL) so as to coincide with the local leaf flush at the beginning of the main growing season. Seeds were planted on May 5, 2016 at Washington University's Tyson Research Center; on June 14, 2016 at The University of Minnesota-Duluth's Research and Field Studies Center; and on October 13, 2016 at The University of Florida-Gainesville's Plant Science Research and Educational Unit (PSREU) in Citra, FL.

Seeds were scarified with fine-grain sandpaper on-site immediately prior to planting, and tubes of pooled seeds from each of the 33 localities were planted in separate 4.5" square pots with the bottoms removed. Pots were embedded in freshly tilled local soil, such that the pots were level with the surrounding ground. Seeds were watered only during the initial planting. White Scrubbie Mesh (Jo-Ann Stores, Inc., Hudson, Ohio) was used to loosely cover pots during the experiment. The mesh mimicked shading that occurs for seedlings that naturally germinate within a matrix of other plants but did not prevent small herbivores from accessing the seedlings; it has been used similarly in a previous white clover germination experiment (Wright *et al.*, 2017).

Cyanotypes among surviving seedlings. To test whether the cyanotype frequencies of surviving seedlings in field experiments displayed an increased frequency of the locally abundant cyanotype, relative to seedlings that survive in a

benign GH environment, we determined cyanotypes for a subset of the surviving seedlings in field experiments and calculated cyanotype frequencies. Specifically, we assayed approximately 50 surviving seedlings from each environment of origin in each germination environment (total N = 477). For each group of ~50 plants, we included representatives from all localities with the same environment of origin; the number of representatives from a given locality was proportional to the number of survivors from that locality in a given germination environment. Cyanotypes were determined as described above.

To determine whether the locally most abundant cyanotypes increased in frequency in each field germination environment, we performed two proportion z-tests to compare GH seedlings to those that survived in different germination environments. Specifically, we tested for increases in the frequency of Acli among surviving seedlings in the DMN and STL environments, relative to their respective GH groups, and we tested for an increase of AcLi in the GFL environment; we performed these three comparisons for each of the seed sets independently (nine comparisons in total).

RESULTS

Seedlings (GH) vs. adults: cyanotype frequency shifts

For the wild adult populations in DMN and STL, the locally most abundant cyanotype was Acli (frequencies of 0.38 and 0.47, respectively); for GFL, the locally most abundant cyanotype was AcLi (0.90) (Figure 3.1, Table S3.3). These most abundant cyanotypes recapitulate those reported in previous cyanogenesis cline sampling performed for North American populations at similar latitudes (Kooyers and

Olsen, 2012). Cyanotype frequencies among wild adult populations in the three environments were all significantly different from one another (DMN vs. STL: χ^2=7.83, df=3, P=0.049; DMN vs. GFL: χ^2=72.75, df=3, P<0.00001; STL vs. GFL: χ^2=51.88, df=3, P<0.00001); the GFL population was most pronounced in its differences.

When wild adult populations were compared to their respective GH seedling populations for each environment, two of the comparisons (STL and GFL) demonstrated significant differences in overall cyanotype frequencies (contingency tables: χ^2=10.78, df=3, P<0.02 and χ^2=9.12, df=3, P<0.03). Notably, in both cases, it was only the locally most abundant cyanotype in adult populations (Acli and AcLi, respectively) that increased in frequency from the seedling to adult life stages, while all other cyanotypes decreased in frequency across life stages (Table S3.3). In STL, the frequency of Acli more than doubled from 0.22 to 0.47 (z=-2.87, P=0.002), and in GFL, the frequency of AcLi increased from 0.69 to 0.90 (z=-2.86, P=0.002). These results demonstrate that there are important differences between benign greenhouse and natural environments that select for the most locally abundant cyanotypes during the seedling-to-adult transition and that reflect latitudinal cline patterns.

Field experiments

In the greenhouse, 399 of the 861 planted seeds germinated (46.3%). In comparison, germinant counts in the field experiment were 334 (38.8%), 350 (40.7%), and 364 (42.3%) for the DMN, STL, and GFL field environments, respectively. Whereas 99.3% of GH germinants survived to 45 days, 243 (72.8%), 341 (97.4%), and 322 (88.5%) survived to 30 days in the field environments (Table S3.1, see Supplementary

Methods and Results in Appendix III). These germination rates confirm that there were no major losses in seed viability during the experiment, and survival rates suggest that the greenhouse environment was more benign than field environments.

Cyanotypes among survivors. For five out of nine comparisons, seedling populations displayed a significant shift in the frequency of the locally most abundant cyanotype in field germination environments, relative to the respective baseline GH frequencies of the same seed group (Table S3.4). For four of these five instances, there was a significant increase in the locally most abundant cyanotype (Figure 3.2). Specifically, the frequency of AcLi increased in the GFL environment for both the DMN and GFL seed groups, (z=-2.04, P=0.02 and z=-2.12, P=0.02) (Figure 3.2A,C). Notably, cyanogenic plants in the DMN seed group, which exhibited the lowest baseline greenhouse frequency (21%) among seed groups, increased to a frequency of 39% in the GFL environment (Figure 3.2A). Additionally, the frequency of Acli increased in both the DMN and STL environments; it was the STL seed group that displayed these shifts in both cases (z=-3.14, P<0.001 and z=-2.09, P=0.02) (Figure 3.2B). These results are consistent with selection favoring the locally adaptive cyanotype at the earliest life stages. For the one case where a significant decrease of the locally most abundant cyanotype occurred (Acli cyanotypes for GFL seed in the DMN environment), this effect was likely due to the fact that the GFL seed population showed the least variability across germination environments (i.e., AcLi was at very high frequency in all environments) (Figure 3.2C). Collectively, these results indicate that the locally most abundant cyanotype increased in frequency in all three environments across the cline for at least one of three seed groups, although it was not always the local seed group

that displayed the shift. These results are, to our knowledge, the first empirical evidence for selection acting on cyanogenesis variation at the seedling life stage to favor locally abundant cyanotypes at multiple environments across a cline.

DISCUSSION

In this study, we assessed the contribution of the seedling life stage for cyanogenesis cline evolution in the perennial legume white clover. We found that for two of three environments spanning a North American latitudinal cline, comparisons of wild-collected greenhouse-grown seed and local wild adults indicate that selection in the wild creates frequency shifts between the seedling and adult life stage towards locally abundant cyanotypes (Figure 3.1). Consistent with this finding, seedling survivorship experiments conducted in the field revealed multiple cases of differential survival for plants of the locally most abundant cyanotype (Figure 3.2). These results suggest that cyanogenesis clines can evolve in just a few generations, and further, they suggest that cyanotype-associated fitness variation at juvenile life stages plays an important role in the evolution of clines in this chemical defense polymorphism. Below we discuss the implications of our findings in the context of the white clover cyanogenesis polymorphism and more broadly for the evolution of chemical defense clines.

Adaptive shifts in chemical defense variation within a generation

Since the earliest documentation of adaptive cyanogenesis clines in white clover (Daday, 1954a, 1954b), researchers have studied mechanisms of natural selection that shape the evolution of these clines (Daday, 1965). However, this study is the first to our

knowledge to assess shifts in cyanotype frequencies from the seedling to adult life stages for different populations across a cyanogenesis cline. Given that adult populations of white clover may largely be composed of two- and three-year-old cohorts (Richards and Fletcher, 2002), our findings suggest that selection on the cyanogenesis polymorphism can lead to significant shifts in population cyanotype frequencies in a single generation. Such an effect is important because persistent selection is needed to maintain adaptive clines in outcrossing species, where interpopulation gene flow and sexual recombination are both expected to introduce locally-maladaptive variants into a population (Ennos, 1982; Lenormand, 2002; Savolainen, Pyhäjärvi and Knürr, 2007). A study in the annual species *A. thaliana* similarly demonstrated differential selection on a defense polymorphism that caused evolution within five generations (Züst *et al.*, 2012); in that case, geographically-structured evolution was related to historical and present day distributions of aphids. The rapid evolution of chemical defense polymorphisms has also been documented for introduced plant populations (Bossdorf *et al.*, 2005). Generally speaking, minimal negative pleiotropic effects of plant secondary metabolites may enable their rapid evolution, relative to primary metabolites, which could explain why clines for multiple classes of defense metabolites readily evolve and are maintained in a wide variety of species (Levin, 1976; Kooyers and Olsen, 2012; Moore *et al.*, 2014; Pratt *et al.*, 2014).

Selection at the seedling stage contributes to chemical defense cline evolution

In white clover, a small number of previous field experiments have revealed significant effects of cyanogenesis variation for fitness at the seedling life stage.

Significant increases in the frequencies of linamarase-producing cyanotypes (AcLi and acLi) were observed during the transition from seeds to established adult plants at a field site in northwest England (R. Ennos, 1981). For another white clover population in the U.K., wide variation in the proportion of cyanogenic plants was documented over a 24-year study period; cyanotype frequencies in a given cohort were determined early and persisted as the cohort matured, suggesting that selection on cyanogenesis variation occurred early in life (Richards and Fletcher, 2002). In a field experiment performed in the U.S. state of Mississippi, cyanogenic seedlings experienced reduced insect damage and increased survival compared to acyanogenic plants when both were planted from seed in bermudagrass sod (Pederson and Brink, 1998). None of these studies were performed in multiple contrasting environments; thus, they did not assess the extent to which selection for differing cyanotypes at the seedling stages contributes to cyanogenesis cline evolution. Our results therefore emphasize the importance of environment for determining cyanotype frequencies of seedling cohorts, leading to clinal patterns across broad geographical ranges.

The production of secondary defense metabolites is known to vary throughout the course of development, and younger tissues often contain them at higher concentrations (Moore *et al.*, 2014; Villamil, Zedillo-avelleyra and Boege, 2015; Barton and Boege, 2017). We would therefore expect defenses against small herbivores to be potent at early life stages and potentially to have a larger effect on the probability of survival than they would at later life stages, when minor losses in vegetative tissue have smaller negative effects. While our data only allow us to speculate as to whether differences in herbivore pressure across the three study sites caused the cyanotype

frequency shifts we observed, previous studies indicate a clear effect of generalist herbivores on cyanotype fitness variation, particularly at the seedling life stage. In a controlled feeding study that used a common slug species and mixtures of cyanogenic and acyanogenic seedlings, lethal damage was inflicted on most of the acyanogenic seedlings by 35 days, while cyanogenic seedlings were largely undamaged (Horrill and Richards, 1986). Other studies that did not directly manipulate herbivores have documented marginally significant preferential feeding by herbivores among transplanted cuttings in field experiments (Dritschilo *et al.*, 1979; Wright *et al.*, 2017) and in wild populations (Whitman, 1973); however acyanogenic plants did not display differential mortality due to herbivory in adult plants. While the benefits of chemical defenses emerge in the presence of herbivores, their production may reduce growth significantly at the seedling stage, when competition for space is high and tied to lifetime fitness (Züst and Agrawal, 2017); thus, environments with reduced herbivore pressure are likely to favor fast-growing seedlings that do not produce costly defenses. Furthermore, because geographically structured chemical defense polymorphisms are known to evolve in response to many varying selective pressures, including multiple herbivores, pathogens, mutualists, competitors, and abiotic stresses (Lankau and Kliebenstein, 2009; Kalske *et al.*, 2012; Erwin, Geber and Agrawal, 2013; Moore *et al.*, 2014), a more thorough assessment of cline evolution would consider which of these factors impact fitness at the juvenile life stage.

Our results suggest that early life stages may play an important role in the evolution of chemical defense clines. While our data provide new evidence that selection at the seedling stage contributes to cyanogenesis cline evolution in white

clover, it does not negate the possibility that this defense polymorphism also experiences natural selection at later life stages or during periods of intense natural selection (e.g., drought, episodic periods of intense herbivore pressure). Future studies focused on the role of chemical defense polymorphisms for local adaptation and clinal evolution should aim to consider the effects of defense variation across the entire lifespan of the plant, and over multiple years, to understand the dynamics of natural selection on this trait.

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FIGURES

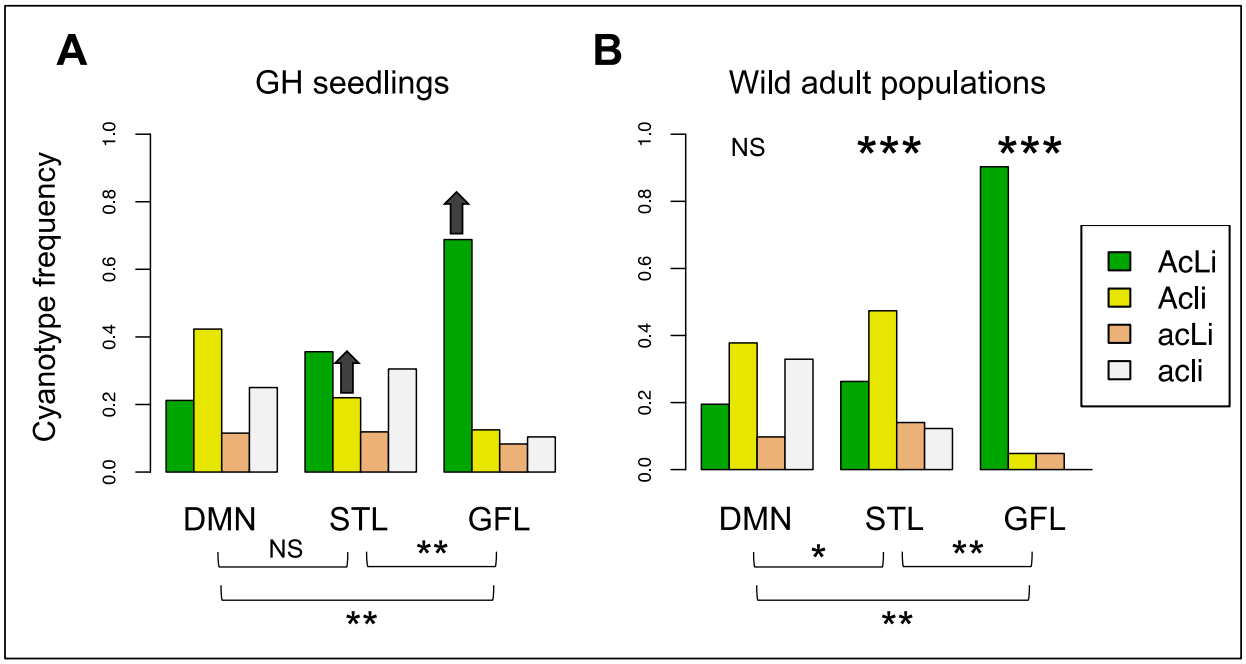

Figure 3.1. Cyanotype frequencies of seedling populations germinated in a benign greenhouse (GH) environment **(A)** versus wild adult populations sampled as stolon cuttings **(B)** for three environments that span a U.S. latitudinal cyanogenesis cline: Duluth, MN (DMN), St. Louis, MO (STL) and Gainesville, FL (GFL) (see also Table S3.3). Asterisks below plots indicate significant overall differences in cyanotype frequencies between adult samples collected from different environments (χ^2 contingency tests), while asterisks and corresponding arrows above plots indicate significant increases in the locally most abundant cyanotype from the seedling to the adult life stage (two proportion *z*-tests). Significance thresholds for both tests: P< *0.05, **0.01, ***0.0003, NS=not significant.

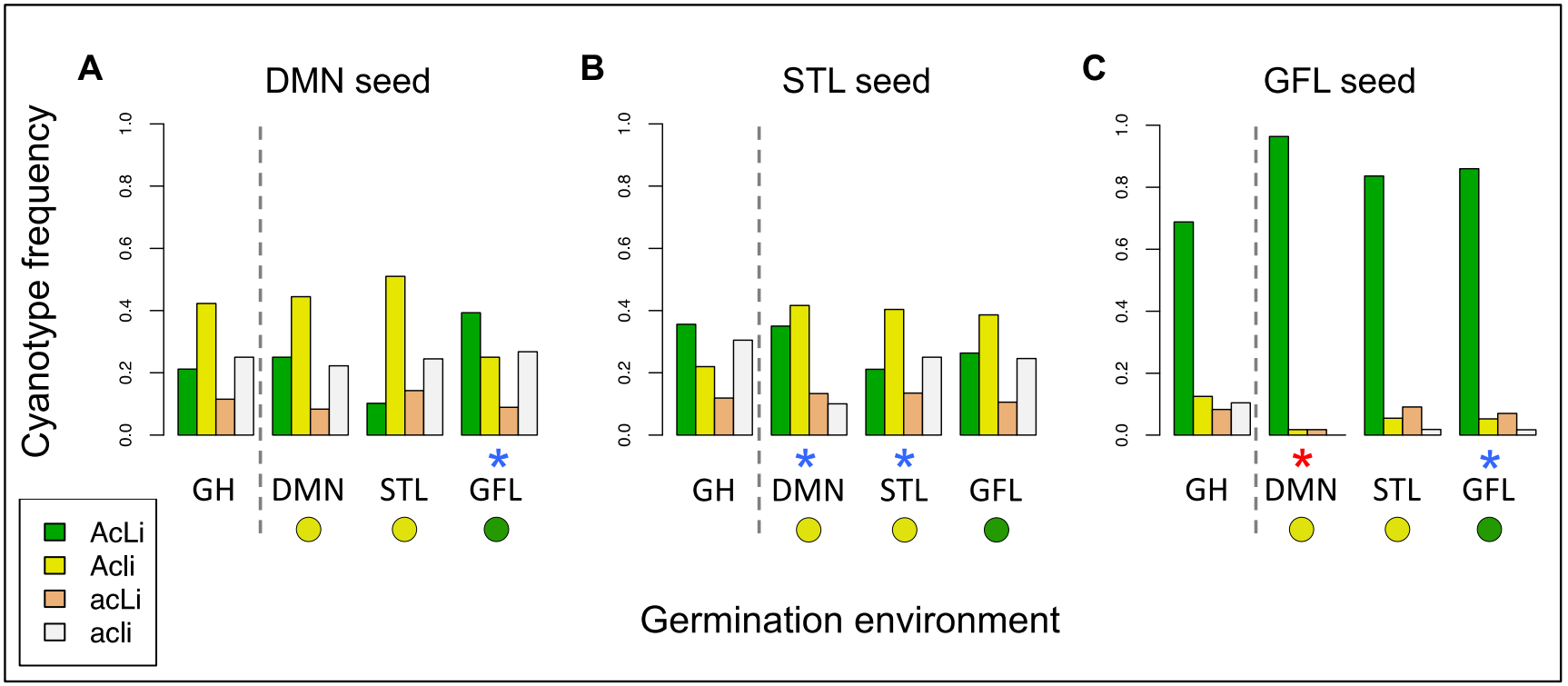

Figure 3.2. Cyanotype frequencies of surviving seedlings that were germinated in three field environments (DMN, STL, and GFL) (see also Table S3.4), compared to baseline frequencies of seedlings from the same seed source that were germinated under benign greenhouse (GH) conditions (Table S3.3). Seeds collected in DMN **(A)**, STL **(B)** and GFL **(C)** were germinated in all three environments and the greenhouse. Colored circles indicate the most locally abundant cyanotype for each field germination environment. Asterisks indicate significant changes from the GH to the field specifically in the locally most abundant cyanotype for that environment. Blue and red asterisks indicate significant increases and decreases, respectively (two proportion *z*-tests, P<0.05).

APPENDIX III

Chapter 3 Supplementary Material

SUPPLEMENTARY METHODS

There is abundant evidence that white clover displays local adaptation across its climatic range in North America, but the extent to which cyanotype variation contributes to seedling fitness in different environments has not been assessed. We therefore performed germination counts and seedling survivor counts among germinants for each locality in each field germination environment to determine whether seedlings showed evidence of home-site advantage with respect to these early life stages.

Measurements

Counts were assessed using digital photographs taken at least twice per week for 30 days after the seeds were planted. After 30 days, seedlings grew too large to distinguish individual plants in photos (Figure S3.1).

Statistical analysis

We analyzed the variation in germination and survival among localities using linear mixed models that we constructed separately for germinant count and seedling survivor count. Models were built using R statistical software and the *lme4* package (Bates *et al.*, 2015). The germinant count model included the environment where seeds were planted (E) and the number of seeds planted as fixed effects. The surviving seedling count model included E and the number of germinants as fixed effects. Locality nested within environment of origin (O) and O×E were included as random effects in both models. The significance of fixed effects was assessed with

ANOVAs, and the significance of random effects was assessed with likelihood ratio tests and the *lmerTest* package (Kuznetsova, Brockhoff and Christensen, 2017).

SUPPLEMENTARY RESULTS

Germination

Germinant counts were 334 (38.8% of the total seeds planted), 350 (40.7%), and 364 (42.3%) for the DMN, STL, and GFL field environments, respectively, compared to 399 (46.3%) in the benign greenhouse environment (Table S3.1). In the germinant count model, germination environment (E) did not explain significant differences in germination counts ($F_{3,6}$=0.74, P=0.5654). These results suggest that there were no major losses in seed viability for the time frame over which the field experiments were conducted (January-November 2016).

Environment of origin (O) was a significant random effect (χ^2=11.71, df=1, P=0.0006); it explained the highest proportion of total variance in germination counts among the 33 localities (0.73), after accounting for the number of seeds planted as a fixed effect ($F_{1,29}$=70.13, P < 0.0001). This suggests that there were differences in germination propensity for seed sets originating from different environments and may reflect genetic differences in dormancy. There were also marginally significant differences in germination between localities from the same O (χ^2=3.87, df=1, P=0.0490).

There was a significant O×E interaction (χ^2=9.62, df=1, P=0.0019). This interaction was evidenced by the fact that all populations had low relative germinant

counts (negative O×E effects) in their native environments and higher relative counts (positive O×E effects) in other environments. Specifically, DMN seed germinated best in the GH and worst in DMN, STL seed germinated best in GFL and worst in the GH, and GFL seed germinated best in STL and worst in GFL (Table S3.5). These findings suggest there is not a home-site advantage with respect to germination; rather, the wild-collected seeds may possess some degree of home-site maladaptation for germination.

Seedling survivorship

Among germinants that emerged within 30 days after planting, 243 (72.8%), 341 (97.4%), and 322 (88.5%) survived to the 30-day mark in the DMN, STL and GFL environments, respectively, compared to 99.3% that survived to the 45-day mark in the benign GH environment (Table S3.1). Unlike germinant counts, seedling survivorship among localities was significantly affected by E ($F_{3,7}$=5.95, P=0.023), after including the number of germinants as a fixed effect ($F_{1,65}$=1344, P<0.0001); thus, the environments imposed differential selection for seedling survival. Seedling survival rates suggest that the DMN environment imposed the strongest natural selection, and the STL environment was relatively benign.

The O×E interaction also significantly affected seedling survivorship (χ^2=15.92, df=1, P<0.0001); it was the random effect that explained the highest proportion of variance in survivorship among localities (0.29). In the DMN environment where survivorship was lowest, the local DMN seed had the highest relative survivorship (positive O×E effects), while seed originating from GFL germplasm had low relative survivorship (negative O×E effects) (Table S3.5). In the STL environment, where

survivorship was highest among the three field environments, this pattern reversed; seed originating from GFL had high relative survivorship and the DMN seed had low relative survivorship. Taken together, these findings provide evidence for home-site fitness advantage (i.e., local adaptation) in the DMN environment. They also suggest that there are survivorship trade-offs for seedlings originating from DMN and GFL in harsher (DMN) vs. more benign (STL) field environments.

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SUPPLEMENTARY TABLES

Table S3.1. Seed sample information and results of the field germination experiment, including germinant and seedling survivor counts in each environment.

SEED SAMPLES						GERMINATION EXPERIMENT COUNTS							
						Germinants*				Surviving Seedlings*			
Environment	**Locality**	**Latitude**	**Longitude**	**No. Samples**	**No. Seeds Planted**	GH	DMN	STL	GFL	GH	DMN	STL	GFL
DMN	D01-Portland Square	46.80	-92.09	8	24	8	5	8	8	8	3	8	5
DMN	D02-Chester Park	46.81	-92.10	8	24	4	4	6	3	4	4	6	3
DMN	D03-UMD baseball fields	46.82	-92.08	10	30	16	3	11	6	15	3	11	6
DMN	D04-8th St. & N. 20th Ave. E.	46.81	-92.08	9	27	11	5	6	6	10	5	6	4
DMN	D05-Lakewalk North	46.81	-92.06	7	21	8	7	3	6	8	6	3	6
DMN	D06-Leif Erikson Park	46.80	-92.08	8	24	8	8	5	6	8	7	4	6
DMN	D07-Canal Park Beach	46.79	-92.10	9	27	16	3	5	8	16	2	5	5
DMN	D08-Marshall High School	46.80	-92.11	6	18	7	8	9	2	7	7	9	2
DMN	D09-57-99 W. Mulberry St.	46.80	-92.13	9	27	13	2	5	9	13	2	5	9
DMN	D10-Arrowhead & Stebner	46.82	-92.20	9	27	18	5	5	5	18	4	4	5
STL	S01-Waterman Blvd	38.65	-90.30	10	30	11	14	10	16	11	8	10	11
STL	S02-Central West End	38.65	-90.26	8	24	8	6	4	7	8	4	4	7
STL	S03-Forest Park Metrolink	38.65	-90.29	10	30	10	11	6	9	10	7	6	9
STL	S04-Good Shepherd offices	38.67	-90.32	10	30	3	7	8	10	3	6	8	10
STL	S05-Olive Blvd	38.66	-90.30	10	30	9	11	14	9	9	9	14	9
STL	S06-Forest Park (location 1)	38.63	-90.30	5	15	4	6	5	3	4	3	5	1
STL	S07-Forest Park (location 2)	38.64	-90.28	10	30	8	12	7	12	8	9	7	12
STL	S08-Forest Park (location 3)	38.64	-90.29	9	27	6	9	8	5	6	3	7	5
STL	S09-Joseph Mooney Park	38.66	-90.33	9	27	9	11	7	12	9	10	7	10
STL	S10-Farmhaus restaurant	38.60	-90.30	9	27	11	2	6	11	11	1	6	9
STL	S11-Heman Park	38.66	-90.32	10	30	8	3	7	9	8	3	7	9
STL	S12-STL Comm. Credit Union	38.63	-90.32	10	30	10	8	9	20	10	6	9	19
STL	S13-University City	38.66	-90.31	5	15	6	4	5	9	6	2	5	5
GFL	G01-U Florida offices	29.63	-82.36	15	45	29	33	33	30	29	30	33	28
GFL	G02-AFRU at U Florida	29.80	-82.41	9	27	16	15	18	17	15	13	16	16
GFL	G03-Beef unit at U Florida	29.74	-82.28	10	30	17	21	19	17	17	17	17	13
GFL	G04-Alachua	29.79	-82.49	6	18	13	17	14	14	13	9	14	13
GFL	G05-U Florida campus	29.64	-82.36	10	30	20	17	25	18	20	12	24	17
GFL	G06-U Florida campus	29.64	-82.36	10	30	23	14	21	24	23	9	20	22
GFL	G07-U Florida campus	29.64	-82.36	6	18	14	15	13	9	14	11	13	9
GFL	G08-U Florida campus	29.64	-82.36	7	21	16	16	12	14	16	4	12	11
GFL	G09-U Florida campus	29.64	-82.36	9	27	23	23	22	16	23	18	22	13
GFL	G10-U Florida campus	29.64	-82.36	7	21	16	9	14	14	16	6	14	13
*30 days after planting			**TOTAL**	287	861	399	334	350	364	396	243	341	322

Table S3.2. Sampling locations and cyanotypes for wild population samples (adult plants).

Environment	Accession	Latitude	Longitude	Cyanotype
DMN	DMN_200	46.803	-92.095	Acli
DMN	DMN_201	46.804	-92.086	acli
DMN	DMN_202	46.805	-92.097	Acli
DMN	DMN_203	46.805	-92.085	acli
DMN	DMN_204	46.805	-92.085	Acli
DMN	DMN_205	46.806	-92.085	acLi
DMN	DMN_206	46.805	-92.085	acli
DMN	DMN_207	46.805	-92.086	acli
DMN	DMN_208	46.805	-92.086	AcLi
DMN	DMN_209	46.805	-92.086	acLi
DMN	DMN_210	46.827	-92.037	Acli
DMN	DMN_211	46.827	-92.037	acli
DMN	DMN_212	46.836	-92.079	AcLi
DMN	DMN_213	46.837	-92.079	Acli
DMN	DMN_214	47.048	-91.631	acLi
DMN	DMN_215	46.813	-92.913	Acli
DMN	DMN_216	46.813	-92.913	acli
DMN	DMN_217	46.813	-92.095	AcLi
DMN	DMN_218	46.815	-92.096	AcLi
DMN	DMN_219	46.815	-92.096	AcLi
DMN	DMN_220	46.815	-92.096	Acli
DMN	DMN_221	46.815	-92.096	acli
DMN	DMN_222	40.742	-93.989	acli
DMN	DMN_223	47.115	-91.651	AcLi
DMN	DMN_224	47.415	-92.259	acli
DMN	DMN_225	47.670	-92.751	acli
DMN	DMN_226	46.814	-92.091	acLi
DMN	DMN_227	46.819	-92.098	Acli
DMN	DMN_228	46.837	-92.008	Acli
DMN	DMN_229	46.730	-92.175	Acli
DMN	DMN_230	46.732	-92.168	AcLi
DMN	DMN_231	46.733	-92.168	acLi
DMN	DMN_232	46.800	-92.010	AcLi
DMN	DMN_233	46.807	-92.074	Acli
DMN	DMN_234	46.821	-92.075	AcLi
DMN	DMN_235	46.822	-92.076	Acli
DMN	DMN_236	46.837	-92.078	AcLi
DMN	DMN_237	46.837	-92.078	Acli
DMN	DMN_238	46.837	-92.080	acLi
DMN	DMN_239	46.837	-92.078	Acli
DMN	DMN_240	46.778	-92.408	acLi
DMN	DMN_241	46.778	-92.408	acli
DMN	DMN_242	46.819	-92.100	Acli
DMN	DMN_243	46.819	-92.100	Acli
DMN	DMN_244	46.819	-92.100	AcLi
DMN	DMN_245	46.820	-92.100	Acli
DMN	DMN_246	46.818	-92.081	Acli
DMN	DMN_247	46.869	-92.052	acli
DMN	DMN_248	46.869	-92.053	acli

DMN	DMN_249	46.869	-92.050	acli
DMN	DMN_250	45.599	-92.992	Acli
DMN	DMN_251	45.599	-92.992	acli
DMN	DMN_252	46.818	-92.079	acli
DMN	DMN_253	46.818	92.091	Acli
DMN	DMN_254	46.819	-92.087	Acli
DMN	DMN_255	46.819	-92.087	Acli
DMN	DMN_256	46.818	-92.080	Acli
DMN	DMN_257	46.818	-92.081	AcLi
DMN	DMN_258	46.867	-92.049	Acli
DMN	DMN_259	46.868	-92.048	Acli
DMN	DMN_260	46.778	-92.408	acLi
DMN	DMN_261	46.849	-92.083	acli
DMN	DMN_262	46.731	-92.190	acli
DMN	DMN_263	46.803	-92.095	AcLi
DMN	DMN_264	46.803	-92.095	Acli
DMN	DMN_265	46.815	-92.101	AcLi
DMN	DMN_266	46.868	-92.049	Acli
DMN	DMN_267	46.726	92.191	acli
DMN	DMN_268	NA	NA	AcLi
DMN	DMN_269	NA	NA	Acli
DMN	DMN_270	46.806	-92.078	acli
DMN	DMN_271	46.822	-92.112	Acli
DMN	DMN_272	46.787	-92.098	AcLi
DMN	DMN_273	46.787	-92.098	acli
DMN	DMN_274	46.815	-92.082	acli
DMN	DMN_275	46.815	-92.082	acli
DMN	DMN_276	46.820	-92.090	Acli
DMN	DMN_277	47.903	-91.867	acli
DMN	DMN_278	46.868	-92.050	Acli
DMN	DMN_279	46.868	-92.050	acli
DMN	DMN_280	46.868	-92.051	acLi
DMN	DMN_281	46.869	-92.050	acli
DMN	DMN_282	46.869	-92.050	acli
STL	STL_0106	38.641	-90.287	Acli
STL	STL_0108	38.641	-90.287	acli
STL	STL_0507	38.641	-90.287	acLi
STL	STL_0701	38.605	-90.267	acli
STL	STL_0905	38.625	-90.450	Acli
STL	STL_1010	38.611	-90.486	Acli
STL	STL_1309	38.638	-90.293	acLi
STL	STL_5000	38.648	-90.288	acli
STL	STL_5001	38.634	-90.303	Acli
STL	STL_5002	38.641	-90.332	Acli
STL	STL_5003	38.639	-90.358	AcLi
STL	STL_5004	38.653	-90.367	Acli
STL	STL_5005	38.656	-90.406	Acli
STL	STL_5006	38.666	-90.396	Acli
STL	STL_5007	38.671	-90.374	Acli
STL	STL_5008	38.675	-90.371	AcLi
STL	STL_5009	38.675	-90.360	Acli
STL	STL_5011	38.673	-90.343	Acli

STL	STL_5012	38.667	-90.333	acli
STL	STL_5013	38.658	-90.319	Acli
STL	STL_5014	38.657	-90.298	AcLi
STL	STL_5015	38.662	-90.304	acLi
STL	STL_5016	38.673	-90.318	Acli
STL	STL_5017	38.645	-90.280	acli
STL	STL_5018	38.644	-90.268	AcLi
STL	STL_5019	38.640	-90.251	Acli
STL	STL_5020	38.640	-90.244	Acli
STL	STL_5021	38.639	-90.239	Acli
STL	STL_5022	38.639	-90.239	Acli
STL	STL_5023	38.644	-90.238	acLi
STL	STL_5024	38.646	-90.246	Acli
STL	STL_5025	38.652	-90.256	acLi
STL	STL_5026	38.650	-90.261	Acli
STL	STL_5027	38.655	-90.273	acLi
STL	STL_5028	38.648	-90.278	AcLi
STL	STL_5029	38.653	-90.284	Acli
STL	STL_5030	38.650	-90.286	Acli
STL	STL_5031	38.623	-90.352	Acli
STL	STL_5032	38.618	-90.363	AcLi
STL	STL_5033	38.617	-90.395	acLi
STL	STL_5034	38.607	-90.394	AcLi
STL	STL_5035	38.603	-90.385	Acli
STL	STL_5036	38.597	-90.380	acli
STL	STL_5037	38.584	-90.378	AcLi
STL	STL_5038	38.592	-90.357	AcLi
STL	STL_5039	38.605	-90.349	acli
STL	STL_5040	38.604	-90.333	Acli
STL	STL_5041	38.604	-90.333	AcLi
STL	STL_5042	38.613	-90.323	AcLi
STL	STL_5043	38.623	-90.287	AcLi
STL	STL_5044	38.608	-90.290	AcLi
STL	STL_5045	38.615	-90.284	Acli
STL	STL_5046	38.628	-90.292	AcLi
STL	STL_5047	38.626	-90.302	Acli
STL	STL_5048	38.620	-90.314	Acli
STL	STL_5049	38.623	-90.325	AcLi
STL	STL_5050	38.623	-90.341	acLi
GFL	GFL_001	29.633	-82.356	AcLi
GFL	GFL_002	29.633	-82.356	AcLi
GFL	GFL_003	29.633	-82.355	AcLi
GFL	GFL_004	29.633	-82.355	AcLi
GFL	GFL_005	29.639	-82.359	AcLi
GFL	GFL_006	29.639	-82.359	AcLi
GFL	GFL_007	29.641	-82.362	AcLi
GFL	GFL_008	29.641	-82.362	AcLi
GFL	GFL_009	29.641	-82.364	AcLi
GFL	GFL_010	29.641	-82.364	AcLi
GFL	GFL_011	29.640	-82.364	AcLi
GFL	GFL_012	29.640	-82.364	AcLi
GFL	GFL_013	29.638	-82.363	AcLi

GFL	GFL_014	29.638	-82.363	AcLi
GFL	GFL_015	29.638	-82.363	AcLi
GFL	GFL_016	29.638	-82.362	AcLi
GFL	GFL_017	29.638	-82.363	AcLi
GFL	GFL_018	29.635	-82.361	AcLi
GFL	GFL_019	29.635	-82.361	AcLi
GFL	GFL_020	29.635	-82.361	AcLi
GFL	GFL_021	29.636	-82.365	AcLi
GFL	GFL_022	29.636	-82.365	AcLi
GFL	GFL_023	29.636	-82.365	AcLi
GFL	GFL_024	29.636	-82.366	AcLi
GFL	GFL_025	29.636	-82.367	AcLi
GFL	GFL_026	29.636	-82.367	AcLi
GFL	GFL_027	29.634	-82.367	AcLi
GFL	GFL_028	29.634	-82.367	AcLi
GFL	GFL_029	29.649	-82.360	Acli
GFL	GFL_030	29.649	-82.360	AcLi
GFL	GFL_031	29.644	-82.349	AcLi
GFL	GFL_032	29.644	-82.349	AcLi
GFL	GFL_033	29.640	-82.360	AcLi
GFL	GFL_034	29.641	-82.361	Acli
GFL	GFL_035	29.641	-82.364	AcLi
GFL	GFL_036	29.643	-82.367	AcLi
GFL	GFL_037	29.642	-82.368	AcLi
GFL	GFL_038	29.638	-82.363	acLi
GFL	GFL_039	29.640	-82.364	AcLi
GFL	GFL_040	29.640	-82.364	AcLi
GFL	GFL_041	29.642	-82.366	AcLi
GFL	GFL_042	29.642	-82.366	AcLi
GFL	GFL_043	29.642	-82.364	AcLi
GFL	GFL_044	29.650	-82.371	AcLi
GFL	GFL_045	29.649	-82.360	AcLi
GFL	GFL_046	29.645	-82.357	AcLi
GFL	GFL_047	29.648	-82.351	AcLi
GFL	GFL_048	29.646	-82.357	AcLi
GFL	GFL_049	29.648	-82.358	AcLi
GFL	GFL_050	29.648	-82.345	AcLi
GFL	GFL_051	29.650	-82.343	acLi
GFL	GFL_052	29.650	-82.341	acLi
GFL	GFL_053	29.649	-82.332	AcLi
GFL	GFL_054	29.643	-82.332	AcLi
GFL	GFL_055	29.644	-82.341	AcLi
GFL	GFL_056	29.644	-82.341	Acli
GFL	GFL_057	29.644	-82.340	AcLi
GFL	GFL_058	29.642	-82.342	AcLi
GFL	GFL_059	29.642	-82.343	AcLi
GFL	GFL_060	29.642	-82.343	AcLi
GFL	GFL_061	29.642	-82.343	AcLi
GFL	GFL_062	29.641	-82.343	AcLi

Table S3.3. Cyanotype counts and frequencies of seedlings grown from wild-collected seeds in a benign greenhouse environment (GH seedlings) vs. wild adult plants from three environments that span a U.S. latitudinal cyanogenesis cline. χ^2 contingency tests compare cyanotype composition between the two cohorts. Significant increases in the frequency of the locally most abundant cyanotype are also indicated (two proportion *z*-tests).

			Cyanotype Counts				**Cyanotype Frequencies**			
Sample origin	**Life Stage**	**N**	AcLi	Acli	acLi	acli	AcLi	Acli	acLi	acli
Duluth, Minnesota (DMN)	GH seedlings	52	11	22	6	13	0.21	0.42	0.12	0.25
(χ^2=0.97^{NS})	Wild adults	82	16	31	8	27	0.20	*0.38*	0.10	**0.33**
St. Louis, MO (STL)	GH seedlings	59	21	13	7	18	0.36	0.22	0.12	0.31
(χ^2=10.78*)	Wild adults	57	15	27	8	7	0.26	***0.47***	0.14	0.12
Gainesville, FL (GFL)	GH seedlings	48	33	6	4	5	0.69	0.13	0.08	0.10
(χ^2=9.12*)	Wild adults	62	56	3	3	0	***0.90***	0.05	0.05	0.00

Bold frequencies indicate the cyanotype that increased in frequency from the seedling to adult life stage.

Italics indicate the most abundant cyanotype in wild populations at or near the study locations (Kooyers and Olsen, 2012; Wright *et al.*, 2017).

Significance thresholds: *P<0.05, **P<0.01

Table S3.4. Cyanotype counts and frequencies for surviving seedling populations in field germination experiments.

Seed Origin	Germination environment	N	Cyanotype Counts				Cyanotype Frequencies			
			AcLi	Acli	acLi	acli	AcLi	Acli	acLi	acli
DMN	DMN	36	9	16	3	8	0.25	0.44^{NS}	0.08	0.22
	STL	49	5	25	7	12	0.1	0.51^{NS}	0.14	0.24
	GFL	56	22	14	5	15	0.39*	0.25	0.09	0.27
STL	DMN	60	21	25	8	6	0.35	0.42*	0.13	0.10
	STL	52	11	21	7	13	0.21	0.40*	0.13	0.25
	GFL	57	15	22	6	14	0.26^{NS}	0.39	0.11	0.25
GFL	DMN	55	53	1	1	0	0.96	0.02*	0.02	0.00
	STL	55	46	3	5	1	0.84	0.05^{NS}	0.09	0.02
	GFL	57	49	3	4	1	0.86*	0.05	0.07	0.02

Two proportion *z*-tests for the locally most abundant cyanotypes: *P<0.05, ***P<0.001, NS=not significant at P=0.05.

Table S3.5. Random effect estimates of O×E (i.e., environment of seed origin × germination environment) interactions included in linear mixed models for germinant and surviving seedling counts among 33 localities. Means presented are least squares means for each germination environment.

		Germination Environment			
Model	**Origin**	GH	DMN	STL	GFL
Germinant Count*	DMN	1.91	-0.93	-0.38	-0.87
	STL	-1.41	0.35	-0.60	1.43
	GFL	-0.50	0.58	0.98	-0.56
	Mean	12.49	10.36	10.93	11.17
Surviving Seedling Count*	DMN	-0.19	1.38	-0.21	0.07
	STL	-0.19	0.07	-0.03	-0.02
	GFL	0.38	-1.44	0.24	-0.05
	Mean	10.97	8.14	10.67	9.70

*30 days after planting

SUPPLEMENTARY FIGURE

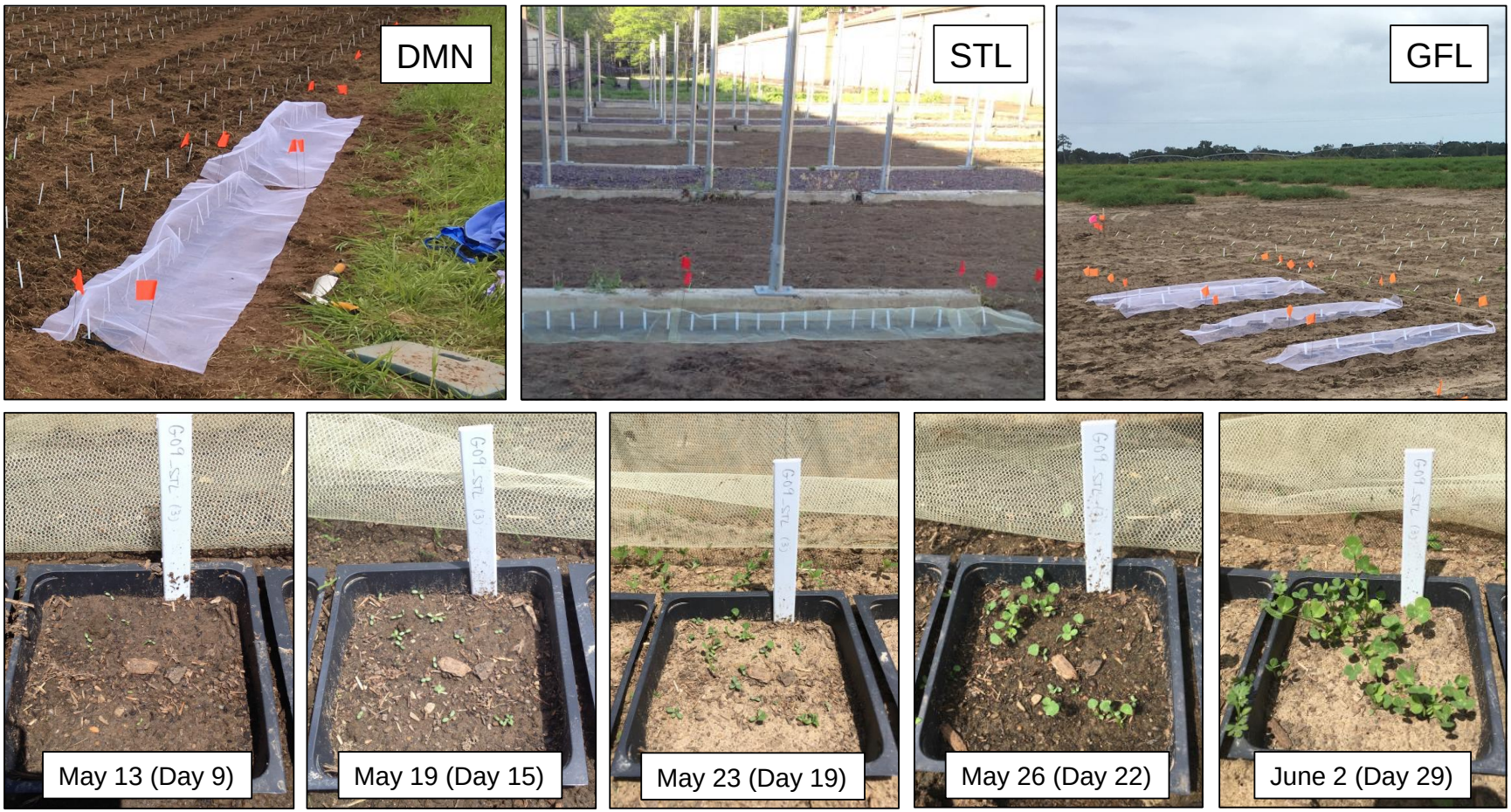

Figure S3.1. Field germination experiments at three field sites that span a latitudinal cyanogenesis cline in North America (DMN, STL, and GFL). Pictures of seedlings at various time points throughout the 30-day experiment are shown for one of the 33 localities planted in STL.

CONCLUSIONS

This thesis was designed to study local climatic adaptation in a widespread herbaceous legume, white clover, which was once considered the “agricultural equivalent of coal” for its importance as a source of nitrogen fertilizer (Kjærgaard, 2003) and which remains one of the most important forage crops worldwide (Abberton and Thomas, 2010). The chapters herein aimed to contribute to a body of evolutionary research concerned with characterizing the genetic architecture of local adaptation (Savolainen, Lascoux and Merilä, 2013) by increasing the breadth of species to include an outcrossing, herbaceous perennial plant. Furthermore, this research has aimed to assess the relative importance of a well-studied, adaptive chemical defense polymorphism for fitness across multiple environments, and for both juvenile and adult life stages.

In Chapter 1, I explored the relationships between vegetative growth, reproductive fitness, cyanogenesis variation, and continent-wide climatic variation for 15 widespread North American populations grown for one year in a central U.S. common garden environment (St. Louis, MO). There were clear correlations related to population of origin for both fitness measures. Specifically, populations originating in climates that were more similar to St. Louis performed better than those that were collected in more dissimilar climates, as would be expected if populations were adapted to local climates (Kawecki and Ebert, 2004; Raabová, Münzbergová and Fischer, 2007). In addition to the evolution of cyanogenesis clines in these populations (Kooyers and Olsen, 2012, 2013), this result provides strong evidence that North American white clover populations have rapidly adapted to local climatic variation since their introduction to this continent in the last 500 years. Further, linear mixed modeling

analyses suggested that maximum summer temperatures and temperature variability at the home-site were the highest weighted climatic predictors for vegetative growth in a continental climate, whereas precipitation, and cyanotype to a lesser extent, were best predictors of reproductive output. These results indicate that different selective mechanisms affect alternate aspects of fitness across heterogeneous environments in white clover, similar to other plant species (Moles *et al.*, 2014; Siepielski *et al.*, 2017). Lastly, while cyanogenic plants showed marginally lower levels of herbivore leaf damage in the St. Louis common garden (as evidenced by a non-significant trend), this effect did not translate into a fitness advantage. Thus, in the absence of intense herbivore pressure, there do not appear to be clear fitness costs or benefits associated with the cyanogenesis polymorphism among adult white clover plants (Hughes, 1991; Züst and Agrawal, 2017). This chapter was published as part of a *Journal of Heredity* special issue on local adaptation (Wright *et al.*, 2017).

In Chapter 2, I created F_2 genetic mapping populations and performed reciprocal common garden experiments over a two-year period across three climates that span a latitudinal cyanogenesis cline in the United States. The results provided further evidence of local adaptation in white clover, indicated by genotypic trade-offs for both vegetative growth and reproductive output traits across reciprocal environments. As predicted by population genetic theory (Anderson, Willis and Mitchell-Olds, 2011; Savolainen, Lascoux and Merilä, 2013), antagonistic pleiotropy (allelic tradeoffs between environments) contributed to local adaptation in this outcrossing herbaceous plant, indicated by QTL × E interactions at fitness QTLs. As in Chapter 1, I found essentially no evidence that cyanogenesis variation contributed to fitness variation or

local adaptation in any of the three common garden environments, in contrast to predictions from worldwide climate-associated clinal variation in this trait (Daday, 1958; de Araújo, 1976; Kooyers and Olsen, 2012, 2013). Instead, we found strong evidence of selection for divergent life history strategies, such that early flowering and rapid life cycles were favored in the warmest environment, and long-term vegetative persistence with delayed flowering was favored in the cooler environments. These results contribute to a body of local adaptation literature that suggests herbaceous plants may commonly employ alternate life history strategies for local adaptation (e.g., Friedman *et al.*, 2015; Kooyers, 2015; Hämälä, Mattila and Savolainen, 2018).

In Chapter 3, I assessed the contribution of the seedling life stage for local adaptation and the evolution of cyanogenesis clines by performing population genetic surveys of adult plants, combined with germination experiments in the greenhouse and in three field environments that span a North American latitudinal cyanogenesis cline. I found that for two of three environments, local wild adult populations exhibited an increased frequency of the locally most abundant cyanotype, relative to seedlings grown in benign greenhouse conditions. Assuming that adult white clover populations are likely to be composed primarily of 2-3 year-old plants (Richards and Fletcher, 2002), this result indicates that within a few generations, regionally-varying selection can contribute to the evolution of cyanogenesis clines; such an effect is important for the maintenance of adaptive clines in outcrossing species, where gene flow and recombination are expected to introduce locally maladaptive variants (Ennos, 1982; Lenormand, 2002; Savolainen, Pyhäjärvi and Knürr, 2007). Seedling survival experiments in the field further indicated differential survival of the locally most abundant cyanotype in all three

environments; this result, to our knowledge, is novel in studies relating cyanogenesis to fitness variation in white clover. It suggests that the juvenile life stage plays a critical role in the evolution of chemical defense polymorphisms, which may have greater costs and benefits during this vulnerable period (Cook, 1979; Züst and Agrawal, 2017).

Together, the three chapters presented in this dissertation provide a working model for the process of local adaptation in white clover, as it relates to the cyanogenesis polymorphism and cline evolution. Selection at juvenile life stages (i.e., in first 30 days post germination) alters the frequency of cyanogenesis variants in seedling populations (Chapter 3), which likely remain relatively constant through the cohort's 2-3 year existence (Richards and Fletcher, 2002). In the first growing season, contrasting environments in different locations select for alternate life histories, leading to the evolution of locally adapted genotypes (Chapter 2). Specifically, early flowering and rapid life cycles appear to be favored in localities that experience periods of long-term heat stress (longer than 3-4 months) (Kooyers, 2015), because prolonged heat stress triggers massive vegetative tissue loss and ultimately mortality (Wright *et al.*, 2017, Chapter 2). In less stressful environments, white clover vegetation flourishes, and most genotypes exhibit low mortality. Energetic trade-offs between vegetative growth and reproduction can exist in the first year, such that genotypes exhibiting reduced flowering in the first year have higher reproductive fitness over a two-year period. Overall, vegetative persistence and a perennial life history appear to be favored in these environments, even at the expense of earlier reproductive output (Chapter 2). At the adult life stage, cyanogenesis does not seem to play a major role in determining fitness (Chapters 1 and 2, Wright *et al.*, 2017). Nevertheless, while I did not capture major

effects of cyanogenesis for adult fitness in these experiments, the results do not rule out the possibility that episodes of intense herbivore pressure or other selective agents may favor alternate cyanotypes in contrasting environments (Hughes, 1991), thereby contributing to the evolution of cyanogenesis clines and potentially to local adaptation if events are consistently associated with heterogeneous environmental variation.

This dissertation provides additional insights into the genetic architecture of local adaptation for an outcrossing, herbaceous perennial plant species. The results indicate that significant, locally-adaptive life history differences can be largely explained by a small number of large-effect loci (Yeaman and Whitlock, 2011); this does not negate the added effect of many smaller-effect loci that may never or rarely be detectable in empirical studies (Rockman, 2012; Yeaman, 2015). White clover also provides a compelling example of antagonistic pleiotropy (i.e., allelic tradeoffs between environments) underlying local adaptation in an herbaceous plant. Although predicted by population genetic theory (Anderson, Willis and Mitchell-Olds, 2011), this result has rarely been documented empirically in plant species studied to date (Savolainen, Lascoux and Merilä, 2013; Wadgymar *et al.*, 2017; Price *et al.*, 2018). Furthermore, the results hint at potential genetic mechanisms for the evolution of rapid, annual life cycles for heat and drought escape (Kooyers, 2015); these findings will be of interest to the clover breeding community (Abberton and Thomas, 2010).

Finally, the resources accumulated during this dissertation set the stage for exciting white clover adaptation research in the next several years. Mentored undergraduate honors theses have provided insight into the potentially adaptive roles of copy number variation at the cyanogenesis loci and the induction of cyanogenic

glucoside synthesis, as they relate to water stress; these explorations contribute to previous studies that have suggested a relationship between cyanogenesis variation and drought (Vickery, Wheeler and Mulcahy, 1987; Hughes, 1991; Kooyers *et al.*, 2014). Genetic mapping populations that were generated for Chapter 2 have been used to map the locations of the *Ac/ac* and *Li/li* polymorphisms; this work will be submitted as a paper to a peer-reviewed journal. Additionally, F_3 mapping populations are being generated from the F_2 lines for use in future genetic mapping experiments. Lastly, wild sample collections that I performed across North America for 43 wild populations, along with complementary genotyping-by-sequencing data that I generated, will be used for population genomic analyses, including environmental association analysis and F_{ST} outlier scans (De Mita *et al.*, 2013; Forester *et al.*, 2016; Ahrens *et al.*, 2018; Price *et al.*, 2018). This analysis will further characterize the genetic architecture of local adaptation in this outcrossing herbaceous perennial, and it will continue to improve our understanding of the relative adaptive importance of the cyanogenesis loci, in comparison to other genome-wide factors, for adaptation to continent-wide climatic variation.

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