Original introduction

\section\*{Introduction}

Because plants are sessile organisms, their ability to monitor and adapt to the environment is essential to their fitness. The light environment is one example of an environmental variable to keep track of because plants require light to photosynthesize. Changes to the light environment can impact fitness and development due to changes in photosynthetic output. Consequently, plants have evolved (1) photoreceptors to sense changes in the light environment and (2) developmental responses to optimize fitness under non-optimal light conditions.

The shade avoidance response (SAR) is an example of a developmental program in response to shading by nearby vegetation. Plant photoreceptors absorb the a wide-range of spectra - e.g. red and blue - but will reflect far-red light. Consequently, shading by neighboring plants or light passing through a canopy will result in reduced red lighting and increased far-red absorption \cite{Casal2012ShadeAvoidance}. This change in the red:far-red ratio (RFR) of light is recognized by phytochromes, and shifts in the phytochrome equilibrium - between a red absorbing form and a far-red absorbing form - elicit downstream transductional changes [A]. These transductional changes result in developmental changes - such as petiole elongation, reduced branching [C], and accelerated flowering [D, E] - that reduce current or future shading [A] \cite{Franklin2005PhytochromesPlants, Green-Tracewicz2011ShadePlant, Halliday1994PhytochromeRatio, Wollenberg2008AccelerationFlowering}.

In \textit{Arabidopsis thaliana}, phytochrome-mediated sensing of shade is well-characterized, and mutant studies have been instrumental in determining the function of each phytochrome. There are five phytochromes in A. thaliana - PHYA - PHYE - with PHYB being the primary regulator of the SAR \cite{Franklin2005PhytochromesPlants}. PHYD has been shown to be redundant, and likely has complementary functions to PHYB [A-C] \cite{Franklin2005PhytochromesPlants, Goosey2002DifferentialGenes, Mathews1997PhytochromeDiversity}. Similarly, the function of PHYE was elucidated through mutant studies, and its role in the SAR was established alongside PHYB and PHYB as one of the primarily regulators of the SAR \cite{Franklin2005PhytochromesPlants, Devlin2007PhytochromeArabidopsis, Franklin2003PhytochromesArabidopsis}, . PHYA, on the other hand, is light labile and is accumulated to high levels only in etiolated seedlings \cite{Quail1994PhytochromeExpression}. Results suggest that the role of PHYA is to antagonize the actions of PHYB [A, G] \cite{Franklin2005PhytochromesPlants, Johnson1994PhotoresponsesArabidopsis}. phyC mutants have been shown to display a constitutive shade response in shade, which suggests that PHYC lacks a role in regulating the SAR [E, H] \cite{Franklin2003PhytochromesArabidopsis, Franklin2003MutantPhotomorphogenesis}. Overall, mutant studies have been important in illuminating the mechanisms of shade sensing and the initiation of the SAR.

While the mechanisms of phytochrome-mediated sensing of shade are well-established, the transductional mechanisms linking shade sensing to developmental rewiring have only recently emerged. Shade sensing leads to decreased levels of active PHYB, and subsequently increased levels of PHYTOCHROME INTEGRATING FACTOR (PIF) proteins. Upregulation of PIF4 and PIF5 increases expression of genes related to hypocotyl elongation, and upregulation of PIF3, PIF4, and PIF7 maintains low levels of phyB to maintain long-term promotion of elongation \cite{Casal2012ShadeAvoidance}. A low R:FR ratio also leads to changes in hormone expression required for hypocotyl elongation. For instance, low R:FR increases free auxin levels and auxin signaling in the cotyledons \cite{Tao2008RapidPlants}, and also increases expression of auxin transporter genes (PIN3, PIN7) \cite{Friml2002LateralArabidopsis, Sieberer2000Post-transcriptionalAXR1, Devlin2007PhytochromeArabidopsis} and other auxin-related genes (IAA1, IAA3, etc.) [E] \cite{Devlin2007PhytochromeArabidopsis}. DELLA protiens - proteins that repress elongation - are also affected by changes in R:FR [A, F, G] \cite{Casal2012ShadeAvoidance, Devlin2007PhytochromeArabidopsis, Feng2008CoordinatedGibberellins}. In low R:FR and low blue light, DELLA proteins are degraded, leading to increased stem and hypocotyl growth.

The flowering pathway is also influenced by shading through regulation of flowering-related genes, and changes in these downstream genes result in accelerated flowering [A, I]. Players in the circadian clock pathway, such as CO and ELF3, are also influenced [A, I-K] \cite{Casal2012ShadeAvoidance, Wollenberg2008AccelerationFlowering, Jimenez-Gomez2010NetworkArabidopsis, Coluccio2011GeneticRegulation}. The number of affected genes and pathways demonstrates the complexity of the transductional mechanisms of the SAR.

While mutant studies have been instrumental in the sensing of shade as well as the transduction pathways of the SAR, mutant studies have limitations: (1) mutant knockouts can have no observable phenotype (2) the mutant phenotype can be context dependent (genotype or environment) and (3) a single knockout can affect multiple phenotypes \cite{Tonsor2005GeneThaliana}. These limitations can hinder the discovery of novel SAR genes and prevent the understanding of a gene’s numerous effects on phenotype \cite{Tonsor2005GeneThaliana}.

Recently, studies examining the molecular basis of natural variation in SAR have emerged, and these studies can potentially overcome the limitations of mutant studies in terms of novel SAR gene discovery [B - F] \cite{Jimenez-Gomez2010NetworkArabidopsis, Coluccio2011GeneticRegulation, Filiault2012AResponse}. Quantitative trait loci (QTL) mapping studies, for instance, have implicated a circadian clock gene (ELF3) in the genetic architecture underlying the SAR \cite{Jimenez-Gomez2010NetworkArabidopsis, Coluccio2011GeneticRegulation}. There has also been overexpression of shade-avoidance related genes (PHYA) to increase the yield of commercial crops like tobacco \cite{Robson1996GeneticGene}. Additionally, the SAR for hypocotyl elongation and flowering time has been shown to have huge natural genetic variation \cite{Botto2002DifferentialAvoidance}, suggesting that the SAR is complex in terms of genetic architecture across multiple developmental stages. Taken together, these results demonstrate a large untapped potential in terms of the genetic architecture underlying the SAR, and further research can provide insight not only into its genetic mechanisms but also provide applications in plant breeding.

Despite this, the genetic mechanisms underlying natural variation in the SAR remain poorly understood, especially for later developmental traits. To date, there have only been a handful of experiments conducted to parse the genetic architecture of the SAR \cite{Jimenez-Gomez2010NetworkArabidopsis, Coluccio2011GeneticRegulation, Filiault2012AResponse}, and only one for later developmental traits \cite{Jimenez-Gomez2010NetworkArabidopsis}. While traditional QTL mapping strategies have been successful in identifying candidate genes responsible for variation in the SAR, these studies are limited in scope due to limitations of genetic variation in the parental accessions.

Instead of a traditional biparental population, we use a nested association mapping population (NAM) to combine the advantages of linkage analysis and association mapping \cite{Yu2008GeneticMaize}. A NAM population has higher genetic diversity due to the increased number of founders; this consequently increases QTL mapping power and can detect QTL that have greater relevance to other populations.

In this mapping population, we find small to moderate variation in later developmental shade responses. We find QTL on chromosomes 4 and 5 that colocalize for multiple phenotypes, suggesting that there is a similar underlying genetic architecture for later developmental SAR. We estimate the effects of detected QTL on traits throughout developmental time, and test the effects of natural and known functional varation in sun and shade path models. We discover that QTL effects are primarily indirect for later developmental traits, suggesting that QTL effects on later developmental shade responses are primarily mediated by effects on earlier developmental traits. We also show that shade and genetic architecture jointly affect trait correlations, which consequently influences indirect QTL effect sizes in later development. These results highlight the importance of an integrated view of the genotype-phenotype relationship, and the need to not only account for genetics and environment, but also phenotype relationships throughout developmental time.