Outline of introduction

* Plants are sessile organisms
  + Light is an example of environmental cue they need to keep track of
* Shade avoidance response is an example of response to change in light environment
  + List some examples of changes
* Shade avoidance in crops and Arabidopsis
* Shade avoidance in Arabidopsis
* How shade avoidance can be linked from Arabidopsis to all plants
* What’s unknown in shade avoidance response, and how can QTL mapping studies help
* How does development potentially affect QTL mapping results? (Indirect effects of QTL)

A few overall thoughts on the intro: - I think you should try to be more clear about Arabidopsis vs all plants. The level of knowlege about mechanisms, genetic variation, developmental stages, and impact on performance are very different between Arabidopsis and other plants. I think you probably should have a section on SAR in all plants, a section on Arabidopsis specifically, and a section that relates your study in Arabidopsis to all plants (if that is a goal) - Justifying the NAM-QTL approach vs a mutant screen is tricky. I think we want to be careful here. NAM is useful for chracterizing genetic architecture of a population, while bi-parenatal is useful for specific differences among two lines. mutant screens are more comprehensive, but maybe less relevant to useful variation. - I think that you should set up the developmental aspect to SAR better since that's a main point to your methods. Responses early in development could cause later developmental effects. Or there could be additional signaling and plasticity late in development. Distinguishing among these is a central goal

Because plants are sessile organisms, their ability to monitor and adapt to the environment is essential to their fitness (Bernhard Schmid 1992, Phenotypic Variation in Plants, Loretta Gratani 2014 Plant Phenotypic Plasticity in Response to Environmental Factors). 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 and carbon allocation (Weinig 2000 Differing selection in alternative competitive environments: shade-avoidance responses and germination timing, Roig-Villanova and Martinez-Garcia 2016). 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 (Kami et al. 2010 Light-regulated plant growth and development).

The shade avoidance response (SAR) is an example of developmental and physiological reprogramming in response to shading (Frankling 2008 Shade avoidance, Casal 2012 Shade avoidance). Plant photoreceptors absorb 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}.

Some SAR mechanisms are similar between the model plant organism Arabidopsis and several crop species. Phytochrome-mediated sensing of shade has been documented in Arabidopsis (Franklin 2003 and Franklin 2005), and phyB has been established as a primary mediator of the shade avoidance response in Arabidopsis, sorghum (Kebron 2006), maize (Sheehan 2007), and tomato (Schrager-Lavelle 2007). There are similar genetic and hormonal mechanisms that control axillary bud growth in shade for both Arabidopsis and sorghum; shade repression on axillary bud growth is controlled by the transcription regulator TB1 in sorghum, and its homologs BRC1 and BRC2 in Arabidopsis (Carriedo 2016). The plant hormones auxin, cytokinin, and strigolactone are known regulate tiller bud growth in Arabidopsis and sorghum (Carriedo 2016). Auxin-related genes have shown to be upregulated in stem transcriptome profiles in tomato (Cagnola 2012). However, there are also differences between Arabidopsis and crop species in SAR mechanisms such as the phytochrome integrating factors (PIFs). In Arabidopsis, the PIFs interact with the phytochromes in the nucleus to control hypocotyl elongation and other traits, whereas PIFs or PIF homologs seem to lack a role in crop species (Carriedo 2016). Overall, there are both similar and distinct mechanisms that govern the SAR in Arabidopsis and crops.

While there are differences between Arabidopsis and crop species in terms of SAR mechanisms, knowledge of the SAR in Arabidopsis could be useful in controlling the SAR in crops. Numerous studies have utilized knowledge gained from Arabidopsis, i.e. the function of phytochromes, to repress the SAR in crops. Robson et al. 1996 uses a mutant that overexpresses PHYA to alter carbon allocation in tobacco (Robson et al. 1996), while Boccalandro et al. (2003) uses a series of mutants that overexpress PHYB to increase tuber yield in potato (Boccalandro et al. 2003). These results suggest that are overlapping mechanisms of the SAR between Arabidopsis and crops. Consequently, if we utilize the extensive genomic resources available to Arabidopsis to gain greater insight into the SAR, we could not only learn more about the SAR but also discover new SAR genes that can be manipulated to increase yield in crops.

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. In Arabidopsis, being shaded leads to decreased levels of active PHYB and 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. A 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.) \cite{Devlin2007PhytochromeArabidopsis}. DELLA protiens - proteins that repress elongation - are also affected by changes in R:FR \cite{Casal2012ShadeAvoidance, Devlin2007PhytochromeArabidopsis, Feng2008CoordinatedGibberellins}. In shade conditions, 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.

Recently, studies examining the molecular basis of natural variation in SAR have emerged, and these studies can complement mutant studies in terms of finding novel SAR genes and novel variants [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}. The SAR for hypocotyl elongation and flowering time have 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.

Shade effects are not strictly limited to the traits we observe, but there can be indirect effects transmitted to later development and other traits. These indirect effects arise from developmental and physiological relationships between traits. A higher leaf area index in tomato leads to increases in yield, due to higher levels of photosynthesis and carbon assimilates for plant growth (Heuvelink et al. 2004). Indirect effects of shade on plant reproduction have also been reported in velvetleaf (\textit{Abutilon theophrasti}) (Weinig 2000). Weinig (2000) shows that elongation is modulated by the light environment, and this has indirect effects on fecundity through biomass. Similarly, genetic effects can also be indirect, and genotype and environment can both influence later developmental trait variation through their effects on earlier development. Fournier-Level et al. (2013) shows that both genetic background and planting location contribute to life history variation, and that indirect QTL effect sizes were modulated by the environment (Fournier-Level et al. 2013). However, indirect effects of QTL have not been quantified in the context of the SAR before. Estimating indirect QTL effects can help us determine if the underlying genetics between early developmental SAR and later developmental SAR are distinct. Coluccio et al. 2011 and Jiminez-Gomez et al. 2010 both detect a SAR QTL overlying ELF3 for hypocotyl elongation and adult traits, respectively. It remains unknown, however, if ELF3 effects on adult traits are mediated by their effects on hypocotyl elongation. Estimating the indirect effects of QTL can help us distinguish true pleiotropy between traits.

Velvetleaf plants grown in competitive environments displayed elongated internodes, which leads to a cost to the plant’s available carbon for allocation. This led to decreased biomass and subsequently lower fruit production than plants grown in noncompetitive environments.

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 co-localize 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.