Germination Responses to Vegetation in Maternal and Progeny Environments

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Abstract:

The conditions in which a seed germinates is crucial to the survival and fitness of the plant. The ability to regulate germination given certain conditions is thus extremely important. This research examines the plastic germination responses to neighbor-associated light cues in *Arabidopsis thaliana* within a natural population. Our results show that light-induced germination responses of seeds from different maternal lineages within a natural population are largely uniform in direction. Although seeds exhibited dormancy loss with after-ripening, seeds imbibed under a canopy had lower germination proportions than those imbibed under white light. With respect to maternal environment, our results associate higher germination proportions with denser, more crowded maternal canopies. The effect of these maternal light cues on germination were most apparent during periods of high dormancy, suggesting that seeds become less selective over time as they after-ripen. Interestingly, the maternal and progeny cues are diametric to each other, with maternal cues seeming to encourage germination among neighbors while progeny cues respond negatively to canopies.

Introduction:

Plants lack the ability to migrate; the location of germination largely fixes a plant's location. Thus, the locale and timing of germination are of great importance. Seeds need not immediately germinate after dispersal, and they can remain viable for long periods of time (Thompson 2000). Germinating in the wrong season or in drought conditions has negative consequences for a seedling. In addition to abiotic factors, the biotic environment of a seed is critical (Goldberg and Barton 1992). The characteristics of surrounding vegetation can change throughout the year, including the identity of the neighbors and their density.[1] Germinating in opportune abiotic and biotic conditions is important not only because the seedling stage is one of the most vulnerable stages, but also because germination can affect selection on later life-stages such as growth and flowering phenotypes (Donohue et al. 2010, Donohue 2002).

Seeds do not sense in the mammalian manner, but natural selection has favored the development of various mechanisms in plants to perceive clues about their surrounding environments and to respond accordingly. This ability of a genotype to express different phenotypes in response to environmental stimuli is known as plasticity (Sultan 1987, Scheiner 1993). The ability to adjust germination in response to neighbors could be adaptive by allowing seedings to synchronize their emergence with periods of low competition (Kalisz 1986, Donohue et al. 2005). Especially for plants in competitive environments, accurate timing, speed, and location of seed germination are crucial for survival and reproduction (Mack and Harper 1977, Weiner and Thomas 1986, Smith 2000, Donohue 2002).

I investigate the germination responses in the model organism *Arabidopsis thaliana* (Brassicaceae) to potential cues of light spectral quality and irradiance that indicate the presence of nearby vegetation. *A. thaliana* inhabits a wide geological range, meaning it experiences a large diversity of environmental conditions to which it may respond and adapt, including climate and neighbor identity (Hancock et al. 2011, Fournier-Level et al. 2011). *A. thaliana* is a widely studied model for genetics and plasticity (Dorn, Pyle, and Schmitt 2007, Callahan and Pigliucci 2002, Pigliucci, Cammmell, and Schmitt 2001).

Light cues are a means by which seeds receive information about the environment and lead to changes in germination (Donohue et al. 2005). Several environmental cues can lead to plastic germination responses in *A. thaliana*, including temperature (Burghardt, Edwards, and Donohue 2015) and moisture (Baskin and Baskin 1983). This research focuses on the effect of light in the seed environment, which is known to influence germination proportion and rate (Botto et al. 1996, Dorn, Pyle, and Schmitt 2000). In the presence of neighboring vegetation, the qualities of light can change. Vegetative canopies not only reduce light irradiance, but also reduce the ratio of red to far-red light (Smith and Whitelam 1997, Casal and Sánchez 1998). Many plant species, including *A. thaliana,* are capable of sensing these changes in the red to far-red light ratio with photoreceptors known as phytochromes. (Smith and Whitelam 1997). Phytochromes are known to regulate several physiological responses, including germination and flowering (Devlin et al. 1996, Casal and Smith 1989). Thus, phytochromes enable *A. thaliana* to sense neighboring vegetation and adjust germination responses accordingly (Smith 2000 and Keller et al. 2011, Franklin and Whitelam 2005).

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[1] Source Needed

In addition to light cues that seeds receive in their immediate environment following dispersal, seeds may receive environmental clues from the maternal plant during seed maturation (Munir et al. 2001, Roach and Wulff 1987). Maternal effects in plants are thought to modify germination and seed performance via various means, including changes in dormancy (Roach and Wulff 1987, He et al. 2014). A large proportion of seedling variation is thought to be the result of maternal control (Roach and Wulff 1987). Although maternal effects in plants are generally thought to occur through cytoplasmic inheritance, there are multiple ways the maternal plant can influence offspring phenotype (Roach and Wulff 1987).

A. thaliana seeds receive information from two sources of light signals: cues experienced by seeds after dispersal and cues endowed from the maternal plant (maternal effects). Although these signals may overlap, they present the possibility of contradicting one another. It is generally thought that the influence of maternal cues diminishes as seeds age (Roach and Wulff 1987), possibly because the ability of the maternal environment to predict seedling fitness decreases with time. Maternal and progeny signals can also oppose one another; progeny fitness may not align with maternal fitness, and research suggests maternal effects are geared primarily to maximize maternal fitness rather than offspring fitness (Marshall and Uller 2007). These cues may also lead to a direct conflict, where progeny may present direct competition with the mother plant; thus, the maternal cue may be to delay germination at the cost of the optimal progeny response (Ellner 1986, Westoby 1981).

Both maternal and progeny light cues may falsely communicate the state of the environment, and thus have fitness consequences. While some maternal cues such as maternal photoperiod may be regarded as accurate cues of the environment (Munir et al. 2001), it is unclear how accurate light cues may be. Previous work has suggested responses to foliage shade can be both adaptive and maladaptive (Dorn, Pyle, and Schmitt 2000). The accuracy (and potential fitness consequences of) light cues is complicated by the differing responses *Arabidopsis* can have with vegetative neighbors.

A. thaliana seeds could respond in different ways to the presence of neighbors. Previous research has suggested two strategies for seeds in competitive environments: the prevention of germination (Lortie and Turkington 2002, Kluth and Bruelheide 2005), or the acceleration of germination in order to out-compete neighbors or minimize the strength of competition from neighbors (Dyer, Fenech, and Rice 2000). While preventing germination offers the possibility of waiting for more favorable conditions, it carries the risk of having the seed remain in the seed bank where it faces decay or predation (Davis and Raghu 2010). Preventing germination also means slower propagation compared to other lineages, thereby reducing frequency.

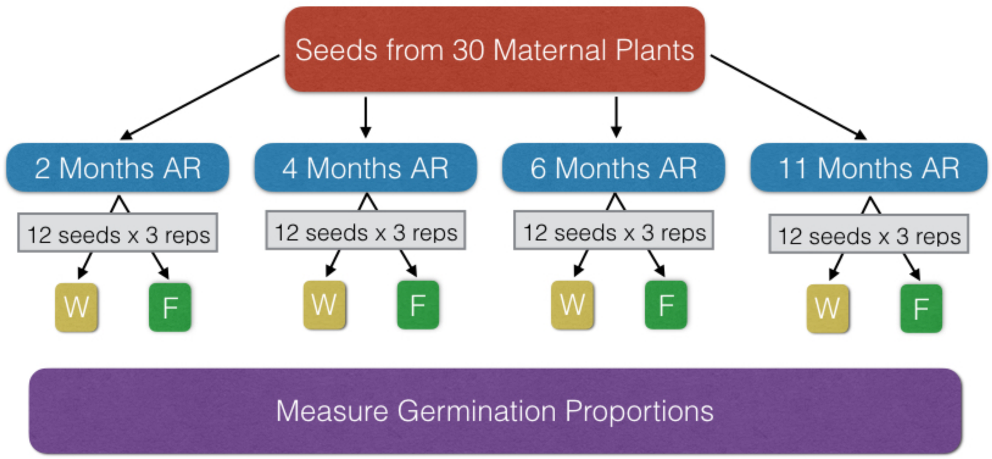
The types of interactions (competition or facilitation) that seedlings will have with their neighbors may vary in time or space. Being a ruderal species (plants which thrive in disturbed environments), *A. thaliana* is predicted to have reduced germination under cues that indicate nearby vegetation because the species often colonizes sparsely populated environments. However, this species is known to inhabit a wide variety of habitat conditions across its geographic range in Europe and North America, meaning germination responses may vary across global populations (Lev-Yadun and Berleth 2009). Populations adapting to different environments and conditions could show differing germination responses to light signals from neighboring vegetation (Yan et al. 2006). For example, some populations may be found in ecosystems with neighbors more likely to provide competition, while another population may experience more stressful abiotic factors such as temperature or precipitation levels that make facilitative effects with neighbors more likely. Neighbor cues may in fact result in more germination in certain populations depending on the relations and interactions with neighboring vegetation. Our previous work has shown that the directional germination response to neighbors does indeed differ across populations found across the global range, with ecotypes exhibiting both lower or higher germination proportions under a vegetative canopy. (Schieder, Leverett, and Donohue, 2014.)

Whether individuals within a single population show varying germination responses to neighbors or if they present uniform responses is unclear (Callahan and Pigliucci 2002). Given the likelihood of high genetic relatedness in a natural population due to high frequencies of self-fertilization in *Arabidopsis* (Bergelson et al. 1998), we hypothesize a single population to show a unidirectional response to light because of highly similar genetics. However, genetic variation can be maintained within a population as well. Preserving different germination strategies through balancing selection alongside low gene flow could maintain differentiation within a population (Tian et al. 2002).

After-ripening is a crucial factor in germination and likely modifies the effect of light cues on germination. After dispersal (or harvest), *Arabidopsis* seeds are known to synchronize dormancy with unfavorable conditions so that germination occurs in the appropriate season (Bewley 1997, Finch-Savage and Leubner-Metzger 2006, Baskin and Baskin 2014, Footitt et al. 2014). The process of dormancy loss after dispersion is known as after-ripening. With this dormancy loss, the conditions in which germination can occur generally expands, depending on additional cues in the environment (Donohue et al. 2005, Holdsworth, Bentsink, and Soppe 2008). We expect that seeds demonstrate increased germination proportions with after-ripening. As germination proportions increase, we expect the effect of light cues to diminish since the selectivity of seeds likely decreases. However, seeds do not necessarily germinate in the first season. Seeds may remain in the seed bank and await more favorable conditions. Thus, dormancy levels may be observed to increase (i.e., the seeds re-enter dormancy) to help ensure that germination occurs within a favorable season (Baskin and Baskin 1985, Bewley and Black 1994, Baskin and Baskin 2014, Footitt et al. 2014, Auge et al. 2015).

In our investigation of a natural population, we characterized the effects of canopy, after-ripening, and maternal effects on germination. Specifically, we seek to examine the direction of response to these effects, the variability of responses within a natural population, and the relation between maternal and progeny light cues.

Methods
Experimental Design:



Schematic 1. Overview of experimental setup. Seeds from 30 maternal plants were collected and stored dry at room temperature. After 2, 4, 6, and 11 months of after-ripening (AR), germination assays were performed in two light treatments, white light (W; control) and filter light (F), which involved a green filter reducing overall light irradiance and adjustment of the red to far-red light ratio to simulate an environmental canopy. Germination assays measured germination proportions on a plate of 12 imbibed seeds. For each light treatment in each after-ripening period, 3 replicates of 12 seeds were used in the germination assays.

- Field site and seed collection

75 maternal plants were selected from a natural population of *Arabidopsis thaliana* (Brassicaceae) located about 5 miles northeast of Durham, NC, USA. In this area, *A. thaliana* grows in a series of agricultural fields that are tilled most years. In the specific location where maternal plants were sampled, nitrate and potassium concentrations were approximately 2.0 and 24.1 ppm, respectively (LD Leverett and CJ Hubbard, unpublished data). The original 75 maternal plants were sampled along 5 transects: 15 plants were sampled at 0.5 m intervals. From the 75 sampled maternal plants, 30 were chosen randomly to be included in the analyses. Seed collections were made in early May 2014 and seeds were stored dry at room temperature until germination assays began.

- Measurements of maternal environments

We recorded characteristics of the maternal neighborhood within a 5 cm radius around each maternal plant at the time of seed collection. Characteristics included the number of neighbors, percent canopy cover, proportion of neighbors in reproductive life history stages, number of neighbors of each species, and composite traits such as species richness (S) and species evenness (E).

The maternal neighborhoods (Table 1) were largely populated by other members of the Brassicacea, such as *Arabidopsis thaliana, Draba verna, Sibera virginica*, and *Cardamine* *hirsuta*. Other common neighbors included *Cerastrium fontanum ssp. vulgare* (Caryophyllaceae), *Lamium amplexicaule* (Lamiaceae), and *Stellaria media* (Caryophyllaceae).

Maternal Environment	mean value	standard deviation	N
# Neighbors	3.53	1.91	30
% Cover	0.23	0.16	30
Shannon Index (H)	0.85	0.41	30
% Neighbors Reproductive	0.27	0.27	30
Species Richness (S)	2.66	0.97	30

Table 1. Summary statistics of the maternal environments from 30 focal plants collected in a local population.

- Experimental design and treatments

To test whether seed germination differs in the presence of filtered light suggestive of a vegetative canopy, seeds were imbibed under two light treatments in Percival Model GR41LX incubation chambers: white light (control) and green filter, which reduced the ratio of red to far-red light from 1.4 to 0.3 and reduced total irradiance (PAR) by approximately 50%. Irradiance was estimated to be approximately 290 µmol $m^{-2}s^{-1}$ for the control treatment. The canopy treatment consisted of a green filter over the seeds which simulated a dense vegetative canopy by reducing light quantity and quality within the range of changes observed in natural vegetation (Smith 1982). Light treatments were kept separate in two separate light chambers, but were switched every 2 days to control for chamber effects.

To examine whether germination under the different light treatments changed as seeds after-ripened, and whether the other effects change with seed maturation, germination assays were performed on seeds when they were about 2, 4, 6, and 11 months old.

For each light treatment 3 replicates of 12 seeds per maternal plant were seeded onto 0.7% agar plates. Seeds were imbibed at 16°C with 12 hours of light. The plates were censused for germination at 2, 4 and 7 days at which point germination had plateaued. Final germination proportion was recorded on day 7 as the number of germinated seeds per plate divided by the total number of seeds per plate.

<u>Data Analysis</u>:

All statistical analyses were performed using the R environment (R Core Team 2014). We used generalized linear mixed-effect models and likelihood ratio tests to evaluate the effects of Light and AR (after-ripening) and their interaction on germination proportion. Specifically, generalized linear mixed-effect models (glmm's) were fit with binomial family and a logit link function using the 'glmer' function in the 'lme4' package (Bates et al. 2014), then likelihood ratio (LR) tests were performed to assess statistical significance of predictors. *P*-values were adjusted for multiple comparisons using the Holm procedure. In all analyses, germination was the dependent variable with seed light treatment and AR treated as fixed factors, while maternal plant was treated as a random effect.

After testing for overall effects on germination proportion (germ=Light+AR+LightxAR), we used sub-models to investigate other possible significant interactions. We tested for the effect of light treatment at each after-ripening period, then tested for the effect of after-ripening across each light treatment.

We next tested whether maternal neighborhood variables influenced germination proportions. Because many of these variables were potentially correlated (especially in the case of composite traits such as species richness and evenness), we used ‘princomp’ (R Core Team 2014) to perform principal component analysis (PCA) and obtain a set of uncorrelated variables describing variation in the maternal environments. The principal components were used to analyze how the maternal environment influenced germination. The germination value used in the analyses testing for maternal effects used the mean germination of all replicates for each maternal plant in a given light treatment and after-ripening time. To test for variation, we used analysis of variance (ANOVA, R Core Team 2014) to examine the effects of light, after-ripening, and maternal environment on mean germination proportion.

After testing for overall effects on mean germination proportions (with factors PC1, PC2, Light, and AR), we observe PC2 to be nonsignificant for all effects and interactions. Upon analyzing sub-models, we restrict our factors to PC1, Light, and AR.

Results

- <u>A vegetative canopy in the seed environment reduces germination and this negative effect changes with after-ripening.</u>

The imbibition light treatment had a significant effect on seed germination proportions, but the strength of its effect changed as seeds after-ripened (Light x AR in Table 2, Figure 1, Table 3).

Seeds imbibed under a simulated vegetative canopy germinated less than those imbibed in white light, and this effect was significant at each observed period of after-ripening (Figure 1, effect of light at different intervals of AR in Table 2, Table 3).

The time spent after-ripening after harvest also had a significant effect on seed germination proportions (Effect of AR in Table 2, Figure 2, Table 3), and this effect was significant in each light treatment (effect of AR in either light treatment in Table 2). Although seeds generally germinated more as they after-ripened, seeds that had been stored for 11 months prior to imbibition germinated less than those stored for 6 months (Table 4, Figure 2, Table 3).

Effect	Df	Chisq	Pr(>Chisq)
Light x AR	3	34.624	1.462e-07 ***
Light (across AR)	1	437.28	<2.2e-16 ***
AR (across light treatments)	3	214.53	<2.2e-16 ***
Light (2 months of AR)	1	142.53	<2.2e-16 ***
Light (4 months of AR)	1	145.3	<2.2e-16 ***
Light (6 months of AR)	1	148.4	<2.2e-16 ***
Light (11 months of AR)	1	60.643	6.842e-15 ***
AR (in control)	3	95.672	<2.2e-16 ***
AR (in light treatment)	3	165.53	<2.2e-16 ***

Table 2. glmer statistical analysis of the effects of canopy imbibition (Light) and after-ripening (AR) on germination changes. Maternal plant is held as a random effect. The reference levels are the white light treatment and 2 months of after-ripening. Germination proportions were analyzed using logit-linked generalized linear mixed-effect models (glmm's), and likelihood ratio tests.

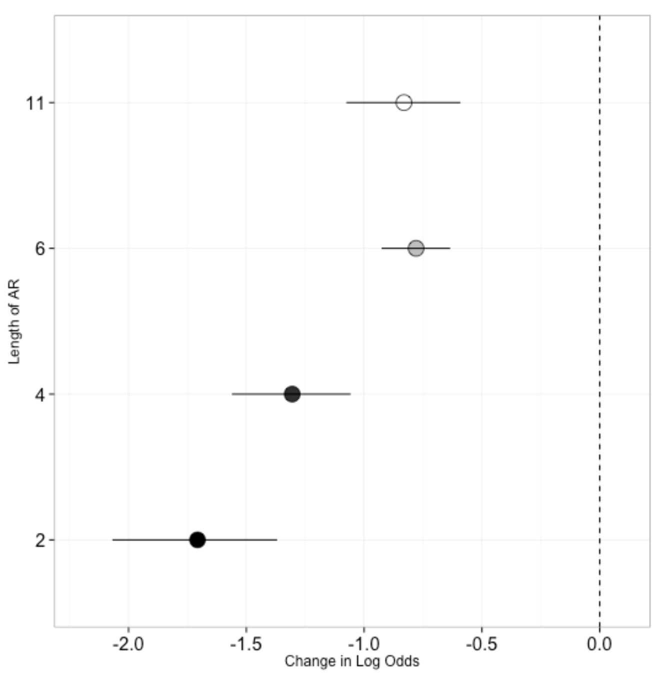


Figure 1. Canopy effect on germination in each after-ripening period. Each dot represents the coefficient estimate of the canopy treatment on germination proportion for a specific after-ripening time. The reference level is the white light treatment. Error bars are graphed as 95% confidence intervals. Negative values of change in log odds characterize negative effects of canopy on germination, i.e., causing lower germination proportions.

Treatment	n	Median Germination Proportion	Mean Germination Proportion	SD
Control (Across AR)	120	0.16670	0.23970	0.2349962
Filter (Across AR)	120	0.05556	0.09977	0.1343563
AR 2 (Across Light Treatment)	60	0.05556	0.10830	0.1666431
AR 4 (Across Light Treatment)	60	0.08333	0.17360	0.208788
AR 6 (Across Light Treatment)	60	0.18060	0.23630	0.2234765
AR 11 (Across Light Treatment)	60	0.08333	0.16060	0.1948202
Control, AR 2	30	0.11110	0.17870	0.2068026
Control, AR 4	30	0.19440	0.26020	0.2538008
Control, AR 6	30	0.2083	0.3051	0.2524487
Control, AR 11	30	0.1389	0.2148	0.2152811
Filter, AR 2	30	0.00000	0.03796	0.05903083
Filter, AR 4	30	0.05556	0.08704	0.09362768
Filter, AR 6	30	0.10420	0.16760	0.1676191
Filter, AR 11	30	0.05556	0.10650	0.1574872

Table 3. Summary statistics for germination proportions in various experimental conditions.

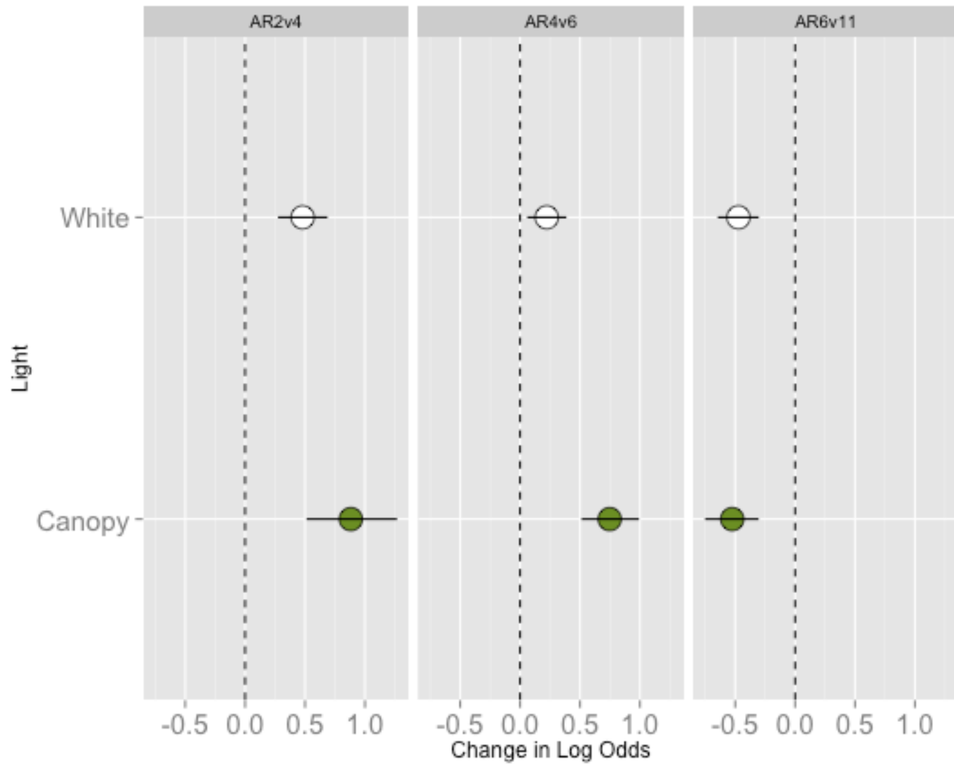


Figure 2. After-ripening effect on germination in each seed imbibition light treatment. Each dot represents the coefficient estimate of the AR treatment on germination proportion for a specific light treatment. The reference level is the earlier after-ripening period of the pair compared. Error bars are graphed as 95% confidence intervals. Positive values of change in log odds characterize positive effects of after-ripening on germination, i.e., causing higher germination proportions.

Effect of AR on Germination Proportion	Df	Chisq	Pr(>Chisq)
AR (2 and 4 months)	1	45.563	1.478e-11 ***
AR (4 and 6 months)	1	42.954	5.603e-11 ***
AR (6 and 11 months)	1	64.211	1.118e-05 ***

Table 4. glmer statistical analysis of effect of after-ripening on germination compared to the previous after-ripening period as reference.

- <u>More dense maternal environments reduce germination, but this effect is likely masked under filter and becomes less pronounced with dormancy loss.</u>

The maternal environment was expressed using principal component analysis. The first principal component (PC1) explained about 56% of the variation (Table 5) and was largely constructed from number of neighbors and number of *Arabidopsis* neighbors (Figure 3). Higher numbers of neighbors and number of *Aradbidopsis* neighbors were represented by lower (more negative) PC1 values. The second component (PC2) explained about 15% of the variation (Table 5) and was constructed from variables such as species richness (Figure 3).

Maternal environments were found to have a significant effect on germination (significant effect of PC1 in Table 6). Specifically, more crowded maternal environments were associated with higher germination proportions (Figure 4). The effect was most pronounced for early AR periods and was lost as seeds after-ripened, but there was a tendency for the effect of PC1 to reemerge when seeds were after-ripened for 11 months (Near significance of PC1 x AR in Table 6, Figure 4). The first principal component was significant in both light treatments (Table 7).

Principal Component	Proportion of Variance	Standard Deviation
Comp.1	0.5642841	2.2381847
Comp.2	0.1458872	1.1380348
Comp.3	0.1092339	0.9847493

Table 5. The proportion of the maternal environment variance explained by the top three principal components and their respective standard deviations.

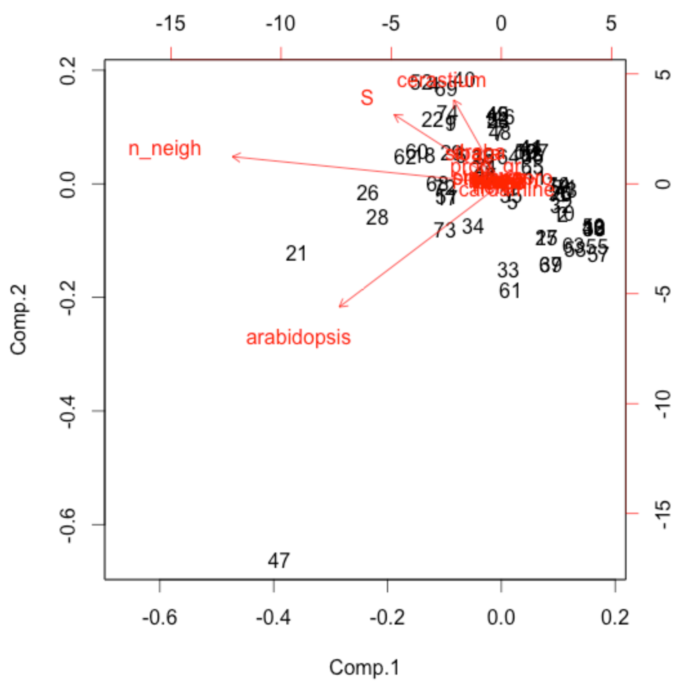


Figure 3. Biplot of Principal Component Analysis using maternal environment variables. The direction of vectors show their loadings within each of the first two principal components. PC1 is mostly comprised of number of neighbors and number of *Arabidopsis* neighbors, while PC2 encompasses species richness and identity of neighbors.

Effect on Average Germination	LR Chisq	df	Pr(>Chisq)
PC1	20.1249	1	7.255e-06 ***
PC2	1.7933	1	0.180519
Light	22.8380	1	1.762e-06 ***
AR	23.9030	3	2.617e-05 ***
PC1 x PC2	9.0841	1	0.002578 **
PC1 x Light	0.8909	1	0.345244
PC2 x Light	0.1934	1	0.660106
PC1 x AR	7.3048	3	0.0662791 .
PC2 x AR	1.2484	3	0.741410
Light x AR	4.8754	3	0.181153
PC1 x PC2 x Light	1.0234	1	0.311717
PC1 x PC2 x AR	2.6010	3	0.457316
PC1 x Light x AR	0.6350	3	0.888373
PC2 x Light x AR	3.1291	3	0.372148
PC1 x PC2 x Light x AR	0.8369	3	0.840628

Table 6. The effects of PC1, PC2, light, and after-ripening time on mean germination proportion. Germination proportions were analyzed using analysis of variance (ANOVA). If the last AR period (11 months, where germination proportions switch direction) is dropped, the PC1 x AR interaction increases significance to a p-value of 0.025973. The Light x AR interaction also increases in significance to a p-value of 0.094222.

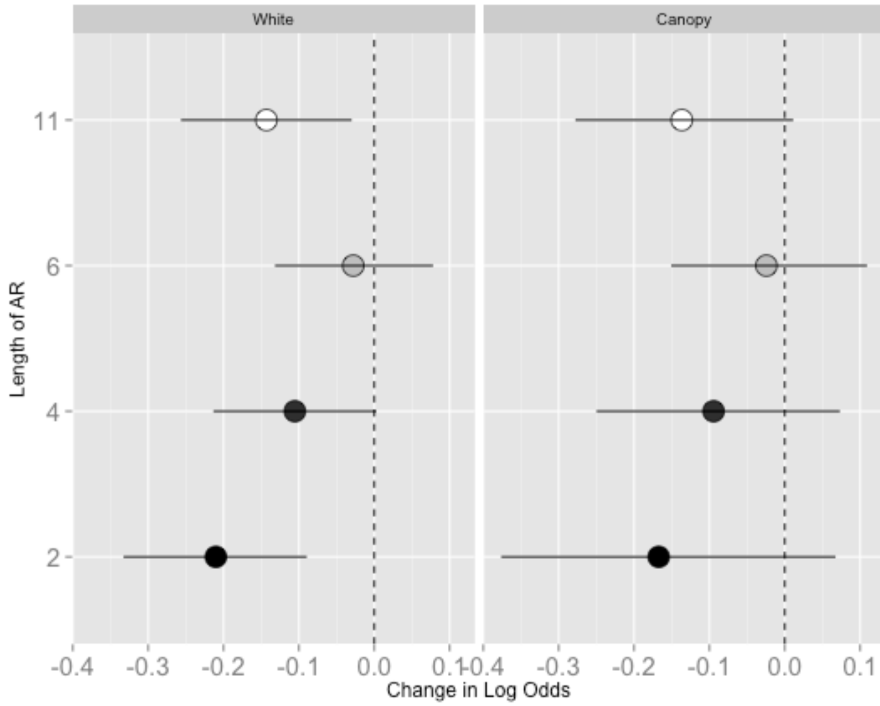


Figure 4. Effect of PC1 on germination in each after-ripening period. Each panel shows the effect in a different light treatment. Each dot represents the coefficient estimate of PC1 on germination proportion for a specific light treatment and after-ripening period. Error bars are graphed as 95% confidence intervals. Negative values of change in log odds characterize negative effects (causing lower germination proportions) of PC1 on germination, and because the value of PC1 is inversely related to neighborhood density, it appears that neighborhood density is positively correlated with germination proportions.

Effect of PC1	Df	Chisq	Pr(>Chisq)
in control (across AR)	1	15.96	6.471e-05 ***
in filter treatment (across AR)	1	4.7445	0.02939 *
in control (2 months AR)	1	11.514	0.0006908 ***
in control (4 months AR)	1	3.6526	0.05598 .
in control (6 months AR)	1	0.27401	0.6007
in control (11 months AR)	1	6.115	0.0134 *
in treatment (2 months AR)	1	2.0347	0.1537
in treatment (4 months AR)	1	1.2708	0.2596
in treatment (6 months AR)	1	0.13523	0.7131
in treatment (11 months AR)	1	3.3007	0.06925 .

Table 7. glmer statistical analysis of effect of principal component 1 (PC1) on mean germination proportion within each light treatment and after-ripening time.

Discussion

- Summary paragraph

A vegetative canopy was found to reduce germination proportions across maternal plants in a natural population of *A. thaliana*. As hypothesized, the direction of response to a vegetative canopy was largely unidirectional (Figure 5). Although seeds generally exhibited decreasing dormancy as they after-ripened, this pattern experienced a notable break between 6 and 11 months, possibly suggesting dormancy cycling (Figure 2). The maternal environment was found to significantly affect seed germination, with more crowded maternal environments associated with increased germination (Figure 4). The strength of this maternal environmental effect appears to be proportional to dormancy levels (Effect of PC1xAR in Table 7, Figure 4).

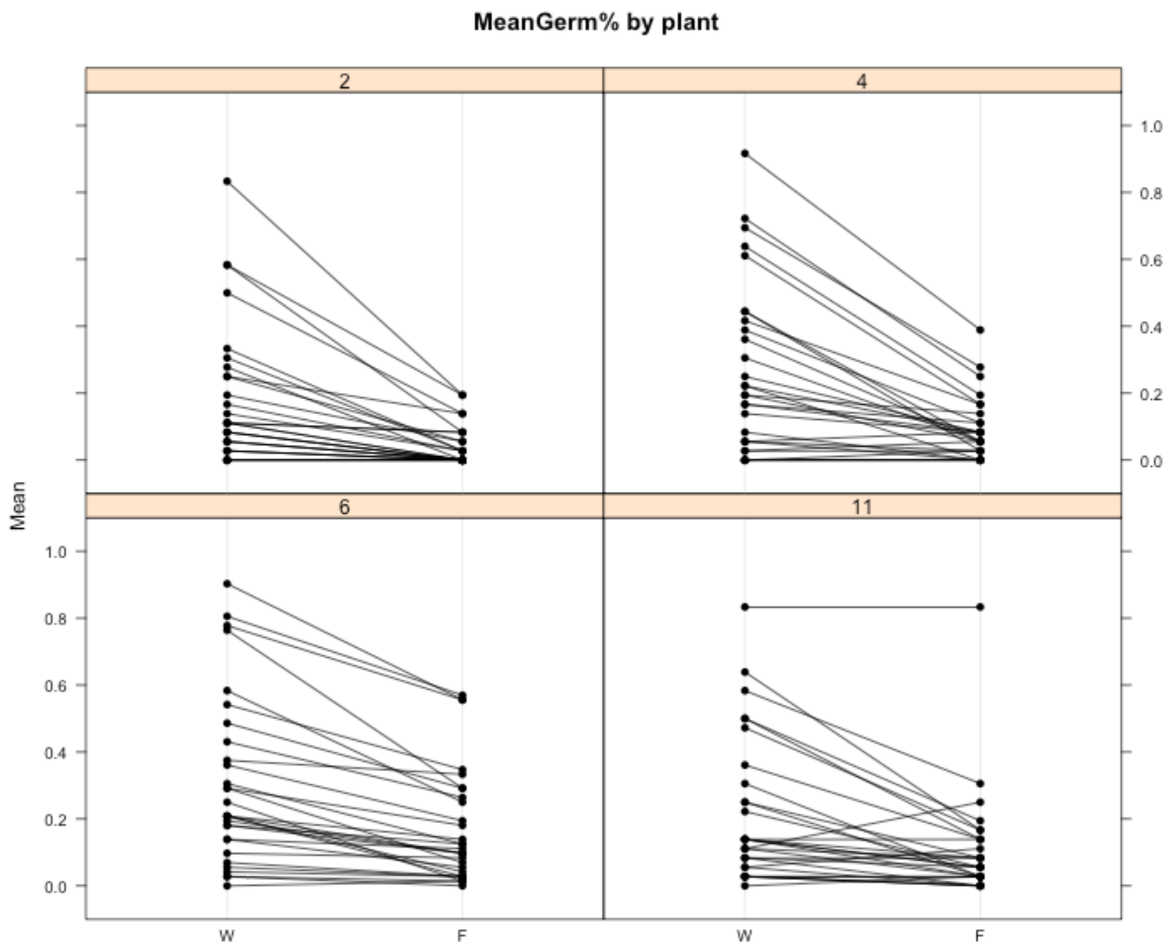


Figure 5. Reaction norms for each maternal plant (sibship), showing mean germination proportion in white light (‘W’) or green filter light (‘F’) in seeds aged for 2, 4, 6, or 11 months. Each line represents a single sibship. Each panel shows the relationship in different periods of after-ripening. Downward sloping lines represent negative responses to a canopy.

- <u>Germination responses to light within a population are largely uniform in direction</u>

Seeds within the natural population exhibited a significant negative response to a simulated vegetative canopy. This effect was observed throughout after-ripening. Virtually all progeny collected in this population appeared to reduce germination in the presence of filtered light (Figure 5). This observation supports our hypothesis that different lineages within a natural population will exhibit largely uniform responses to neighboring light cues. Our previous work showed that global ecotypes differ in their directional response of neighboring vegetation (Schieder, Leverett, Donohue 2014). This suggests the possibility that plastic germination responses in different global populations adapt to their local communities in unique ways. This inference would suggest that seedlings in this population emerging with neighbors will have lower fitness - possibly as a result of competitive interaction - hence the (assumed adaptive) response to avoid germination in crowded environments. If germinating in the presence of neighbors was beneficial, we would expect higher germination proportions in the canopy treatment. Follow-up work is currently determining the relationship between this population and natural neighbors, specifically identifying whether it is always beneficial to avoid germination with neighbors. It is likely that changing variables such as abiotic conditions alter the interactions plants have with their ecological neighbors. It is important to note that our filter treatment affected both light irradiance and the red to far-red ratio, thus we cannot conclude which aspect was the effector of germination responses. Current work is researching how each component may contribute to germination responses in seeds.

- <u>After-ripening decreases dormancy and modulates the effect of light on germination.</u>

Seeds largely exhibited increasing germination proportions over after-ripening time, supporting previous findings. After-ripening time also modulated the effect of a vegetative canopy over time, weakening the effect of a canopy as the seeds after-ripen. This result suggests that seeds are most selective about the conditions in which they will germinate upon dispersal, and as the seeds mature, the selectivity against germinating among neighboring vegetation weakens. Germinating prematurely increases the likelihood of seedling death[2], however, this strategy might be worth the risk should the environment be unoccupied and thus lacking competitive interactions which would otherwise diminish the fitness of the plant. However, this ignores the possibility of facilitative effects.

- <u>Seeds may exhibit dormancy cycling as the seeds after-ripen</u>

Despite the general trend of seeds losing dormancy with after-ripening, a notable exception to the tendency to increase germination occurred after 11 months of after-ripening. Between 6 and 11 months of after-ripening, germination proportions decreased in both light treatments. This could be interpreted as increasing dormancy. Dormancy in *A. thaliana* has been well documented to cycle yearly to synchronize germination with optimal conditions (Auge et al. 2015). Should a seed not germinate - possibly as a result of neighbor cues - a mechanism needs to exist so that the seed may exit the seed bank later in the appropriate season and conditions. Germinating too early or late has dire consequences for the plant, and thus, dormancy cycling may provide a mechanism for appropriately timing germination by keeping track of time beyond the immediate season. However, it is worth noting that this observation could also be the result of seed death, with lower germination proportions the result of fewer

[2] Source Needed!

viable seeds. Seed viability was not experimentally tested at 11 months, although visually the seeds did not appear to be dead and remained firm.

- Maternal effects on germination are unexpected and contradictory to seed responses to light

The maternal environment, modeled using principal component analysis, correlates more crowded maternal environments with higher seed germination proportion. This effect has been previously observed in *A. thaliana*. (Leverett et al. 2015) The association between more crowded maternal environments with higher germination proportions contradicts our hypothesis. This directionality seems to be in contradiction with lower germination proportions being associated with canopy filtered light. That is, this work suggests that maternal neighbor light signals increase the likelihood of seeds germinating in a more crowded environment, while seed neighbor light signals decrease the likelihood. One possibility is that maternal plants may strategize to produce fewer numbers of seeds with higher quality in the presence of neighbors. Seed size is known to affect germination phenotype (Riefler et al. 2006, Venable and Brown 1987), but we can not conclude anything in this experiment because we did not measure seed mass. However, it is important to note that we can not conclude whether these responses are caused just by light signals. Other cues such as resource limitation may be significant factors.

Dueling cues may be the effect of different abilities of the seed or maternal environment to predict conditions (DeWitt, Sih, and Wilson 1998). This inconsistency could be interpreted in several ways. There could an important spatial aspect to neighbor presence, in that the seed may be dispersed a distance away from the maternal plant. Temporally, the maternal plant would be making a prediction about the progeny environment, rather than directly responding. Relative size of neighbors may also be important. Neighboring plants filtering light to maturing seeds are likely larger than neighbors filtering light to germinating seeds. It could be that smaller plants are more likely to compete within the niche of *A. thaliana*, thus producing a more negative germination response. Or larger neighbors may be more likely to confer facilitation. Further complicating the matter is the concept of micro-site quality. It is expected that areas offering good conditions and soil nutrients for plants will be more densely populated than resource-poor areas. Thus, although neighbors may present competition for resources, their presence may indicate higher quality environments and thus cue higher germination proportions in progeny seeds.

The observed maternal effect on germination was observed to inversely correlate with germination proportions, i.e., maternal effects were most pertinent in periods of high dormancy. Previous work has suggested that maternal effects decrease in importance after dispersal.[3] From harvest to 6 months of after-ripening, the importance of PC1 is seen to diminish in predicting germination (Table 8). However, During the apparent induction of dormancy between 6 and 11 months, the significance of the maternal effect appears to increase in significance (Table 8). This suggests that maternal effects may carry through secondary dormancy, which has been previously implicated (Auge et al. 2015).

- Significance of variation within a population; genetic or plastic.

It is important to note that our conclusions about the effect of maternal environment on germination are complicated by the unknown relations of the maternal plants. Plants within a

[3] Source NEEDED

single population of *Arabidopsis* could be identical or very similar due to high incidence of self-fertilization and inbreeding (Bergelson et al. 1998). If the genetic relation was high, most of the variation could be attributed to plastic germination responses to the environmental conditions. It has been hypothesized that there exists an inverse relationship between heterozygosity and phenotypic plasticity (Schlichting 1986). However, without concrete knowledge of the genetic relatedness of the sibships we used in this study, we cannot conclude for certain how much of the variance in germination responses to canopy light cues is due to maternal environmental as opposed to genetic effects. Even in a population, large genetic differences can persist given the low gene flow caused by self-fertilization (Tian et al. 2002).

- Future direction of projects

With knowledge of the effect of neighbor cues on germination in this population, the next step is to determine whether these responses are adaptive. We expect negative plastic germination responses to neighbors to be an adaptation to largely negative interactions of neighbors on fitness given the effect of light imbibition treatment. However, neighbor interactions are likely highly context-dependent effects. The effect sizes of neighbor competition, along with possible facilitative effects, change in context to different conditions and across the life cycle. (Callaway and Walker 1997, Miriti 2006). Generally, it is thought that more stressful environments may tend to promote more positive interactions via drought tolerance and other mechanisms (Bertness and Callaway 1993, Maestre et al. 2009, Callaway et al. 2002) Current work is testing whether this population always experiences negative effects of neighbors, specifically addressing whether neighbors of different sizes in stressful conditions can change the adaptive fitness of the neighbor response.

Our previous work identified global ecotypes responding in different ways to a vegetative canopy. This natural population exhibited a largely uniform response to neighbor light cues. Because some of the global populations exhibited a propensity for higher germination proportions under a vegetative canopy, perhaps this suggests that these genotypes have adapted to neighbors presenting less competition and/or more facilitation. How plastic germination responses of neighbors affect fitness in different populations of *A. thaliana* is an interest of our future work.

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