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Maternal effects alter natural selection on phytochromes through seed germination

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Summary

1. Phytochromes regulate seed germination in response to light and temperature, and different phytochromes contribute to germination under different environmental conditions.

2. Using *Arabidopsis thaliana* mutants with different combinations of non-functional phytochromes, we tested which phytochromes contribute to germination and other life-history traits under field conditions and whether that contribution changes with seed-maturation temperature. We also quantified natural selection on phytochrome variants through their influence on seed germination.

3. We found that some phytochromes contributed to germination under field conditions and that the phytochrome that contributed most strongly depended on seed-maturation temperature. Specifically, when seeds were matured under warm temperature, *phyA* and *phyE* null plants had the most strongly reduced germination, with *phyA* not able to germinate late in the season. In contrast, when seeds were matured under cool temperature, *phyB* nulls had the most reduced germination, and effects of the *phyA* mutation were apparent only on a *phyB* background.

4. These effects on germination translated to effects on total lifetime fitness, such that selection on phytochromes that contributed to germination sometimes depended on seed-maturation conditions.

5. *Synthesis*. Natural selection on phytochromes occurs through their effects on seed germination, and maternal effects alter phytochrome contributions to germination. Therefore, maternal effects can alter natural selection on phytochromes. The results demonstrate a novel role of maternal effects in contributing to variable natural selection on specific genes associated with plant responses to climatic conditions.

Key-words: dormancy, germination, life-history traits, maternal effects, natural selection, phytochrome, plant development, plasticity

Introduction

Phytochromes are among the most important environmental sensors in plants, and they regulate numerous aspects of plant growth and development from germination to floral induction (Chen *et al*. 2004). Being photoreceptors of red and far-red light, they perceive cues of seed burial, competition from a vegetative canopy and day length (Ballare *et al*. 1987; Casal & Sanchez 1998; Casal *et al*. 2003; Mathews 2006). As such, phytochromes are essential for regulating morphological and phenological responses to complex ecological conditions.

The phytochrome apoproteins are encoded by a small family of genes, and the Brassicaceae, which includes *Arabidopsis thaliana*, has five phytochrome genes (Sharrock & Quail 1989; Clack, Mathews & Sharrock 1994; Mathews & Sharrock 1997). An early duplication resulted in two phytochrome clades, one of which contains *PHYA* and *PHYC* genes and the other contains *PHYB*, *PHYD* and *PHYE*. *PHYD* is a recent duplication, similar in sequence to *PHYB*, which occurs only in the Brassicaceae. These phytochrome genes differ in both coding and regulatory sequences.

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The ecological significance of the duplication of genes that encode environmental sensors is of great interest. In the case of phytochromes, the different duplicated genes often contribute to the same basic process, but each copy does so under somewhat different conditions. This sort of functional diversification of the phytochromes has been documented for both germination and flowering.

Regarding germination, phyA protein promotes germination after low levels of far-red light, whereas phyB promotes germination under red light, and acts antagonistically to phyA under far-red light (Shinomura *et al*. 1994; Poppe & Schafer 1997; Shinomura 1997; Ritchie & Gilroy 1998; Koornneef, Bentsink & Hilhorst 2002; Holdsworth, Bentsink & Soppe 2008). PhyE contributes to germination in continuous FR light (Hennig *et al*. 2002), and phyD has a role in red light and in inhibiting phyA-mediated germination in FR light (Hennig *et al*. 2001). Thus, the phytochromes have diversified with respect to the light conditions under which they contribute to germination.

Phytochromes also differ in their temperature-dependent contributions to germination (Donohue *et al*. 2007, 2008; Heschel *et al*. 2007, 2008). Specifically, *PHYA* and *PHYB* appear to be most important for promoting germination at warm temperatures, but *PHYE* is most important for promoting germination at low temperature. *PHYD* contributes to germination most strongly after imbibition at warm temperature or when seeds are matured under cool temperatures. Temperature-dependent contributions of different phytochromes to flowering have also been reported in *A. thaliana* (Franklin *et al*. 2003; Halliday & Whitelam 2003; Halliday *et al*. 2003; Franklin & Whitelam 2004).

Such environment-specific contributions of different phytochromes to plant development are significant for two reasons. First, this sort of functional diversification can provide a potential mechanism for extremely precise responses to different combinations or sequences of environmental conditions. Second, it suggests that effects of phytochrome variation would be apparent only under specific ecological conditions and that natural selection on specific phytochromes would be highly environment-dependent; some phytochromes would be exposed to selection in some environments, but others would be under selection in other conditions.

Evidence for variable natural selection on phytochromes is mainly indirect, consisting of the presence of natural genetic variation in phytochrome genes. Functionally significant sequence variation in individual phytochrome genes of *A. thaliana*, including natural null mutations, has been detected through phenotypic screens of hypocotyl elongation and flowering time (Aukerman *et al*. 1997; Maloof *et al*. 2001; Balasubramanian *et al*. 2006; Filiault *et al*. 2008; Atwell *et al*. 2010). Other evidence consists of molecular signatures of natural selection on phytochrome genes in diverse taxa (e.g. White, Hamblin & Kresovich 2004; Ikeda, Fujii & Setoguchi 2009; Ikeda & Setoguchi 2010). Thus, functionally significant natural variation in phytochromes does exist, but its adaptive significance is not known.

Here, we measure environment-dependent natural selection on phytochrome nulls across the life cycle of *Arabidopsis thaliana*, beginning with seed germination. Past studies of natural selection in *A. thaliana* showed that selection on germination timing is intense and that adaptive germination in the autumn strongly affects projected population growth rates (Donohue *et al*. 2005; Donohue 2009; Huang *et al*. 2010). Thus, genes that influence germination are likely to be subjected to natural selection in *A. thaliana*.

Maternal effects on germination are extremely common and often very strong (reviewed in Donohue 2009). Such maternal effects on seed traits have been shown to influence life-history expression, population dynamics and even the genes that are associated with the expression of seed traits (Galloway 2001; Galloway & Etterson 2007; Donohue 2009). For example, studies of germination in *A. thaliana* have shown pronounced effects of seed-maturation conditions on germination and on the genetic basis of germination, including the contributions of phytochromes to germination (Donohue *et al*. 2008). Thus, seed-maturation conditions are expected to influence the strength of natural selection on phytochromes through their effects on germination.

We tested whether maternal effects altered natural selection on phytochromes using a set of phytochrome null mutations. Replicate plants with mutations in *PHYA*, *PHYB*, *PHYD* and *PHYE* were grown under different temperatures that represent temperatures during different seasons of seed maturation, and then fresh seeds were dispersed into the field. These seeds were followed throughout their lives, and germination, flowering and fitness were monitored. In this manner, we were able to (i) measure the effects of phytochrome disruption on different life stages, including germination, (ii) measure the fitness consequences of phytochrome disruption and (iii) test whether differences in germination accounted for any fitness effects of phytochrome disruption. In this manner, we tested whether seed-maturation temperature altered phenotypic effects of and natural selection on phytochromes.

Materials and methods

Under field conditions, we compared the germination of a sample of *Arabidopsis thaliana* phytochrome mutants to those of their background ecotype, Landsberg *erecta* (L*er* hereafter). Table 1 lists the mutants used in this study and their original sources. With the exception of *phyD-1*, all *phy* mutations in Table 1 were isolated in the L*er* genetic background. The *phyD-1* mutation was identified as a natural null mutation in the Wassilewskija (Ws) ecotype (Aukerman *et al*. 1997) and introgressed onto the L*er* background. The corresponding backcrossed L*er* line, which contained the wild-type L*er PHYD* allele (as opposed to the *phyD* allele), was used for all comparisons of mutants containing *phyD*. However, this background genotype did not differ significantly from the standard L*er* genotype, so it is not presented in the figures in this paper. We also used the same L*er* line from which the *phyE* mutant was isolated (L*er*' hereafter) for all comparisons to *phyE* (Devlin, Patel & Whitelam 1998). This background differed slightly from the standard L*er*, so it is included in the figures.

Seeds were matured under two temperatures in Conviron E7/2 growth chambers (Controlled Environment, Ltd., Winnipeg, MB,

Table 1. List of genotypes and their sources. 'Reference name' is the name given to the line in this paper

Reference name	Type of mutation	Allele	Source-stock number
L*er*	'Wild type'	Landsberg *erecta*	ABRC-CS20
phyA	Deficient (null)	*phyA-201*	ABRC-CS6219
phyB	Null	*phyB-5*	ABRC-CS6213
phyD	Natural null (Ws ecotype)	*phyD-1*	RAS
phyA/phyB		*phyA-201/phyB5*	ABRC-CS6224
phyB/phyD		*phyB-1/phyD-1*	RAS
L*er'*	'wild type'	Landsberg *erecta*	CS20 used in the construction of *phyE,* GCW
phyE	Null	*phyE-1*	GCW

ABRC, Arabidopsis Biological Resource Center at Ohio State University. RAS, R. A. Sharrock; GCW, G. C. Whitelam.

Canada): The 'Warm' treatment imposed a 10-h light/14-h dark cycle at 22 °C and resembles the seasonal conditions during seed maturation in mid-spring. The 'Cool' treatment imposed a 10-h light/14-h dark cycle at 10 °C and resembles the seasonal conditions during seed maturation when plants mature seeds in the autumn or very early spring in New England. Plants were grown in four blocks (chamber compartments) per treatment, with two replicates of each genotype in each compartment. Plantings were staggered so that seeds of all genotypes in all treatments matured simultaneously. Seeds were harvested over a period of 1 week, and seeds were pooled over blocks for use in the field. Seeds were kept dry at room temperature during this processing time and were deposited in the field within 1 week after harvesting.

The field plot was in an old-field site at the Concord Field Station of Harvard University, in Bedford, MA, USA. Ten blocks were established, with two replicates of each genotype × seed-maturation combination per block. Seeds were deposited into peat pots filled with Metromix 360 (Scotts Sierra, Marysville, OH, USA); this was carried out to minimize the variation in soil conditions and to focus on effects of the seasonal environment on germination phenology. Twelve seeds of a given genotype × seed-maturation temperature combination were placed in a given pot. The pot was the unit of analysis. Seeds were dispersed in early July, which was 2–3 weeks after the natural dispersal season. Temperature and precipitation data from the nearest weather station (Hanscom Air Force Base, Bedford, MA, USA) for the duration of the experiment are provided in Fig. S1. While the experimental seeds would not have experienced any potential late cold spells, as naturally dispersed seeds may have, the experimental seeds did experience the full cycle of warm summer conditions followed by autumn conditions of fluctuating temperatures.

Within each block, one of the two genotype × seed-maturation replicates was designated for germination censuses only. During weekly censuses, all germinants were recorded and then plucked from the pot to avoid any suppression of germination by the rosettes. These pots were used for estimates of germination timing and germination proportions. The mean germination timing of all germinants within a pot was calculated, and the total proportion of seeds that germinated during the course of the experiment in each pot was also recorded. Because spring germination was so uncommon ($< 1\%$), we focused our analysis on germination timing in the autumn (before February). The second replicate pot was used for post-germination life-history data. Within these pots, the germination timing of each seed was recorded through weekly monitoring, and a single random focal individual was followed in each pot. The random individual was the germinant (older or newer) that was closest to the centre of the pot, and all other germinants were plucked from the pot during each census. The focal individual was followed throughout its life, and survival from germination to bolting (initiation of reproduction), day of bolting, size at bolting (rosette diameter and number of leaves) and the total number of siliques produced (zero if it died before reproducing) were recorded. Total lifetime fitness was estimated as the probability of germination (proportion of seeds that germinated in the pot, based on the pots used for the germination censuses only), times the total number of siliques produced.

To test for significant differences among genotypes, seed-maturation conditions and their interactions, we first conducted analyses of germination, life-history traits and fitness with all genotypes in a full model, with genotype, seed-maturation treatment and block as fixed factors in analysis of variance (SAS proc GLM). We analysed the pot-mean germination day in autumn and pot-mean germination proportion, and for focal individuals, we analysed the bolting time, rosette diameter at bolting, number of leaves at bolting, total silique production and total lifetime fitness. Survival to bolting was analysed using logistic regression (SAS proc Catmod). To test for effects of specific phytochromes, we compared each mutant to that of the appropriate background, as well as differences between specific mutant pairs, using *a priori* contrasts in a model that included all lines. Because *phyE* was compared to a different background (L*er'*) than the other mutants, it was analysed separately. Because data were not always normally distributed, we verified the significance of ANOVA results with a series of nonparametric Kruskal–Wallis tests. To interpret the interactions between genotype and seed-maturation treatment, we tested differences between mutants and their background genotype within each seed-maturation treatment separately.

Because phytochromes regulate both germination and flowering phenology, we estimated the relative contributions of germination and flowering phenology to total lifetime fitness by conducting phenotypic selection analysis (Lande & Arnold 1983) within each seed-maturation treatment. We tested for significant differences in selection gradients between seed-maturation treatments by testing for significant trait × treatment interactions using ANCOVA. To test whether effects of phytochrome nulls on fitness could be accounted for by their effects on germination, we tested for significant differences between mutants and their background with and without germination traits in an analysis of covariance; if fitness differences between the genotypes were no longer significant when covariates (germination measures) were also included in the model, then this is evidence that the effects of phytochrome nulls on those traits can account for the effects of phytochrome nulls on fitness.

Results

EFFECTS OF PHYTOCHROME DISRUPTION ON GERMINATION AND FLOWERING IN THE FIELD

Mutations in some phytochromes influenced germination timing in the field, but the effects of particular phytochromes depended on seed-maturation conditions (Fig. 1a,b). First, on average across all genotypes, seeds matured under warmer temperatures had earlier germination and germinated to higher percentages than seeds matured under cooler

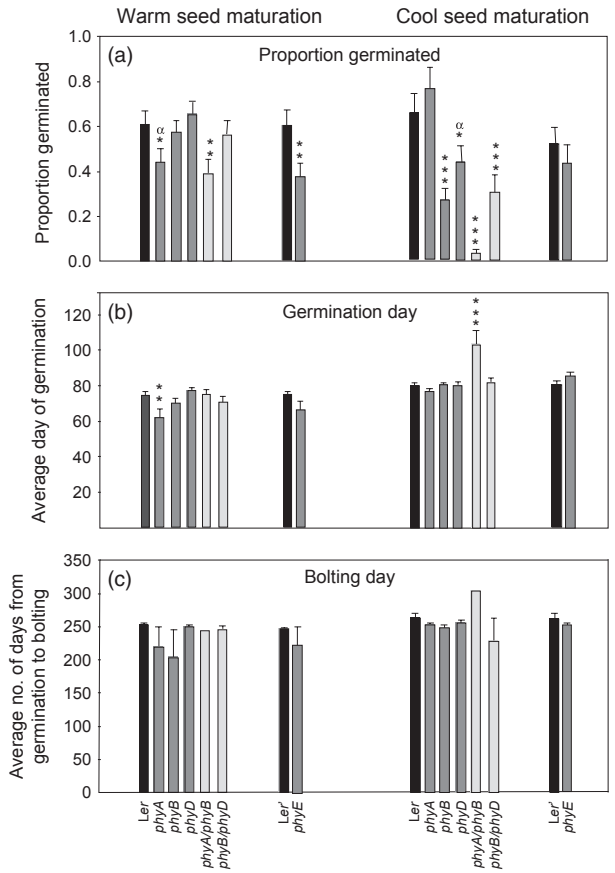

Fig. 1. Genotype mean and standard errors for germination proportion (a), germination time (b), and bolting time (c) of seeds matured under warm (22 °C) and cool (10 °C) temperature. Asterisks indicate significant difference from the appropriate background genotype in planned contrasts from ANOVA. 'L*er*' is the background for *phyA*, *phyB*, *phyD*, *phyA/phyB* and *phyB/phyD*. L*er*' is the background for *phyE*. $*P<0.05$, $**P<0.01$, $***P<0.001$. $^{\alpha}$Not significant in Kruskal–Wallis test.

temperatures (for *phyA, phyB, phyD* comparisons: F(proportion: temperature) = 16.16, $P < 0.001$, d.f. = 1; F(timing: temperature) = 46.12, $P < 0.001$, d.f. = 1; for *phyE* comparisons: F(proportion:temperature) = 2.48, $P > 0.05$, d.f. = 1; F(timing:temperature) = 22.21, $P < 0.001$, d.f. = 1). However, some genotypes differed significantly in their response to seed-maturation temperature (for *phyA, phyB, phyD* comparisons: F(proportion: genotype × temperature) = 3.66, $P = 0.0035$, d.f. = 5; F(timing: genotype × temperature) = 9.86, $P < 0.001$, d.f. = 5; for *phyE* comparisons: F(proportion: genotype × temperature) = 1.25, $P > 0.05$, d.f. = 1; F(timing: genotype × temperature) = 6.35, $P = 0.014$, d.f. = 1). For seeds matured under warmer temperatures, *phyA* germinated earlier than its wild type, and its total germination percentage was lower (significant in ANOVA but not Kruskal–Wallis), suggesting that *phyA* seeds did not germinate well late in the season. *phyE* also had lower total germination than its corresponding wild type, but its germination timing did not differ, suggesting that *phyE* mutants had lower germination throughout the season.

In contrast, in seeds matured under cool temperature, disruption of *PHYB* had the largest effect on germination; *phyB* and its double mutants had greatly reduced germination percentages. The *phyB* mutant had no effect on germination timing, suggesting *phyB* seeds were less able to germinate throughout the season. In cool-matured seeds, *phyD* mutants also had lower germination proportions (significant in ANOVA but not Kruskal–Wallis). The effect of the *phyA* mutation was only apparent on a *phyB* background, and the *phyA/phyB* double mutant had delayed germination compared with wild type. The *phyE* mutant no longer had a significant effect on germination proportion, but *phyE* seeds germinated slightly later than wild type (significant only in Kruskal–Wallis). Thus, the phytochrome that had the strongest effect on germination depended significantly on seed-maturation temperature.

Surprisingly, while cool seed-maturation temperature delayed flowering (for seeds on L*er* but not L*er*' backgrounds; F(maturation temperature) = 3.99, $P = 0.05$, d.f. = 1), phytochrome disruption had no significant effect on flowering time in the field (Fig. 1c). Phytochrome genotype also did not significantly affect rosette diameter at bolting ($F < 2.18$, $P > 0.05$ for all contrasts; figures not presented) or number of leaves at bolting ($F < 2.87$, $P > 0.05$ for all contrasts except *phyE*: F(genotype) = 4.33, $P = 0.0616$ for warm-matured seeds; Fig. S2).

NATURAL SELECTION ON PHYTOCHROME NULLS VIA THEIR EFFECTS ON GERMINATION

As expected, many phytochrome mutants had reduced fitness compared with their respective wild-type genotypes. *phyB* and its double mutants had reduced survival to bolting (Fig. 2a; significant in warm seed-maturation treatment), and these effects were not significantly different across seed-maturation temperature [chi-square(temperature × genotype) < 2.79 and non-significant in all CATMOD models].

All mutants except *phyE* had reduced silique production compared with wild type when seeds were matured in warm conditions (Fig. 2b; *phyA* and *phyD* not significant in Kruskal–Wallis tests). When seeds were matured under cool temperature, *phyA/phyB* and *phyB/phyD* mutants had lower silique production than wild type, but *phyA* seeds actually had higher fruit production [F(genotype × temperature) = 6.06, $P < 0.001$, d.f. = 5, $N = 123$]. In addition, the reduction of silique production of the single *phyB* and *phyD* mutants was no longer significant in cool-matured seeds.

Mutants containing *phyA* and/or *phyB* had reduced total lifetime fitness compared with the wild type when seeds were matured under warm temperature (Fig. 2c; single mutants not significant in Kruskal–Wallis tests, and *phyB* was only marginally significant with $P < 0.1$ in ANOVA), and the magnitude and direction of the effect on fitness depended on seed-maturation temperatures (*phyA*, *phyB*, *phyD* comparisons: F(genotype × temperature) = 6.05, $P < 0.001$, d.f. = 5, $N = 123$). The *phyE* mutant had slight but non-significant reduced total fitness in warm-matured seeds (significantly lower than L*er*, but not L*er*'), and the fitness reduction did not differ signifi-

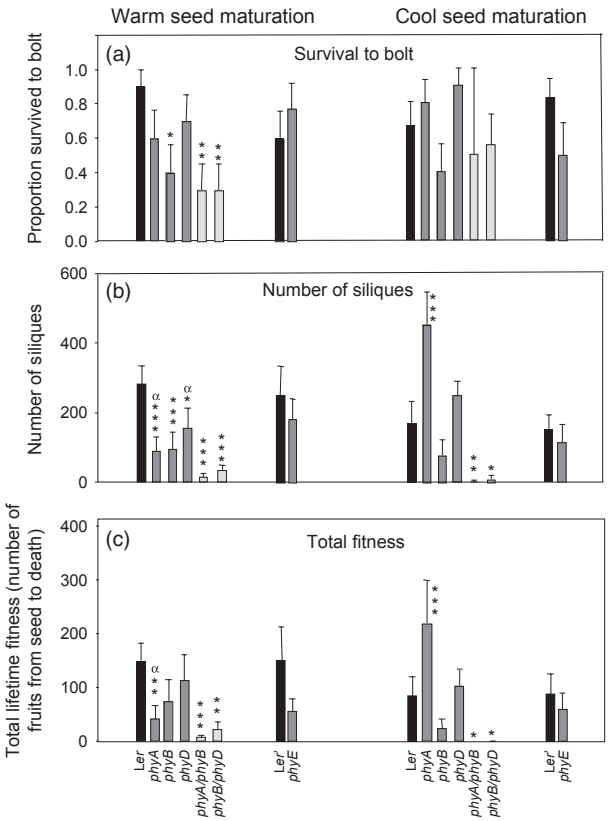

Fig. 2. Genotype mean and standard errors for survival to bolting (a), number of siliques produced (b), and total fitness (c) of seeds matured under warm (22 °C) and cool (10 °C) temperature. Asterisks indicate significant difference from the appropriate background genotype in planned contrasts from ANOVA (see Fig. 1 for further details.) $*P<0.05$, $**P<0.01$, $***P<0.001$. $^{\alpha}$Not significant in Kruskal–Wallis test.

cantly across seed-maturation treatments [F(genotype × temperature) = 0.63, $P > 0.05$, d.f. = 1, $N = 41$]. Cool-matured seeds of the *phyA* mutant had higher fitness than the wild type, reflecting its higher silique production. In summary, selection on phytochrome nulls was detectable in the field, but the strength and direction of selection on some phytochromes depended on seed-maturation temperature.

Significant selection was detected on germination but not bolting time (Table 2). Earlier germination was favourable, especially when seeds were matured under cool conditions, although selection on germination time was not significantly different across seed-maturation treatments. Percentage germination was a significant contributor to total fitness in both seed-maturation treatments.

Because phytochrome disruption influenced the germination, and germination influenced the fitness, we tested whether the observed effects of phytochrome disruption on fitness could be accounted for by its effects on germination (Table 3). For seeds matured in warm temperature, the effects of *PHYA* disruption on germination timing accounted for fitness differences between wild type and *phyA* mutants. Effects of *PHYE* disruption on germination timing and proportion accounted for fitness differences between *phyE* and the L*er* background.

Table 2. Phenotypic selection analysis of germination time, germination proportion and bolting time. Traits were standardized to have a mean = 0 and SD = 1. The strength of selection, or selection gradients, β (multiple regression coefficients), are given for each seed-maturation temperature separately. 'Trait × temperature' gives the F-ratio of analysis of covariance to test whether selection differs significantly depending on whether seeds were matured in cool vs. warm temperature

Trait	β-Warm	β-Cool	Trait × temperature
Germination day	−0.18	−0.74**	0.18
Per cent germination	0.91***	1.46***	2.37
Bolting day	0.81	0.33	0.02

$**P<0.01$, $***P<0.001$.

Table 3. Test of whether mutational effects on germination account for differences in fitness between mutants and wild types for each contrast. Results are given for seeds matured under warm (22 °C: A) and cool (10 °C: B) temperature. Total lifetime fitness was estimated as the probability of germination (proportion of seeds that germinated in the pot) times the total number of siliques produced. The table shows least-squares (LS) means for the effect of the mutant on fitness, with and without germination covariates. 'Difference between genotypes' is the LS mean difference between genotypes with no covariates in ANOVA. 'Germination day' is the LS mean difference between genotypes when the day of germination is included in the model. 'Percentage germinated' is the LS mean difference between genotypes when the total germination proportion is included in the model. 'Both' is the LS mean difference between genotypes when both germination day and total per cent germination are included as covariates. Asterisks indicate significant differences between mutants and wild types. When significant differences are apparent without covariates but disappear when covariates are included, effects of mutation on the covariate are interpreted as accounting for effects of mutation on fitness. Only contrasts with significant effects of the mutation are shown

Contrast	Difference between genotypes	Germination day	Proportion germinated	Both
(A) Warm seed maturation				
L*er* vs. *phyA*	92.5*	73.9	96.5*	68.1
L*er* vs. *phyE*†	92.2*	73.4	40.9	−2.6
L*er* vs. *phyA/phyB*	141.8***	140***	128.1***	130.3**
L*er* vs. *phyB/phyD*	126.3**	124**	123.5**	114.5**
(B) Cool seed maturation				
L*er* vs. *phyA*	−207.8*	−191.3*	−198.9*	−182.2*
L*er* vs. *phyA/phyB*	85.4*	49.1	61.5	27.6
L*er* vs. *phyB/phyD*	84.6*	86.2*	77.7	75.6

$*P<0.05$, $**P<0.01$, $***P<0.001$.
†Significant difference from L*er* but not L*er*′. Comparisons here are to L*er*.

However, fitness differences between wild type and double mutants with *phyB* were not accounted for by differences in germination.

In seeds matured under cool conditions, effects of phytochrome disruption on germination timing and germination proportion accounted for fitness differences between wild type and *phyA/phyB* mutants (Table 3). Differences in germination proportion accounted for fitness differences between wild type and *phyB/phyD* mutants. The increase in fitness of *phyA* seeds relative to wild type was independent of germination.

Thus, effects of phytochrome disruption on germination accounted for some fitness effects of phytochrome disruption. Some phytochromes that had environment-dependent effects on germination also had environment-dependent contributions of germination to fitness, indicating that maternal effects on phytochrome contributions to germination can contribute to environment-dependent selection on phytochromes.

Discussion

Multiple phytochromes contributed to germination under field conditions, but which phytochromes contributed depended on the seed-maturation temperature. Phytochrome disruption did not significantly alter the timing of reproduction (i.e. flowering) under the conditions of this experiment. Thus, germination appears to be an especially important developmental transition regulated by phytochromes under field conditions, perhaps even more important than later life stages.

Effects of phytochrome disruption on germination in the field were consistent with some of their documented effects under laboratory conditions (Heschel *et al.* 2007, 2008; Donohue *et al.* 2008). For instance, the effect of *phyD* and *phyB* was more pronounced in the laboratory when seeds were matured at cool as opposed to warm temperature, consistent with these results from the field. However, while *phyE* mutants did have lower germination overall in the field, there was no evidence that the *phyE* mutation preferentially prevented germination under cooler conditions (presumably later in the season), nor that the *phyA* mutation preferentially prevented germination under warmer conditions (presumably earlier in the season), as would be expected based on laboratory studies (Heschel *et al.* 2007). Thus, the effects of seed-maturation temperature were more predictable from laboratory results than effects of post-dispersal temperature in this study.

The lower total germination percentage of phytochrome nulls is likely caused by the inability of phytochromes to germinate under specific conditions, as was observed in laboratory studies of these mutants (Heschel *et al.* 2007, 2008), especially in those mutants with impaired ability to germinate at specific times of the season. For mutants that had impaired germination throughout the season, seed viability could have been reduced, or the appropriate conditions for the germination of those mutants may not have occurred in the field. This reduction of the ability to germinate contributed to a reduction in total fitness. It is possible that ungerminated seeds could be able to germinate in future years, however. If so, those potentially dormant genotypes may have somewhat higher fitness than estimated here, depending on when they germinated.

As expected, phytochrome disruption also reduced fitness in many cases. Effects of phytochromes on germination fully accounted for those fitness reductions of *phyA/phyB* and *phyB/phyD* mutants under cool seed-maturation conditions, and they accounted for the fitness reduction of *phyA* and *phyE* mutants under the warm seed-maturation conditions. Therefore, while other traits may also contribute to fitness reduction of phytochrome mutants, phytochrome-mediated germination appears to be a significant potential source of natural selection on these phytochrome nulls. In contrast, germination did not account for the fitness effects of the double mutants containing *phyB*, namely *phyA/phyB* and *phyB/phyD*, under warm seed-maturation conditions. *PHYB* is known to play a role in germination under laboratory conditions even under warm seed-maturation temperatures (Shinomura *et al.* 1994; Shinomura 1997; Poppe & Schafer 1997; Hennig *et al.* 2001; Donohue *et al.* 2008; Heschel *et al.* 2008), and it is frequently cited as the most important phytochrome that regulates germination. However, results presented here indicate that under field conditions, its primary fitness effects seem to be through traits other than germination when seeds are matured under warm, but not cool, conditions.

As with germination, the effects of the disruption of some phytochromes on fitness depended on seed-maturation temperature. While the *phyA/phyB* and *phyB/phyD* mutants had significantly reduced fitness regardless of the maturation temperature, *phyA* and, to some degree, *phyE* had reduced fitness only under the warmer seed-maturation condition. This was also the condition in which *phyA* and *phyE* influenced germination. In fact, effects of *PHYA* and *PHYE* disruption on germination of warm-matured seeds fully accounted for the reduced fitness of these nulls (although the lower germination success of *phyE* mutants compared to its wild-type background was obscured by high variation in other aspects of fitness of the wild type). Likewise, *phyB* significantly altered germination only in cool-matured seeds, and the fitness reduction of mutants containing *phyB* was accounted for by germination only in cool-matured seeds. These results indicate that seed-maturation temperature can contribute to variable natural selection on phytochromes through its effects on germination.

Such maternal effects on germination, imposed by variation in seed-maturation temperature, could be manifest in the field via two processes. First, some plants may experience cooler seed-maturation temperatures simply because they inhabit cooler climates. Such geographic variation in seed-maturation temperature could result in geographic variation in selection on phytochromes, with phytochrome nulls of *phyA* and possibly *phyE* being exposed to selection via germination in warmer climates but masked from it in cooler climates.

The second process associated with variation in seed-maturation temperature is genetic variation in the tendency to flower under cooler conditions (as in earlier in spring or in autumn). Natural variation for flowering time is well documented in *A. thaliana* (e.g. Efmertova 1967, Alonso-Blanco *et al.* 2003, Nordborg & Bergleson 1999, Simpson & Dean 2002, Shindo *et al.* 2005, Werner *et al.* 2005, Korves *et al.* 2007). Genotypes that flower only under warmer conditions would have seeds in which phytochrome nulls of *phyA* and possibly *phyE* would be exposed to significant negative selection

via germination, but not those that flower under cooler temperatures. In genotypes that flower under cool conditions, selection could even favour the *phyA* null. Epistasis for fitness would then result from combinations of phytochrome and flowering alleles. Such epistasis could contribute to variable natural selection on phytochromes.

This study demonstrates that phytochromes significantly affect germination under field conditions and that their effects are influenced by maternal effects on germination. Such maternal effects can contribute to variable natural selection on some phytochromes, via geographic climatic variation or genetic variation in the season of flowering. Further study on the geographic distribution of phytochrome variants could test whether, for example, nulls of *phyA* and *phyE* are more apparent in cooler climates, where their effects on germination are masked. Likewise, tests for linkage disequilibrium between phytochrome and flowering alleles would also be informative for testing how epistasis for fitness, caused by maternal effects, might influence patterns of natural selection on individual phytochrome loci. Such potential contributions of maternal effects to variable natural selection on life-history loci should be considered when interpreting patterns of natural variation in life-history traits and its adaptive significance.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Weather data throughout the experiment, gathered from Hanscom Air Force Base weather station (Bedford, MA).

Figure S2. Genotype mean and standard errors for number of leaves (A) and rosette diameter (B).

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