New Phytologist

Research

A new role for phytochromes in temperature-dependent germination

M. Shane Heschel[1,4], Jessica Selby[1], Colleen Butler[1], Garry C. Whitelam[2], Robert A. Sharrock[3] and Kathleen Donohue[1]

[1]Department of Organismic and Evolutionary Biology, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA; [2]Department of Biology, University of Leicester, Leicester LE1 7RH, UK; [3]Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717-3140, USA; [4]Present address: Department of Biology, Colorado College, 14 East Cache La Poudre Street, Colorado Springs, CO 80903, USA

Author for correspondence:
M. S. Heschel
Tel: +1 719 389 6406
Fax: +1 719 389 6940
Email: Shane.Heschel@coloradocollege.edu

Received: *20 November 2006*
Accepted: *21 January 2007*

Summary

- Germination timing is a fundamental life-history trait, as seedling establishment predicates realized fitness in the wild. Light and temperature are two important cues by which seeds sense the proper season of germination. Using *Arabidopsis thaliana*, we provide evidence that phytochrome-mediated germination pathways simultaneously respond to light and temperature cues in ways that affect germination.
- Phytochrome mutant seeds were sown on agar plates and allowed to germinate in lit, growth chambers across a range of temperatures (7°C to 28°C).
- phyA had an important role in promoting germination at warmer temperatures, phyE was important to germination at colder temperatures and phyB was important to germination across a range of temperatures.
- Different phytochromes were required for germination at different temperatures, indicating a restriction or even a potential specialization of individual phytochrome activity as a function of temperature. This temperature-dependent activity of particular phytochromes reveals a potentially novel role for phytochrome pathways in regulating the seasonal timing of germination.

Key words: *Arabidopsis thaliana*, ecology, germination, life history, phytochrome, temperature.

New Phytologist (2007) **174**: 735–741

doi: 10.1111/j.1469-8137.2007.02044.x

Introduction

The timing of germination determines the environment experienced by plants throughout their lives (Donohue, 2003, 2005). Therefore, accurate assessment of a seasonal environment by seeds is crucial to the lifetime fitness of plants. Light and temperature are two major cues through which seeds sense the proper season of germination (Spalding & Folta, 2005), but little is known about the integration of light and temperature information to elicit germination (Fielding *et al.*, 1992; Yamaguchi & Kamiya, 2000; Steckel *et al.*, 2004). In particular, the genetic basis of germination responses to temperature is poorly understood. Temperature varies geographically and is also predicted to change with shifts in the global climate. Therefore, appropriate germination responses to temperature are necessary for successful establishment after long-distance dispersal and could be crucial for preserving adaptive life-history phenology under a changing climate. Understanding the genetic basis of germination sensitivity to temperature is fundamental for dissecting the interactions between ecology and germination and predicting responses to climate change scenarios. Using *Arabidopsis thaliana*, we demonstrate that phytochrome-mediated germination pathways are sensitive to both light and temperature, revealing a potentially novel role for phytochrome pathways in regulating the seasonal timing of germination.

Arabidopsis thaliana (Brassicaceae) is a highly selfing, weedy annual. The typical life history displayed by *A. thaliana* is a 'winter annual' where seeds germinate in the autumn, overwinter as rosettes, and reproduce in the spring. Some populations display a 'spring annual' life history in which seeds germinate in the early spring and reproduce later in the spring. An 'autumn annual' life history has also been observed in some populations, in which plants germinate and flower in the autumn (Thompson, 1994; Griffith *et al.*, 2004). The mechanism determining these different life history strategies in *A. thaliana* is influenced by both germination timing and vernalization requirements for flowering (Napp-Zinn, 1976; Nordborg & Bergelson, 1999). Therefore germination is a key determinant of life-history strategy and predicates realized fitness in the wild.

Global climate models predict changes to the environment that would directly alter the conditions that influence germination. Global climate change is predicted to influence temperature and precipitation and the variation of these factors (Watson *et al.*, 1997). This study focuses particularly on the predicted changes in temperature, because temperature represents one of the most important germination cues for seeds (Pons, 2000). Germination cueing to temperature has the potential to influence successful climate tracking and thereby influence species distributions. Since phytochrome genes have known importance to germination and have been shown to have temperature-dependent effects on flowering time (Halliday & Whitelam, 2003), phytochromes are likely to be important influences on phenological changes accompanying climate change and range expansion.

Phytochromes regulate germination responses to light. In *A. thaliana*, the genes *PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE* encode five distinct phytochromes, which are photoreversible biliproteins. Phytochromes are synthesized in the inactive red-absorbing (Pr) form (Casal & Sanchez, 1998; Franklin & Whitelam, 2004), and red light converts them to the bioactive far-red absorbing (Pfr) isomer that is involved in light-stimulated germination (Whitelam & Devlin, 1997); Pfr reverts to Pr in the dark. *PHYA* encodes light-labile phyA whereas the other phytochrome genes (*PHYB-E*) encode apoproteins that are more light-stable upon photoconversion to Pfr (Whitelam & Devlin, 1997). Active phytochrome regulates the synthesis of gibberellin (GA), which promotes germination (Toyomasu *et al.*, 1998; Yamaguchi *et al.*, 1998; Yamaguchi & Kamiya, 2000; Garcia-Martinez & Gil, 2002; Peng & Harberd, 2002; Ogawa *et al.*, 2003). Because temperature affects GA concentration, sensitivity to GA (Derkx & Karssen, 1993; Yamaguchi *et al.*, 2004), rate of dark reversion (Kristie & Fielding, 1994; Hennig & Schafer, 2001), and possibly the rate of phytochrome photoconversion (Pons, 1986), phytochromes may have temperature-dependent effects on germination. Therefore, we examined the relative contributions of different phytochromes to germination across a range of temperatures, focusing on phyA, phyB, and phyE after preliminary observations. We first assessed the germination frequencies of single and multiple loss-of-function phytochrome mutants at 10°C and 22°C. We then assessed their germination at a range of temperatures (7°C, 13°C, 16°C, 19°C, 25°C and 28°C). The range of temperatures used in the experiment spans that of different seasonal temperatures across a range of latitude. The temperature intervals were chosen to represent the degree of temperature alterations expected based on models of global climate change. The study therefore aims to investigate the changing role of different phytochromes in responding to changing environmental temperatures due to global climate change and range expansion.

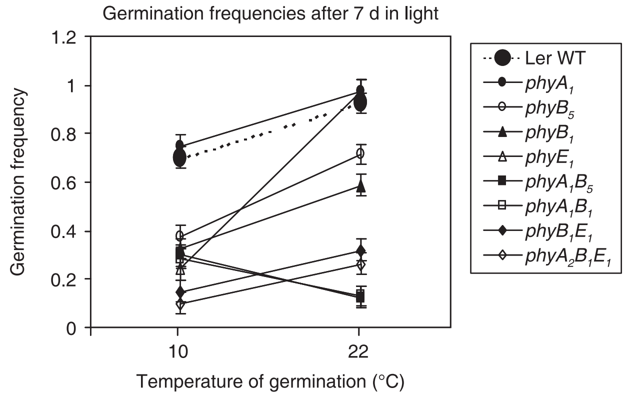

Fig. 1 Germination frequency after 7 d in the light for *Arabidopsis thaliana* single and multiple phytochrome loss-of-function mutants compared with wild type (Ler WT). The data represent mean values pooled across blocks in Expt 1. Large closed circles, Landsberg *erecta*; small filled circles, *phyA-201*, stock #CS6219; open circles, *phyB-5*, #CS6213; *filled squares*, *phyA-201/phyB-5*, #CS6224; filled triangles, *phyB-1*; open squares, *phyA-201/phyB-1*; open triangles, *phyE-1*; closed diamonds, *phyB-1/phyE-1*; open diamonds, *phyA-2/phyB-1/phyE-1*.

Materials and Methods

Plant material

Phytochrome mutants were generated by chemical mutagenesis and gamma radiation of the Landsberg *erecta* (henceforth Ler) background of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (see Fig. 1 for mutant list). Phytochrome mutant lines on the Ler background were supplied from three sources. Lines obtained from TAIR (The *Arabidopsis* Information Resource) included the monogenic *phyA* and *phyB* mutants as well as a double *phyA/phyB* knockout. These TAIR line mutations were created through chemical mutagenesis (Nagatani *et al.*, 1993). Lines generated by the R. A. Sharrock laboratory represented the monogenic *phyA* and *phyB*, and the double mutant *phyA/phyB* (Fig. 1). Mutants with a phyE deficiency (*phyE*, *phyB/phyE* and *phyA/phyB/phyE*) were generated by the G. C. Whitelam laboratory (Fig. 1). The *PHYA* allele in the triple mutant differs from the *PHYA* allele in the other lines.

Experimental conditions

All lines were grown at 22°C and 12 h of light to generate experimental seeds. The seeds from these maternal plants were first used to assess germination frequency at two temperatures (10°C and 22°C: Expt 1), and then at six temperatures (7°C, 13°C, 16°C, 19°C, 25°C, and 28°C: Expt 2). Twelve seeds per line were individually placed onto replicate Petri plates containing 0.5% agar, dark- and wet-stratified at 4°C for 5 d to break dormancy and then transferred to light.

For the first experiment, seeds were transferred after cold stratification to either 10°C or 22°C in Percival germination incubators (Percival Scientific, Inc., Perry, IA, USA). Seeds were given a 12-h photoperiod of white florescent light with a photon flux density (PFD) of approx. 90 μmol m^{-2} s^{-1}. The germination experiment was conducted during December 2003, with eight replicate plates per line per temperature treatment. Germination was scored after 0 d and 7 d in the light and seed viability was determined after 7 d.

For the second experiment, seeds were transferred after cold stratification to 7°C, 13°C, 16°C, 19°C, 25°C, or 28°C in Conviron E7/2 growth chambers (Controlled Environment Ltd., Winnipeg, Manitoba, Canada). Seeds were given a 12-h photoperiod of white florescent light set to half intensity, with a photon flux density (PFD) of approx. 100 μmol m^{-2} s^{-1}, such that light quality and quantity were comparable across the two experiments. The experiment was conducted in two temporal blocks, during August and September 2004. Each temporal block had 14 lines and four replicate plates per temperature treatment for a total of 672 plates. At each temperature, germination was scored after 0, 7, and 10 d in the light. Viability of seeds was assessed after 7 and 10 d in the light.

Data analysis

All statistical analyses were performed with the Statistical Analysis System (version 8.2; SAS Institute, Cary, NC, USA). The frequency of germination in the light was calculated as the number of germinants at 7 d, for example, minus the number of 0-d germinants, divided by the total number of viable seeds (the total number of seeds minus the number of dead seeds). Dead seeds were assessed by testing the firmness of hydrated seeds with microforceps; seeds without active embryos become less turgid as the endosperm degrades with time. To test whether the response of each mutant to temperature differed significantly from that of the wild type (Ler), we conducted a series of ANOVAs that included one mutant and the wild type in a model, with fixed factors of block, line, and temperature and the interaction between line and temperature. A significant interaction between line and temperature would indicate that the response of the mutant differed from that of the wild type. We next tested across blocks for a significant response to temperature for each line separately with a two-factor ANOVA (treatment effect was significant at $P = 0.05$ across all lines, results not presented), and then tested for significant differences between the mutant and wild type at each temperature separately using nonparametric Kruskal–Wallis tests. We also used Kruskal–Wallis nonparametric contrasts to test for significant differences in germination between specific pairs of mutants in each temperature treatment. To test whether *PHYA*, *PHYB*, and *PHYE* allelic effects were additive, we scored wild type, single mutants, and double mutants for functionality in the three phytochromes using dummy variables, with 1 being a functional state, and 0 being a null state. Using ANOVA, we tested for significant interactions between allelic states of the different phytochromes, which would indicate nonadditive contributions of different phytochromes to germination – namely that the effect of functionality of a given phytochrome depends on the functional state of the other phytochrome. Thus, we defined additive effects as independent effects of phytochrome alleles on germination. We tested for these interactions at both temperatures (10°C, 22°C) in Expt 1 and at the extreme temperatures (7°C, 28°C) in Expt 2 (the temperatures in which the mutational effects were most pronounced).

Results

Expt 1: 10°C and 22°C

The only mutants that did not differ from wild type were the *phyA* single mutant at both temperatures and the *phyE* single mutant at 22°C (Fig. 1, see the Supplementary Material, Table S1). All lines, including the wild type, responded significantly to temperature ($P < 0.05$ in all cases), with most lines germinating to higher proportions at 22°C than at 10°C. However, both *phyA*/*phyB* double mutants had lower germination at 22°C than at 10°C. The *phyE* single mutant had strongly reduced germination at 10°C, and therefore had a greater response to temperature than the wild type.

At 22°C, disrupting phyB function significantly reduced germination, as indicated by the significantly lower germination of both *phyB* single mutants. While the single *phyA* mutant did not have reduced germination compared with wild type, both *phyA*/*phyB* double mutants had lower germination than the single *phyB* mutant (see the Supplementary Material, Table S2), indicating that phyA also contributed to germination at 22°C. The *phyA*/*phyB* double mutants had up to 80% reduced germination at 22°C compared with the wild type (Fig. 1), and this difference was statistically significant (Table S1). The contributions of *PHYA* and *PHYB* to germination at 22°C were nonadditive (Table 1), with the contribution of *PHYA* being much more pronounced on a *phyB* background. However, germination of the *phyA*/*phyB*/*phyE* triple mutant was the same as that of the *phyB*/*phyE* double mutant, indicating that disruption of phyA in addition to both phyB and phyE did not significantly reduce germination further.

Germination was most strongly influenced by *PHYB* at 22°C, followed by nonadditive contributions by *PHYA*, and then *PHYE*. Disruption of phyE function alone did not influence germination at 22°C (Table S1; Fig. 1), but disrupting it on a *phyB* background did significantly reduce germination,

Table 1 Tests of phytochrome additive gene contributions to germination at different temperatures

Source	7°C	10°C	22°C	28°C
$PHYA_1$	28.4816***	0.0074	39.1784***	705.6000***
$PHYB_1$	19.3251***	54.1101***	329.3414***	705.6000***
$PHYA_1 \times PHYB_1$	26.5087***	0.6234	60.7350***	739.6000***
$PHYA_1$	0.1415	3.8321+	59.1005***	142.0201***
$PHYB_5$	17.1180***	95.8029***	230.9361***	219.0258***
$PHYA_1 \times PHYB_5$	0.0509	9.0097**	83.1370***	150.7371***
$PHYB_1$	0.5158	21.8211***	145.5002***	15.1317**
$PHYE_1$	164.4737***	40.1909***	7.0217*	15.1317**
$PHYB_1 \times PHYE_1$	0.0105	8.0437**	14.9333***	18.4850**

Results are given from both temperatures from Expt 1 (10°C and 22°C) and temperature extremes from Expt 2 (7°C and 28°C). The upper portion tests for interactions between *PHYA* and *PHYB*. The lower portion tests for interactions between *PHYB* and *PHYE*. *F*-values from ANOVA models run at each temperature are presented.
***, **, *, +, Significant at $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.10$, respectively.

as indicated by the reduced germination of the *phyB/phyE* double mutant compared with the *phyB* single mutant (Table S2). Thus the contributions of *PHYB* and *PHYE* to germination were nonadditive (Table 1), such that the role of phyE might be conditionally redundant with phyB. The effect of disrupting phyE was not as strong as the effect of disrupting phyA, as indicated by the significantly lower germination of the *phyA/phyB* mutant than the *phyB/phyE* mutant. Interestingly, the germination frequency of the *phyA/phyB/phyE* triple mutant was greater than that of the *phyA/phyB* double mutant, suggesting that disruption of phyE on backgrounds with disrupted phyA and phyB activity may actually increase germination. However, the *PHYA* allele differed between the double and triple mutant, so this increased germination may be due to a weaker effect of the particular *PHYA* allele in the triple mutant.

At 10°C, all mutants with disrupted function of phyE (*phyE*, *phyBE* and *phyABE*) had the lowest germination frequencies, with 45% to 55% reduced germination compared with the wild type (Fig. 1), and these differences were statistically significant (Table S1). Disruption of phyB alone also reduced germination, suggesting additive function (Table 1), but it did not disrupt germination significantly on the *phyE* background, as indicated by equivalent germination of the *phyE* single mutant and the *phyB/phyE* double mutant (Table S2). Thus the contributions of *PHYE* and *PHYB* to germination were potentially nonadditive, with phyB having a larger effect on germination in the background with functional phyE.

Functional phyA is not critical for germination at 10°C; disruption of phyA had no effect on germination at 10°C, even on a *phyB* background, as indicated by the equivalent germination of *phyB* and *phyA/phyB* (Table S2). Also, disruption of phyA had no effect on a *phyB/phyE* background, as indicated by the equivalent germination of the *phyB/phyE* and *phyA/phyB/phyE* (Table S2). Thus, at 10°C, phyE had the strongest contribution to germination, followed by phyB, with no detectable contribution of phyA.

Expt 2: temperature range

All the mutants responded significantly to temperature treatment, but the wild type, *phyA* and one of the *phyB* mutants had consistently high germination across the entire temperature range (Fig. 2, Table S1). Many lines responded to temperature by having decreased germination at the highest and/or lowest temperatures, but incremental changes in temperature of only 3°C significantly changed the germination frequency of most mutants, especially after 7 d in the light. Mutant germination responses to warm and cold conditions were consistent with the direction of responses in the first experiment, but differences in the degree of germination responses were present; these differences were most likely attributable to growth condition differences between the two experiments. All lines exhibited their maximum germination at 19°C, and only the *phyA/phyB* double mutants had germination that was significantly lower than wild type at this temperature. Thus, phytochrome contributions to germination are maximally redundant at intermediate temperatures.

At higher temperatures such as 28°C, mutants with disrupted phyB function (*phyB* single mutant, *phyA/phyB* double mutants, *phyB/phyE* double mutant and the triple mutant) had significantly reduced germination, as in Expt 1 (Table S1; Fig. 2). Also, as in Expt 1, the contribution of phyA to germination was contingent upon the loss of phyB function, since the *phyA* and wild type had statistically equivalent germination (Table S1), but the germination of *phyA/phyB* double mutants was significantly lower than that of the *phyB* single mutants (see the Supplementary Material, Table S3). Thus the contribution of *PHYA* and *PHYB* to germination at high temperature was nonadditive (Table 1). Moreover, the germination frequency of *phyA/phyB* was significantly lower than that of *phyB/phyE* at 28°C (Fig. 2; Table S3), indicating a larger contribution of phyA to germination at high temperature than phyE. Therefore, phyB had the largest contribution to

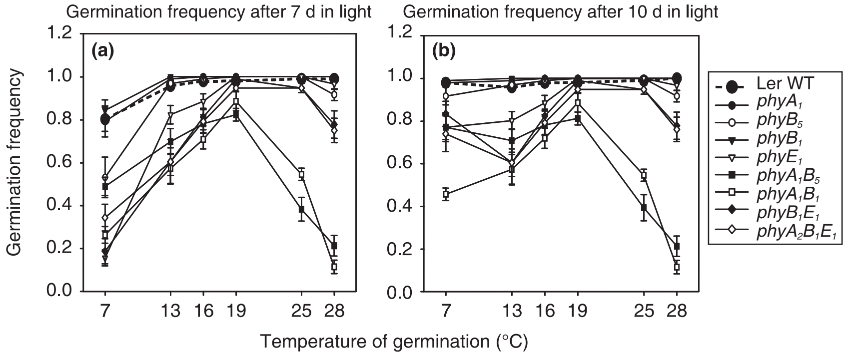

Fig. 2 Germination frequency after 7 d (a) and 10 d (b) in the light for *Arabidopsis thaliana* single and multiple phytochrome loss-of-function mutants relative to wild-type (Ler WT). The data represent mean values pooled across blocks in Expt 2. The germination frequency of each mutant line responded significantly to temperature across blocks (two-factor ANOVA, significant treatment effect, $P = 0.05$). Large closed circles, Landsberg *erecta*; small filled circles, *phyA-201*, stock #CS6219; open circles, *phyB-5*, #CS6213; *filled squares*, *phyA-201/phyB-5*, #CS6224; filled triangles, *phyB-1*; open squares, *phyA-201/phyB-1*; open triangles, *phyE-1*; closed diamonds, *phyB-1/phyE-1*; open diamonds, *phyA-2/phyB-1/phyE-1*.

germination at the highest temperature, followed by phyA, then phyE, as in Expt 1.

At the coldest temperature, mutants deficient in phyE function (*phyE*, *phyB/phyE* and *phyA/phyB/phyE*) had strongly reduced germination (Table S1; Fig. 2), indicating a strong contribution of phyE to germination at low temperature. The *phyE* single mutant had a lower germination frequency than single *phyA* and *phyB* mutants, indicating a greater contribution of phyE to germination at low temperature than phyA or phyB (Fig. 2; Table 1). phyB also contributed to germination at low temperature, as indicated by the significant reduction of germination in one *phyB* single mutant and in the *phyA/phyB* double mutants relative to wild type (Table S1). Interestingly, disrupting phyB function reduced germination more strongly at low temperature in Expt 1 than Expt 2. Nonetheless, as in Expt 1, disrupting phyB function had no effect on a *phyE* background, since germination of *phyE* was already very low (Table S3), leading to potentially nonadditive contributions of *PHYB* and *PHYE* to germination at low temperature. It was found that phyA did not strongly contribute to germination at low temperature, even on a *phyB* or *phyB/phyE* background, as indicated by the equivalent germination of the *phyB5* and *phyA1/phyB5* mutants and equivalent germination of the *phyB/phyE* and *phyA/phyB/phyE* mutants (Table S3). However, phyA did affect germination frequency at 7°C in the *phyB1* background (Table S3), indicating that the effect of *PHYA* at low temperature is variable, depending on the particular *PHYB* allele present. This result explains why there were significant nonadditive and additive effects of *PHYA* and *PHYB* at low temperature (Table 1).

After 10 d (in contrast to 7 d) in the light at 7°C, germination percentages of *phyE* and *phyBE* increased, indicating a reduced contribution of phyE to germination in cold temperatures as time proceeds. After 10 d, mutants deficient in both phyA and phyB had the lowest germination. Germination of the *phyA/phyB* double mutants was significantly lower than the single *phyB* mutants, and germination of the triple *phyA/phyB/phyE* mutant was significantly lower than that of the *phyB/phyE* double mutant (Table S3), indicating that phyA can contribute to germination at low temperature after prolonged exposure to cold.

Discussion

Germination frequencies of the phytochrome mutants depended on temperature, indicating a novel ability of phytochrome to respond to both light and temperature during germination. Specifically, at warm temperatures, phyB contributed the most to germination, followed by phyA then phyE. At cool temperatures, phyE contributed most strongly, followed by phyB, with phyA contributing only after prolonged cold. Thus, phyA contributes to germination primarily in warm temperatures, phyE contributes primarily in cold temperatures, and phyB contributes across a broad range of temperature.

The results indicate that phyB and phyA would be important for germination in late spring or early summer conditions, whereas phyE and phyB would be most important for germination in late autumn or early spring, with phyE being most important in cooler seasons. A study by Hennig *et al.* (2002) indicates that phyE can also contribute to germination in warm conditions on a *phyA/phyB* background (i.e. in the *phyA/phyB/phyE* mutant) under light and stratification conditions different from those used in this study, suggesting that the contribution of phyE to germination also depends on light and/or stratification conditions. Most significantly, the very strong contribution of phyE to germination at cool temperatures indicates that it has an ecologically important function that has not been previously recognized.

The contribution of phyB and phyE to germination diminished with prolonged exposure to cold. This effect may be because abscisic acid (ABA), which represses germination, is degraded at temperatures below 13°C (Ali-Rachedi *et al.*, 2004);

three additional days of degrading ABA at 7°C may have been enough to promote greater germination for *phyE* mutants after 10 d. Therefore, phyE-mediation of germination may involve ABA.

An intermediate optimum germination temperature of 19°C existed for nearly all of the mutant lines. A common optimum value indicates that phytochrome function can be redundant and that phyA, phyB and phyE play a minimal role in promoting germination at intermediate temperatures. In fact, at 19°C, loss of all three of these phytochromes was not enough to reduce germination significantly. Moreover, high germination of *phyA/phyB/phyE* at intermediate temperatures indicates that phyC and phyD may be involved with germination at this temperature, or that germination does not depend strongly on phytochrome at 19°C. Since phytochromes are thought to regulate germination via gibberellic acid (GA), this suggests a new potential role for phyC and/or phyD in affecting GA concentration or sensitivity (Yamaguchi *et al.*, 2004). Interestingly, recent evidence from natural populations of *Arabidopsis* indicates that allelic variants of phyC affect GA-mediated hypocotyl responses (Balasubramanian *et al.*, 2006).

In addition to hormonal mechanisms, the effect of temperature on phytochrome-mediated processes may result from the influence of temperature on the threshold level of Pfr needed for dormancy breakage, the reversion rate of Pfr in darkness, or a combination of these (Kristie & Fielding, 1994; Pons, 2000). Temperature has been shown to affect the response of seeds to the ratio of red to far-red light through the effect on the Pfr threshold required for germination (Pons, 1986; Senden *et al.*, 1986; van der Toorn & Pons, 1988). Furthermore, a study by Fielding *et al.* (1992) suggests that Pfr levels in seeds influence germination by affecting the upper temperature limit for germination. Our data indicate that this upper temperature limit may be established by Pfr levels of phyA and phyB, and that a temperature threshold may exist around 25°C.

Overall, our experiments demonstrated that phyA has a role in promoting germination at high temperatures, phyE has a previously unknown role in promoting germination at low temperatures, and phyB is important to germination across a wide range of temperatures. Even temperature changes as small as 3°C had significant effects on phytochrome contributions to germination, especially at intermediate and high temperatures. At different temperatures, different phytochromes contribute significantly more to germination than others, indicating a restriction, or even a potential specialization of individual phytochrome activity as a function of temperature (see also Halliday & Whitelam, 2003). Moreover, the phytochromes were maximally functionally redundant near 19°C. This temperature occurs during the peak season of germination in the field, suggesting that functional redundancy may be greatest under favorable conditions for germination, and that the loss of function of some phytochromes would still not prevent germination under favorable conditions. A temperature of 19°C is also similar to the temperature at which circadian clock mutants are maximally redundant (Gould *et al.*, 2006), suggesting that disruption of diurnal and seasonal affecters of germination timing may be less important at intermediate temperatures.

Conclusion

Our data suggest a new role for phytochrome in temperature-dependent germination; the activities of different phytochromes are sensitive to both temperature and light – two very different environmental cues. In particular, three phytochrome genes, *PHYA*, *PHYB* and *PHYE* may provide an efficient and flexible system that allows a seed to sense different seasonal cues with the same set of proteins. Specifically, the downregulation or inactivation of particular phytochromes or their pathways can cause temperature-dependent germination. Even small changes in temperature had a large effect on the contribution of particular phytochromes to germination. Thus, the changes in temperature that may accompany global climate change or long-distance dispersal have the potential to alter which of the three phytochromes may be most important in regulating germination. Generally, by affecting seasonal germination timing, these three phytochromes may influence the selective environment experienced in the wild.

Acknowledgements

We thank Suzen Huang, George Chiang, Andrew Wheeler, Lauren Griffen, Jocelyn Hall, Tracy Tisdale, and Julian Boggs for their assistance. We also thank TAIR for access to the mutants used in this study. This research was funded by NSF grant IBN-0235662 to K.D.

References

Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, Jullien M. 2004. Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. *Planta* **219**: 479–488.

Balasubramanian S, Sureshkumar S, Agrawal M, Michael TP, Wessinger C, Maloof JN, Clark R, . Warthmann N, Chory J, Weigel D. 2006. The phytochrome *c* photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. *Nature Genetics* **38**: 711–715.

Casal J, Sanchez RA. 1998. Phytochromes and seed germination. *Seed Science Research* **8**: 317–329.

Derkx MPM, Karssen CM. 1993. Effects of light and temperature on seed dormancy and gibberellin-stimulated germination in *Arabidopsis thaliana*: studies with gibberellin-deficient and – insensitive mutants. *Physiologia Plantarum* **89**: 360–368.

Donohue K. 2003. Setting the stage: phenotypic plasticity as habitat selection. *International Journal of Plant Science* **164**: S79–S92.

Donohue K. 2005. Niche construction through phonological plasticity: Life-history dynamics and ecological consequences. *New Phytologist* **166**: 83–92.

Fielding A, Kristie DN, Dearman P. 1992. The temperature dependence of Pfr action governs the upper temperature limit for germination in lettuce. *Photochemistry and Photobiology* **56**: 623–627.

Franklin KA, Whitelam GC. 2004. Light signals, phytochromes and cross-talk with other environmental cues. *Journal of Experimental Botany* **55**: 271–276.

Garcia-Martinez JL, Gil J. 2002. Light regulation of gibberellin biosynthesis and mode of action. *Journal of Plant Growth Regulation* **20**: 354–368.

Gould PD, Locke JCW, Larue C, Southern MM, Davis SJ, Hanano S, Moyle R, Milich R, . Putterill J, Millar AJ, Hall A. 2006. The molecular basis of temperature compensation in the *Arabidopsis* circadian clock. *Plant Cell* **18**: 1177–1187.

Griffith C, Kim E, Donohue K. 2004. Life-history variation and adaptation in the historically mobile plant *Arabidopsis thaliana* (Brassicaceae) in North America. *American Journal of Botany* **91**: 837–849.

Halliday KJ, Whitelam GC. 2003. Changes in photoperiod or temperature alter the functional relationships between phytochromes and reveal roles for phyD and phyE. *Plant Physiology* **131**: 1913–1920.

Hennig L, Schafer E. 2001. Both subunits of the dimeric plant photoreceptor phytochrome require chromophore for stability of the far-red light-absorbing form. *Journal of Biological Chemistry* **276**: 7913–7918.

Hennig L, Stoddart WM, Dieterle M, Whitelam GC, Schafer E. 2002. Phytochrome E controls light-induced germination of *Arabidopsis. Plant Physiology* **128**: 194–200.

Kristie DN, Fielding A. 1994. Influence of temperature on the Pfr level required for germination in lettuce cv. Grand Rapids. *Seed Science Research* **4**: 19–25.

Nagatani A, Reed J, Chory J. 1993. Isolation and initial characterization of *Arabidopsis* mutants that are deficient in phytochrome A. *Plant Physiology* **102**: 269–277.

Napp-Zinn K. 1976. Population genetical and geographical aspects germination and flowering in *Arabidopsis thaliana. Arabidopsis Information Service* **13**.

Nordborg M, Bergelson J. 1999. The effect of seed and rosette cold treatment on germination and flowering time in some *Arabidopsis thaliana* (Brassicaceae) ecotypes. *American Journal of Botany* **86**: 470–475.

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. 2003. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell* **15**: 1591–1604.

Peng J, Harberd NP. 2002. The role of GA-mediated signaling in the control of seed germination. *Current Opinion in Plant Biology* **5**: 376–381.

Pons T. 1986. Response of *Plantago major* seeds to the red/far-red ration as influenced by other environmental factors. *Physiologia Plantarum* **68**: 252–258.

Pons TL. 2000. Seed responses to light. In: Fenner M, ed. *Seeds – The Ecology of Regeneration in Plant Communities*, 2nd edn. Wallingford, UK: CAB International, 237–260.

Senden J, Schenkeveld A, Verkaar H. 1986. The combined effect of temperature and red: far-red ratio on the germination of some short-lived chalk grassland species. *Acta Oecologica: Oecologia Plantarum* **7**: 251–259.

Spalding EP, Folta KM. 2005. Illuminating topics in plant photobiology. *Plant, Cell & Environment* **28**: 39–53.

Steckel L, Sprague C, Stoller EW, Wax L. 2004. Temperature effects on germination of nine. *Amaranthus* species. *Weed Science* **52**: 217–221.

Thompson L. 1994. The spatiotemporal effects of nitrogen and litter on the population dynamics of *Arabidopsis thaliana. Journal of Ecology* **82**: 63–68.

van der Toorn J, Pons T. 1988. Establishment of *Plantago lanceolata* L. and *Plantago major* L. among grass. II. Shade tolerance of seedlings and selection on time of germination. *Oecologia* **76**: 341–347.

Toyomasu T, Kawaide H, Mitsuhashi W, Inoue Y, Kamiya Y. 1998. Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. *Plant Physiology* **118**: 1517–1523.

Watson RT, Marufu ZC, Moss RH, eds. 1997. *The Regional impacts of climate change: an assessment of vulnerability*. Cambridge, UK: Cambridge University Press.

Whitelam GC, Devlin PF. 1997. Roles of different phytochromes in *Arabidopsis* photomorphogensis. *Plant, Cell & Environment* **20**: 752–758.

Yamaguchi S, Kamiya Y. 2000. Gibberellin biosynthesis: Its regulation by endogenous and environmental signals. *Plant Cell Physiology* **41**: 251–257.

Yamaguchi S, Smith MW, Brown RGS, Kamiya Y, Sun T. 1998. Phytochrome regulation and differential expression of gibberellin 3b-hydroxylase genes in germinating *Arabidopsis* seeds. *Plant Cell* **10**: 2115–2126.

Yamaguchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S. 2004. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell* **16**: 367–378.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 The χ^2 values and *P*-values for Kruskal–Wallis tests for significant differences between mutant and wild-type genotypes for germination frequency after 7 d in light and 10 d in light

Table S2 The χ^2 values for Kruskal–Wallis tests comparing germination frequency of different mutants after 7 d in the light in Expt 1

Table S3 The χ^2 values for Kruskal–Wallis tests comparing germination frequency of different mutants after 7 d in the light in Expt 2

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02044.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to *New Phytologist* Central Office.