RESEARCH ARTICLE

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# Photoperiod throughout the maternal life cycle, not photoperiod during seed imbibition, influences germination in *Arabidopsis thaliana*[1]

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**PREMISE OF THE STUDY:** Plants adjust their phenology in response to seasonal cues experienced both by their parents and by themselves, and coordinating responses to these cues is necessary for expressing adaptive phenology. We investigated how cues are integrated across time to influence an important progeny phenotype, i.e., seed germination.

**METHODS:** We used *Arabidopsis thaliana* to investigate how the photoperiod experienced by maternal parents and by progeny influences seed germination. We examined when maternal photoperiod effects on germination are imposed and how long they persist in progeny.

**KEY RESULTS:** The photoperiod experienced by maternal plants more strongly influenced germination than the photoperiod experienced during seed imbibition. In addition, the photoperiod experienced at the prereproductive stage frequently influenced germination as strongly as that experienced during reproduction. In general, seeds from plants grown under short days had higher seed germination percentages than seeds from plants grown in longer days. These maternal effects diminished with after-ripening, but reappeared in seeds induced into secondary dormancy.

**CONCLUSIONS:** We found no evidence that the effect of photoperiod systematically attenuates in proportion to the time that elapsed between the cue and the timing of seed germination. Moreover, more recently experienced cues did not override the effects of cues experienced previously. Instead, specific sequences of photoperiods experienced at the prereproductive and reproductive stages appear to influence germination behavior.

**KEY WORDS** Brassicaceae; dormancy; germination; maternal effect; phenotypic plasticity; photoperiod; seasonal cues; secondary dormancy

The seasonal timing of phenological transitions has major consequences for fitness and organismal responses to variable environments (Walther et al., 2002; Menzel et al., 2006; Parmesan, 2006; Chuine, 2010; Willis et al., 2008). For phenological transitions that occur early in development, such as seed germination, organisms adjust their phenology in response to seasonal cues experienced both by their parents and by themselves (Roach and Wulff, 1987; Donohue and Schmitt, 1998; Schlichting and Pigliucci, 1998; Gutterman, 2000; Herman and Sultan, 2011; Snell-Rood, 2013). Coordinating responses to cues experienced within and across generations is necessary for expressing adaptive phenology.

What is the relative contribution of environmental conditions experienced by parents vs. progeny in regulating progeny phenotypes? One expectation is that cues experienced directly by progeny may be more accurate predictors of progeny environmental conditions than cues experienced by parents; consequently, responses of progeny to cues experienced by themselves may be expected to be stronger, or to override, responses to cues experienced by parents (DeWitt et al., 1998; Schlichting and Pigliucci, 1998). Alternatively, parents may be able to enable responses of progeny to conditions that progeny cannot yet perceive, or cues perceived by parents may predict conditions that progeny are likely to experience in the future, even if those conditions are not yet present. If so, then responding to parental cues may be adaptive (reviewed in Mousseau and Fox, 1998; Herman and Sultan, 2011), and effects of parental environments may be as strong as or stronger than effects of the progeny environment. Finally, the specific combination of conditions experienced over time—first by maternal parents, then by progeny—may be the most accurate indicator of suitable conditions. For instance, the combination of photoperiod experienced first during seed maturation and then after dispersal may provide the most accurate information of seasonal time of year. If so, then parental

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and progeny environments would interact to determine progeny phenotypes.

The accuracy with which cues perceived by parents predict progeny environments may diminish over time, as environments change. If so, then it is pertinent to know when parental environmental effects are imposed within the lifetime of the parent, and how long those parental effects persist in progeny. Cues experienced early in the lifetime of parents may lose accuracy in predicting progeny environments compared to cues experienced late in life. Likewise, parental environmental effects that influence seeds soon after dispersal may more accurately match the environment experienced by seeds than parental effects that persist over longer periods of time.

Here we investigate the effects of the photoperiod experienced by maternal parents and by progeny (seeds) on the important phenological trait of seed germination in *Arabidopsis thaliana* (L.) Heynh. The timing of seed germination is frequently under intense natural selection (Kalisz, 1986; reviewed in Donohue et al., 2010). In *A. thaliana*, seed dormancy and germination, and the genes that control them, have been shown to be subject to variable natural selection and to contribute to local adaptation (Donohue et al., 2005; Huang et al., 2010; Kronholm et al., 2012; Montesinos-Navarro et al., 2012; Akiyama and Ågren, 2014; Postma et al., 2016). Seed germination often responds to environmental conditions experienced by maternal plants during seed maturation (Gutterman, 2000; Donohue, 2009; Baskin and Baskin, 2014). This is because primary dormancy is induced at the late stages of seed maturation, through maternally regulated hormonal pathways (Bewley, 1997; Holdsworth et al., 2008; Donohue, 2009).

The depth of seed dormancy influences the breadth of environmental conditions under which a seed can germinate (Vleeshouwers et al., 1995). Primary dormancy, induced during seed maturation, is gradually lost in dry seeds through the process of "after-ripening," such that after-ripened seeds that have lost dormancy can germinate under a wider range of environmental conditions. Seeds can also regain dormancy ("secondary dormancy") in response to adverse conditions, such as hot temperature (Auge et al., 2015), leading to natural seasonal cycles in the level of dormancy (Baskin and Baskin, 1983; Footitt et al., 2011, 2013, 2014).

An important seasonal cue for plants is photoperiod. The photoperiod experienced during seed maturation has been shown to influence germination in diverse species, including *Arabidopsis thaliana* (Gutterman et al., 1975; Gutterman, 1978, 1996; Munir et al., 2001). In *A. thaliana*, the photoperiod experienced during seed maturation is likely to vary because of variation in life-history, and in particular, flowering time (Ratcliffe, 1965, 1976; Baskin and Baskin, 1972, 1983; Thompson, 1994; Donohue, 2009). *A. thaliana* is an annual mustard (Brassicaceae), and in its native and introduced ranges (Sharbel et al., 2000) it is typically a winter annual, germinating in autumn and flowering under lengthening days of spring. It also commonly expresses a spring-annual life cycle, in which it germinates in spring and flowers under long days of midspring; less commonly, it exhibits a rapid-cycling life history in which it can germinate and flower in shortening days of autumn (Thompson, 1994; Donohue, 2009). Thus *A. thaliana* is likely to experience diverse day lengths before reproduction, during seed maturation, and after dispersal.

This work investigates germination responses of *Arabidopsis thaliana* to the seasonal cue of photoperiod experienced both by maternal parents and by seeds after dispersal. We first compare the relative magnitude of germination responses to the photoperiod experienced by maternal parents vs. progeny (seeds). We then examine when maternal photoperiod effects on germination are imposed, and how long they persist in progeny. We address the following specific questions: (1) Does germination respond more strongly to the photoperiod experienced by the maternal plant or the photoperiod experienced by the progeny during seed imbibition? (2) Are effects of maternal photoperiod imposed at the prereproductive stage or only at the reproductive stage when seeds are maturing? (3) Do effects of maternal photoperiod persist in the progeny throughout after-ripening and secondary dormancy induction?

## MATERIALS AND METHODS

***Experiment 1: Comparison of effects of maternal vs. seed photoperiod***—To compare the effect on germination of the photoperiod experienced by maternal plants to that experienced during seed imbibition, we grew plants under three photoperiods (8 h, 12 h, and 16 h) and used those same three photoperiods for germination assay.

*Growth conditions of maternal plants*—Seeds of the standard accessions, Columbia (Col-0, or "Col" hereafter) and Landsberg *erecta* (L*er*), of *Arabidopsis thaliana* were sown in 0.6% w/v agar plates, stratified at 4°C for 5 d in darkness, and then transferred to pots with potting soil (Metromix 360 soil, Scotts Sierra, Ohio, USA). Pots were placed into EGC Model GC8-2 growth chambers (Environmental Growth Chambers, Chagrin Falls, Ohio, USA) into a 16 h photoperiod (16 h light/8 h dark) at 22°C until the initiation of reproduction, indicated by the initiation of elongation of the reproductive inflorescence stalk (termed "bolting") and initiation of the formation of flower buds. At the time of transfer to the experimental treatments, plants had reproductive stems that were 2-4 cm high and had begun to produce flower buds. After the initiation of reproduction ("during reproduction"), plants were transferred to 15°C and into one of three maternal photoperiods: short day (SD hereafter) = 8 h light/16 h dark; medium day (MD hereafter) = 12 h light/12 h dark; and long day (LD hereafter) = 16 h light/8 h dark. Plants were grown at 15°C during reproduction because it reflects common temperatures of seed production under natural conditions and because it promotes the induction of an intermediate level of dormancy in these ecotypes, which facilitates the detection of both positive and negative treatment effects (Burghardt et al., 2016; L. Blair, Duke University, unpublished data). Plants were fertilized every 14 d with a 300 ppm nitrogen solution of Blossom Booster Fertilizer (JR Peters, Allentown, Pennsylvania, USA). Twelve maternal plants of each genotype were grown at each photoperiod, with replicate plants randomly distributed within a chamber. Watering was withheld one week before harvest to induce the drying of siliques. Plants from all treatments were harvested simultaneously within two days. After harvesting, seeds were permitted to after-ripen by storing them dry at room temperature (22 ± 2°C) in a desiccator (Secador 4.0 Auto-Desiccator Cabinets, Bel-Art Products, Pequannock, New Jersey, USA) until they were used for each germination assay. Seeds from each of the 12 maternal plants grown at each photoperiod were kept separate for germination assays, such that maternal plants served as biological replicates.

*Germination assays*—For each accession (Col and L*er*), fresh seeds from each of 12 biological replicates (different maternal plants) from each of the maternal-plant photoperiods (SD, MD, and LD) were placed in each of three seed imbibition photoperiods (SD = 8 h light/16 h dark; MD = 12 h light/12 h dark; LD = 16 h light/8 h dark), and two temperatures (10°C or 22°C), in a factorial design (2 accessions × 3 maternal photoperiods × 3 seed photoperiods × 2 temperatures × 12 replicates = 432 total plates). The temperature manipulation was used both to induce a second seasonal cue of temperature and to facilitate the detection of treatment effects by exposing seeds to more (10°C) and less permissive (22°C) germination conditions, thereby minimizing the chance of extreme germination proportions of 0% and 100% germination across all photoperiod treatments that would mask treatment effects. Each replicate comprised 20 seeds sown in 35 mm petri dishes with 0.6% w/v agar and sealed with Parafilm. Plate positions were randomized within a treatment after each census to avoid position effects. Germination was scored regularly, with germination indicated by the protrusion of the radicle from the seed coat. The number of seeds that germinated was counted until germination reached a clear plateau (usually after 14 d). The final proportion of viable seeds that germinated was used for analysis. Seed viability was assessed as the absence of severe fungal infection and obvious degradation of the embryo. Most of the seeds used in this study appeared to be viable, and this was reflected by the ability of seeds to attain 100% germination in some treatments in both ecotypes (see Results).

*Statistical analysis*—All analyses were performed in R version 3.3.1 (R Development Core Team, 2016). Each accession was analyzed separately. Final germination proportions (number of germinants/number of viable seeds at the last census date) were analyzed with generalized linear models with a quasi-binomial error, to correct for over-dispersion, using "glm" in the "stats" package. Analysis of deviance based on *F*-values was performed using "Anova" in "car". We used a full model that included germination proportion as the dependent variable, and maternal photoperiod ("Maternal"), seed photoperiod ("Imbibition"), and temperature ("Temp") as fixed factors.

***Experiment 2: Comparison of effects of maternal photoperiod before vs. after the initiation of reproduction***—To evaluate when maternal effects on dormancy are imposed, we manipulated the photoperiod under which maternal plants were grown both before and after the initiation of reproduction, defined as above under Experiment 1, *Growth conditions of maternal plants*, and monitored the germination of their seeds. To test whether maternal effects on dormancy persisted throughout after-ripening and secondary-dormancy induction, we assessed germination of seeds that were after-ripened for different durations, and assessed germination of seeds induced into secondary dormancy by different durations of hot stratification (preincubation in the dark at 35°C).

*Growth conditions of maternal plants*—Seeds of the Col and L*er* accessions were prepared as described above under Experiment 1, *Growth conditions of maternal plants*, and germinants were placed into different photoperiod treatments. Before the initiation of reproduction ("before reproduction"), plants were grown at one of three photoperiods at 22°C: Short day (SD = 8 h light/16 h dark); medium day (MD = 12 h light/12 h dark); and long day (LD = 16 h light/8 h dark). After the initiation of reproduction ("during reproduction"), plants were transferred to 15°C to either SD or LD, in a factorial design; the medium-day treatment was not used because of space limitation. This resulted in six photoperiod treatments. Twelve maternal plants (biological replicates) were grown at each photoperiod treatment, with replicate plants randomly distributed within a chamber. Growth conditions, harvesting procedures, and seed storage were as described above under Experiment 1, *Growth conditions of maternal plants*.

*Germination assays*—For each accession (Col and L*er*), seeds from 12 biological replicates (different maternal plants) from each of the six maternal photoperiod treatments were allowed to imbibe in each of two temperatures (10°C or 22°C), in either a 12 h photoperiod (12 h light/12 h dark) or in darkness (2 accessions × 6 maternal photoperiods × 2 seed temperatures × 2 seed light treatments × 12 replicates = 576 total plates). Each replicate comprised 20 seeds per petri dish, as described above under Experiment 1, *Germination assays*. Seeds assigned to the dark treatment were immediately transferred to boxes covered in foil after sowing in the light, and boxes were kept in the same growth chambers as seeds in the light treatments. For seeds with no dark treatment, germination was assessed regularly after being placed into their treatments, and the number of seeds that germinated was counted until germination reached a clear plateau (usually after 14 d). For seeds in the dark treatment, germination was recorded after 14 d. The final proportion of viable seeds that germinated was used for analysis, with each plate being the unit of analysis.

To assess whether maternal effects on germination proportions persisted over the course of after-ripening, the above assays were conducted on seeds after-ripened for 0 (fresh), 4, and 12 wk. As a pilot study, seeds harvested for Experiment 1 (plants grown under SD, MD, and LD) were also assayed under these conditions, using seeds after-ripened for 0, 4, 10, and 35 wk.

To assess whether maternal effects on germination proportions persist through the process of secondary dormancy induction, 12-wk after-ripened (nondormant) seeds were sown in 35 mm petri dishes with 0.6% w/v agar, then were preincubated in the dark at 35°C for 0, 1, 3, 5, or 7 d to induce secondary dormancy before being transferred to either 10°C or 22°C in the light (12 h light/12 h dark, as above). As a pilot study, seeds harvested for Experiment 1 that had after-ripened for 35 wk were sown in 35 mm petri dishes with 0.6% w/v agar and preincubated in the dark at 35°C for 0 or 5 d before being transferred to 10°C or 22°C in the light (12 h light/12 h dark).

*Statistical analysis*—Final germination proportions were analyzed with generalized linear models with a quasi-binomial error as described above under Experiment 1, *Statisctical analysis*. Each accession was analyzed separately. We first used a full-model that included germination proportion as the dependent variable, and photoperiod before reproduction ("Before"), photoperiod during reproduction ("During"), temperature of imbibition ("Temp"), duration of after-ripening ("AR"), and light vs. dark ("Light"), as fixed factors, including all interactions. To interpret significant interactions among maternal treatments, AR, Temp, and Light, we next tested for effects of after-ripening, and maternal treatments before ("Before") and during ("During") reproduction, and their interactions for each imbibition treatment (temperature and light) separately. To further interpret interactions with after-ripening, we next conducted multiple comparisons, using "glht" in the "multcomp"

package to test for effects of maternal treatments before and during reproduction for each combination of after-ripening, temperature, and light treatment separately. Germination proportions in these submodels were analyzed with binomial generalized linear models with bias reduction methods, using "brglm" in the "brglm" package, and Z-values are given for the multiple comparisons.

To investigate whether effects of maternal photoperiod before and during reproduction persisted into secondary dormancy induction, we first used a full model that included the photoperiod experienced before ("Before") and during ("During") reproduction, temperature of imbibition ("Temp"), and duration of hot preincubation ("Hot"). We next tested how maternal photoperiod influenced secondary dormancy induction by testing for effects of "Before," "During," "Hot," and their interactions in each imbibition temperature separately. To further interpret interactions, we conducted multiple comparisons, using "glht" in the "multcomp" package to test for effects of "Before" and "During" within each "Temp" and "Hot" treatment separately.

## RESULTS

***Effects of photoperiod experienced by maternal plants and during seed imbibition on the germination proportions of fresh seeds***—To assess the relative contribution of photoperiod when experienced by different generations, we assessed the germination of seeds that had developed under different photoperiods and allowed those seeds to imbibe under different photoperiods. The photoperiod experienced during reproduction in the maternal generation significantly influenced germination proportions for both accessions, but the photoperiod during seed imbibition did not (Fig. 1; Table 1). In general, the effect of maternal photoperiod was consistent across all imbibition photoperiods, and the maternal photoperiod did not alter the response to imbibition photoperiod, as indicated by a nonsignificant interaction between the photoperiod experienced during maternal reproduction and during seed imbibition (Table 1).

Col seeds incubated at 10°C did not respond to maternal photoperiod, because all seeds were highly nondormant (Fig. 1, Table 1). At 22°C, seeds of Col that had matured under SD (i.e., seeds of Col from plants grown under SD during reproduction) germinated more than seeds that had matured under MD and LD, and the photoperiod during seed imbibition did not significantly affect germination proportions (Fig. 1).

In L*er* seeds incubated at 10°C, seeds that matured under SD germinated more than those that matured under MD or LD (Fig. 1, Table 1). At 22°C as well, seeds that matured under SD germinated more than those that matured under the other photoperiods, although the germination proportion was lower at this temperature than at 10°C. The photoperiod during imbibition had no significant effect on germination proportion.

In summary, seeds responded to the photoperiod experienced by the maternal plant during reproduction but not to the photoperiod they experienced during seed imbibition.

***Effects of maternal photoperiod experienced before and after the initiation of reproduction on germination proportions of fresh seeds***—We next compared the effect of maternal photoperiod experienced before vs. during reproduction. As before, seeds germinated more when they imbibed at 10°C than at 22°C, and imbibition temperature interacted significantly with maternal treatments in both Col (Before × Temp $F_{2, 792} = 7.49$, $P < 0.001$; During × Temp $F_{1, 792} = 17.96$, $P < 0.001$) and L*er* (During × Temp $F_{1, 792} = 36.14$, $P < 0.001$; Fig. 2). Seeds lost dormancy (had higher germination proportions) as they after-ripened (Fig. 2), and after-ripening duration interacted significantly with maternal photoperiods under some conditions (Appendix S1, see the Supplementary Data with

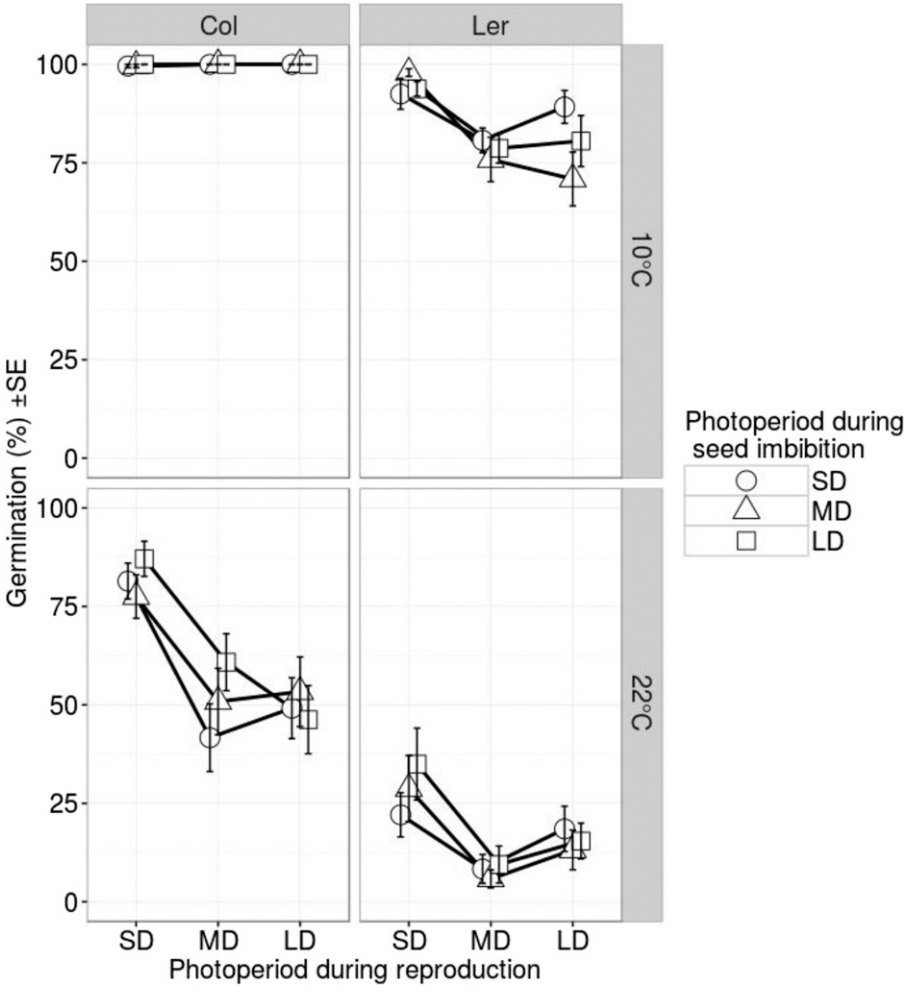


**FIGURE 1** Effects of photoperiod experienced by maternal plants and by imbibing seeds on the germination percentage of fresh seeds. Mean germination percentages (± SE) of Col (left panel) and L*er* seeds (right panel) that imbibed at 10°C (top row) or 22°C (bottom row). Seeds from maternal plants that experienced short days (SD), medium days (MD), or long days (LD) during reproduction (x-axis) and imbibed in either short days (SD, circles), medium days (MD, triangles) or long days (LD, squares).

**TABLE 1.** The effect of photoperiod experienced by maternal plants and imbibing seeds on germination proportions of fresh seeds (Experiment 1). Results are presented for Col and L*er* accessions separately. Germination proportions were analyzed with quasi-binomial generalized linear models, and analysis of deviances was performed based on *F*-values. "Reproduction" indicates photoperiod during reproduction of the maternal parents; "Imbibition" indicates photoperiod during seed imbibition; "Temperature" indicates temperature during seed imbibition. The residual degrees of freedom for Col and L*er* accessions were 198. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

| | | *F*-values | |
|---|---|---|---|
| **Source of variation** | ***df*** | **Col** | **L*er*** |
| Reproduction | 2 | 35.72*** | 22.59*** |
| Imbibition | 2 | 1.56 | 0.88 |
| Temperature | 1 | 471.48*** | 502.77*** |
| Reproduction × Imbibition | 4 | 1.59 | 1.43 |
| Reproduction × Temperature | 2 | 1.49 | 1.28 |
| Imbibition × Temperature | 2 | 0.17 | 0.31 |
| Reproduction × Imbibition × Temperature | 4 | 0 | 0.61 |

this article). We therefore analyzed effects of maternal photoperiods separately for each imbibition treatment and each duration of after-ripening, beginning with fresh seeds (Table 2).

In fresh seeds of the Col accession, the photoperiod experienced before and during reproduction interacted to influence germination proportions for seeds that imbibed in the light (Fig. 2; Appendix S1). When fresh seeds imbibed at 10°C, maternal plants grown under SD before reproduction, compared to MD or LD, produced seeds that had higher germination proportions (Fig. 2; Table 2). Plants grown under MD before reproduction produced seeds with lower germination proportions than seeds from plants grown under LD, provided those seeds matured under SD (Fig. 2; Table 2), indicating that increasing photoperiod length before reproduction did not reduce germination proportion in a monotonic manner, i.e., did not reduce it consistently. The photoperiod experienced during reproduction influenced dormancy, such that seeds that matured under SD had higher germination proportions than seeds that matured under LD, but this effect was significant only for seeds that had experienced LD before reproduction (Table 2).

When fresh Col seeds imbibed at 22°C, seeds from plants grown under SD before reproduction had higher germination proportions than seeds of plants grown under MD and LD, and this effect was most pronounced for seeds from plants that experienced LD during reproduction (Fig. 2; Table 2). Reproduction under SD (compared to LD) actually decreased germination proportions if SD or MD was experienced before reproduction, but it increased germination proportions if LD was experienced before reproduction (consistent with Experiment 1). Thus, the effect of maternal photoperiod switched direction depending on whether plants experienced SD before or during reproduction in this experiment. In the Col accession, therefore, maternal photoperiod did not exhibit a monotonic effect on dormancy, but exhibited strong effects of the photoperiod experienced before reproduction, and variable effects of the photoperiod experienced afterwards, depending on the temperature of seed imbibition.

In fresh seeds of the L*er* accession, the photoperiod experienced before and during reproduction interacted to influence germination proportions (Fig. 2; Appendix S1). The photoperiod experienced before reproduction had a subtle effect on germination proportions (Fig. 2; Table 2); a SD compared to MD or LD photoperiod before reproduction produced seeds with slightly higher germination proportions at both temperatures, when plants subsequently experienced LD. The photoperiod experienced during reproduction strongly influenced germination proportions only when seeds imbibed at 10°C, such that seeds that matured at SD had higher germination proportions than seeds that matured under LD (Fig. 2; Table 2). Seeds that imbibed at 22°C had low germination proportions, and maternal photoperiod during reproduction did not influence the germination proportions of those seeds.

***Effects of interactions between after-ripening and maternal photoperiods on germination proportions***—To investigate the persistence of the effects of maternal photoperiod on progeny germination proportions, we assessed germination after different durations of after-ripening. After-ripening reduced dormancy and altered effects of maternal photoperiods on germination proportions (Fig. 2; Table 2; Appendix S1). See also Appendices S2-S6 for discussion of germination in the dark.

In Col, differences among photoperiod treatments were diminished by after-ripening as seeds lost dormancy and attained high levels of germination (Fig. 2; Table 2). All after-ripened seeds that imbibed at 10°C germinated, so they showed no effect of maternal photoperiod. In seeds that had after-ripened for 4 wk and that subsequently imbibed at 22°C, SD experienced before reproduction slightly increased germination of seeds from plants exposed to LD during reproduction. Likewise, SD experienced during reproduction slightly increased germination of seeds that had experienced MD or LD before reproduction (Fig. 2; Table 2). Similar patterns were observed in seeds that had after-ripened for 12 wk and that were subsequently imbibed at 22°C; namely seeds from plants grown at SD before or during reproduction showed more germination. Therefore, even in after-ripened seeds, the photoperiods experienced before and during reproduction influenced germination proportions. Similarly, in Col seeds from Experiment 1, seeds from plants grown under LD, compared to MD or SD, had lower germination proportions for seeds that had after-ripened for 4 wk and that were subsequently imbibed in light at 22°C, but not for seeds that had after-ripened for 10 or 35 wk (Appendices S5 and S6).

In L*er*, after-ripening decreased dormancy and interacted with photoperiod to influence germination proportions (Fig. 2; Appendix S1). For seeds that had after-ripened for 4 wk, the effect of maternal photoperiods reflected that observed in fresh seeds. Specifically, SD experienced before reproduction increased the germination proportions of seeds that matured under LD; seeds that matured under SD all had very high germination proportions, masking any potential effects of photoperiod experienced before reproduction (Table 2). Likewise, SD experienced during reproduction increased germination proportions (an effect also observed in 4-wk after-ripened seeds from Experiment 1 that imbibed at 22°C; Appendices S5 and S6). In seeds with 12 wk of after-ripening, only seeds from plants that experienced MD before reproduction responded to the photoperiod during reproduction, with higher germination proportions of seeds that matured in SD (Table 2), suggesting that MD before reproduction may be a cardinal day length for dormancy induction.

In summary, for both ecotypes, SD before or during reproduction sometimes produced seeds with higher germination proportions even in after-ripened seeds. Therefore, the photoperiod experienced by maternal plants had persistent effects on seed dormancy even after prolonged periods of seed after-ripening.

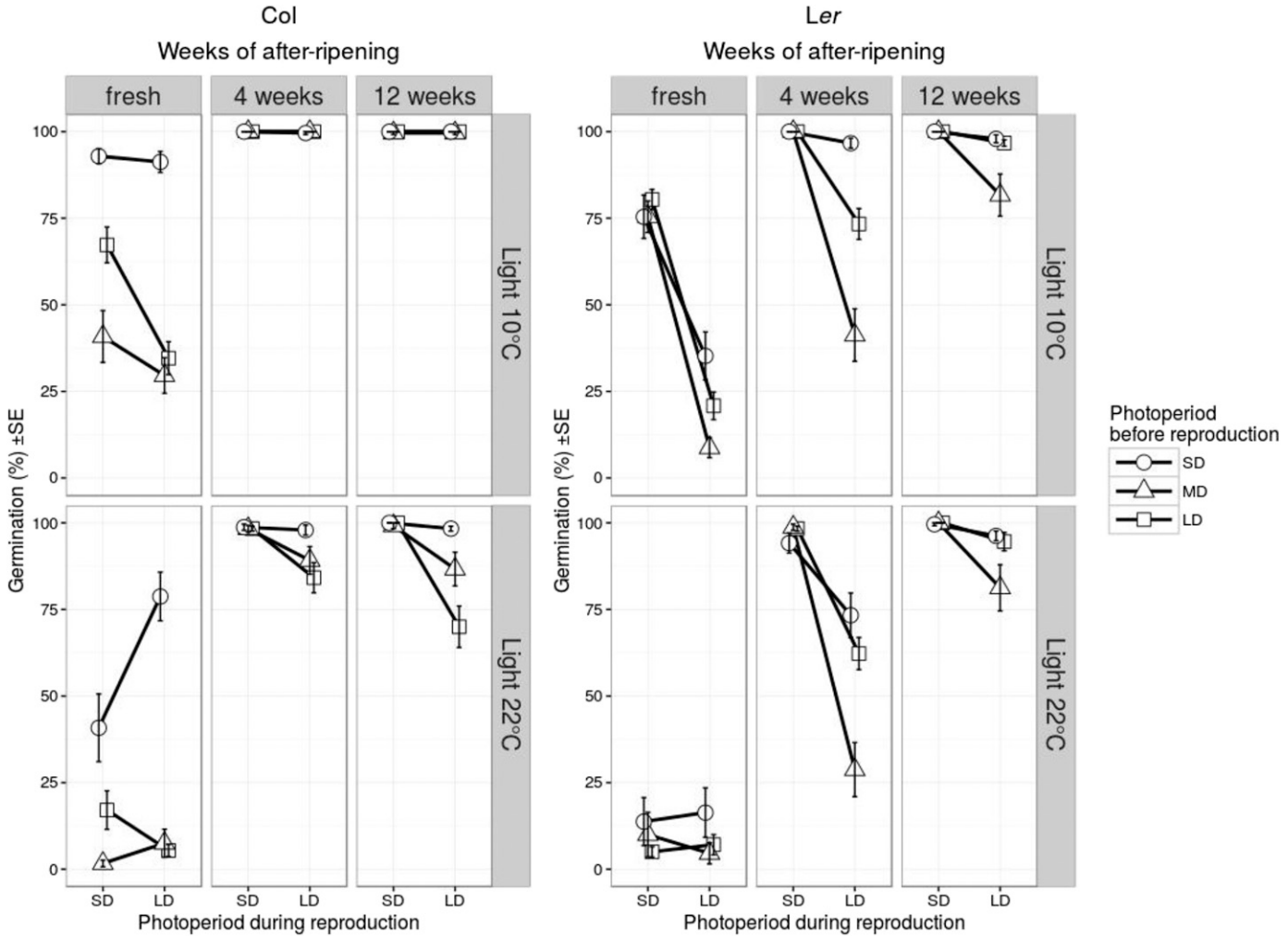


**FIGURE 2** Effects of maternal photoperiod experienced before and during reproduction on the germination of fresh and after-ripened seeds. Maternal plants experienced either short days (SD = circles), medium days (MD = triangles), or long days (LD = squares) before reproduction ("Photoperiod before reproduction"), and then experienced either short (SD) or long (LD) during reproduction ("Photoperiod during reproduction," x-axis). Results of Col (left panels) and L*er* (right panels) accessions are given for seeds that imbibed at either 10°C (top row) or 22°C (bottom row). Seeds were incubated in a 12 h light/12 h dark photoperiod.

***Effects of interactions between maternal photoperiods and induction of secondary dormancy***—Given that maternal photoperiod has persistent effects on progeny germination proportions throughout after-ripening, we explored whether it also affected germination proportions after secondary dormancy induction. Preincubation at 35°C in the dark imposed secondary dormancy in after-ripened seeds, but the degree to which it did so depended on the photoperiods experienced before and during reproduction (Fig. 3; Appendix S7). The effect of secondary dormancy induction also varied with imbibition temperature (see legend of Appendix S7). We therefore analyzed each imbibition temperature separately.

Secondary dormancy induction reduced germination proportions more in seeds incubated at 22°C than at 10°C (Fig. 3). In Col seeds incubated at 10°C, preincubation at 35°C (hot stratification) resulted in the least induction of secondary dormancy in seeds from plants that experienced SD either before or during reproduction (Fig. 3; Table 3). When incubated at 22°C, hot-stratified seeds from plants that experienced SD before reproduction had higher germination proportions (less dormancy induction) when plants were subsequently exposed to LD after reproduction. Curiously, especially after only one day of hot stratification, seeds from plants that experienced SD before reproduction had lower germination proportions when exposed to SD during reproduction than when exposed to LD during reproduction. This response contrasted with that of seeds exposed to MD or LD before reproduction, which had higher germination proportions when exposed to SD during reproduction than LD during reproduction, as was seen in Experiment 1 (Appendices S8 and S9). In summary, Col seeds from maternal plants that experienced SD before or during reproduction had the least secondary dormancy induction in most instances.

In L*er* seeds incubated at 10°C, seeds from maternal plants that experienced SD both before and during reproduction had the least secondary dormancy induction (Fig. 3; Table 3). The effect of photoperiod experienced before reproduction was apparent only in seeds that matured in LD, since all seeds that matured in SD germinated to high percentages. Likewise, the effect of photoperiod during

**TABLE 2.** Comparisons of germination proportions of seeds from plants that experienced different photoperiods before and during reproduction over the course of after-ripening (Experiment 2). Results are presented for Col and L*er* accessions separately. Germination proportions were analyzed with binomial generalized linear models with bias reduction methods, and Z-values are given for specific contrasts. Maternal plants experienced short days (SD), medium days (MD), or long days (LD) before reproduction (first column) and then experienced short (SD) or long (LD) during reproduction (second column). Contrasts compare specific photoperiods experienced before reproduction for a given photoperiod experienced during reproduction, and vice versa. Seeds were incubated in a 12 h photoperiod at either 10°C or 22°C (Light 10°C or Light 22°C). Results are given for seeds after-ripened for 0, 4, or 12 wk, for each incubation condition separately. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

| | Maternal photoperiod | | Fresh | | 4 wk | | 12 wk | |
|---|---|---|---|---|---|---|---|---|
| Ecotype | Before Reproduction | During Reproduction | Light 10°C | Light 22°C | Light 10°C | Light 22°C | Light 10°C | Light 22°C |
| Col | SD vs. MD | SD | −10.37*** | −7.26*** | 0 | −0.36 | −0.67 | −1.04 |
| | SD vs. LD | SD | −6.45*** | −5.57*** | 0 | −0.36 | 0 | 0 |
| | MD vs. LD | SD | 5.71*** | 4.72*** | 0 | 0 | 0.67 | 1.04 |
| | SD vs. MD | LD | −11.94*** | −13.11*** | 0.67 | −3.48** | −0.67 | −4.10*** |
| | SD vs. LD | LD | −11.21*** | −12.82*** | 0.67 | −4.50*** | 0 | −6.27*** |
| | MD vs. LD | LD | 1.17 | −0.91 | 0 | −1.61 | 0.67 | −4.31*** |
| | SD | SD vs. LD | −0.67 | 8.16*** | −0.67 | −0.67 | 0 | −1.48 |
| | MD | SD vs. LD | −2.56 | 2.76* | 0 | −3.60** | 0 | −4.06*** |
| | LD | SD vs. LD | −7.00*** | −3.82** | 0 | −4.54*** | 0 | −3.74** |
| L*er* | SD vs. MD | SD | 0 | −1.25 | 0 | 2.43 | 0 | 0.67 |
| | SD vs. LD | SD | 1.31 | −3.13* | 0 | 2.21 | 0 | 0.67 |
| | MD vs. LD | SD | 1.31 | −2.01 | 0 | −0.36 | 0 | 0 |
| | SD vs. MD | LD | −6.52*** | −3.99*** | −9.8*** | −9.37*** | −4.91*** | −4.68*** |
| | SD vs. LD | LD | −3.48** | −3.11* | −6.07*** | −2.58 | −0.80 | −0.85 |
| | MD vs. LD | LD | 3.60** | 1.14 | 6.93*** | 7.19*** | 4.72*** | 4.20*** |
| | SD | SD vs. LD | −8.54*** | 0.86 | −1.97 | −5.65*** | −1.63 | −2.12 |
| | MD | SD vs. LD | −12.67*** | −2.20 | −4.58*** | −9.18*** | −3.28** | −3.30** |
| | LD | SD vs. LD | −12.04*** | 0.94 | −3.63** | −7.00*** | −1.96 | −2.32 |

reproduction was more apparent in seeds that had experienced MD or LD before reproduction, since those that experienced SD before reproduction had consistently high germination proportions. When incubated at 22°C, the photoperiod experienced before reproduction had only subtle effects on germination proportions, with seeds exposed to MD having lower germination proportions

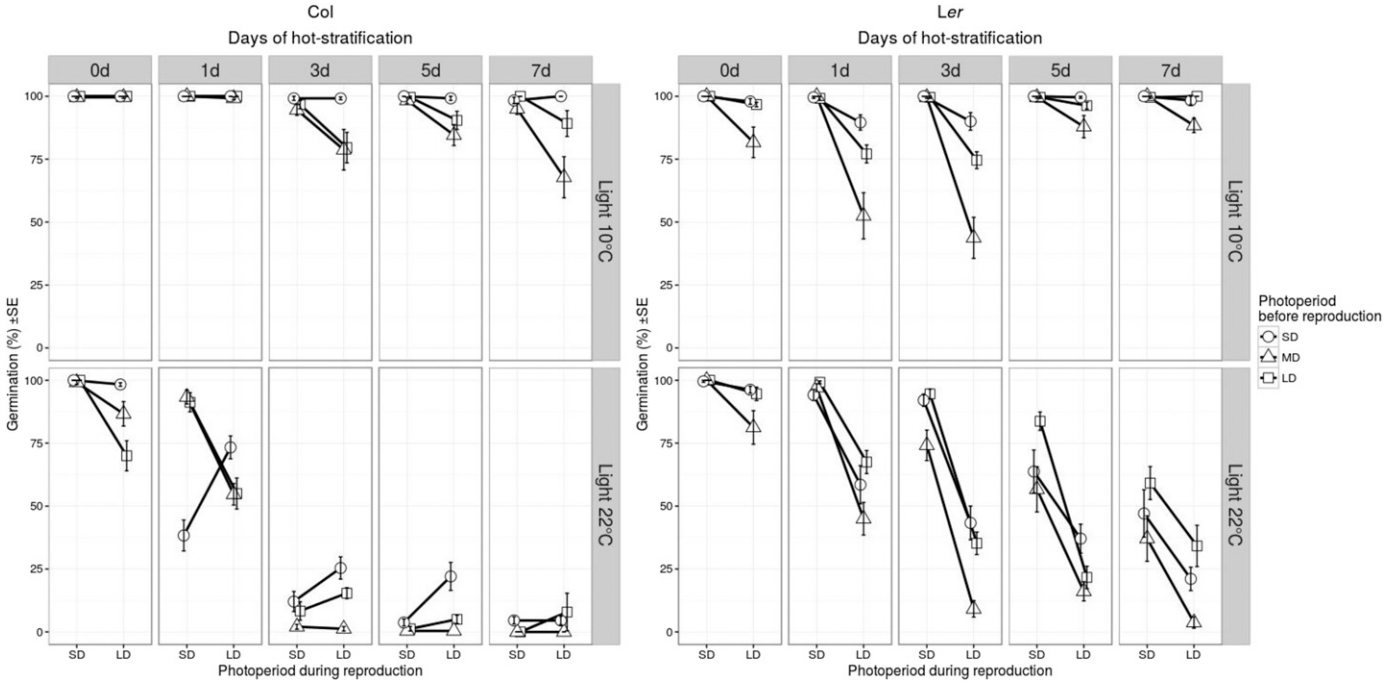


**FIGURE 3** Effects of maternal photoperiod experienced before and during reproduction on secondary dormancy induction. Maternal plants experienced either short days (SD = circles), medium days (MD = triangles), or long days (LD = squares) before reproduction ("Photoperiod before reproduction"), and then experienced either short (SD) or long (LD) during reproduction ("Photoperiod during reproduction," x-axis). Seeds were preincubated at 35°C in the dark to induce secondary dormancy for 0, 1, 3, 5, or 7 wk (left to right for each accession). Results of Col (left panels) and L*er* (right panels) accessions are given for seeds incubated at either 10°C (top row) or 22°C (bottom row). Seeds were incubated in a 12 h light/12 h dark photoperiod.

**TABLE 3.** Comparisons of secondary dormancy induction of seeds that experienced different photoperiods before and during reproduction (Experiment 2). Results are presented for Col and L*er* accessions separately. Seeds were preincubated in the dark at 35°C for 0, 1, 3, 5, or 7 d to induce secondary dormancy and then incubated in a 12 h photoperiod at either 10°C or 22°C. Germination proportions were analyzed with binomial generalized linear models with bias reduction methods, and Z-values are given for specific contrasts. Maternal plants experienced short days (SD), medium days (MD), or long days (LD) before reproduction (first column) and then experienced short (SD) or long (LD) during reproduction (second column). Contrasts compare specific photoperiods experienced before reproduction for a given photoperiod experienced during reproduction, and vice versa. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

| | Maternal photoperiod | | 0d | | 1d | | 3d | | 5d | | 7d | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ecotype | Before Reproduction | During Reproduction | Light 10°C | Light 22°C | Light 10°C | Light 22°C | Light 10°C | Light 22°C | Light 10°C | Light 22°C | Light 10°C | Light 22°C |
| Col | SD vs. MD | SD | −0.67 | −1.04 | 0 | 10.71*** | −2.49 | −3.76** | −1.48 | −2.12 | −1.89 | −2.19 |
| | SD vs. LD | SD | 0 | 0 | 0 | 10.66*** | −1.72 | −1.34 | −0.67 | −1.62 | 1.48 | −2.19 |
| | MD vs. LD | SD | 0.67 | 1.04 | 0 | −0.84 | 1.08 | 2.82* | 1.17 | 0.87 | 2.26 | 0 |
| | SD vs. MD | LD | −0.67 | −4.1*** | −1.04 | −4.19*** | −4.97*** | −5.62*** | −4.33*** | −4.57*** | −3.76** | −2.18 |
| | SD vs. LD | LD | 0 | −6.27*** | 0 | −4.13*** | −4.89*** | −2.68 | −3.46** | −5.01*** | −2.78* | 1.44 |
| | MD vs. LD | LD | 0.67 | −4.31*** | 1.04 | 0.05 | 0.22 | 4.45*** | 1.92 | 2.49 | 5.47*** | 2.60 |
| | SD | SD vs. LD | 0 | −1.48 | 0 | 7.51*** | 0.01 | 3.65** | −1.04 | 5.29*** | 1.42 | 0 |
| | MD | SD vs. LD | 0 | −4.06*** | −1.04 | −8.45*** | −4.73*** | −0.67 | −4.46*** | 0 | −6.75*** | 0 |
| | LD | SD vs. LD | 0 | −3.74** | 0 | −8.14*** | −5.08*** | 2.34 | −3.35** | 2.14 | −2.85* | 2.60 |
| L*er* | SD vs. MD | SD | 0 | 0.67 | 0.67 | 1.5 | −0.68 | −4.95*** | −0.67 | −1.58 | 0 | −2.21 |
| | SD vs. LD | SD | 0 | 0.67 | −0.5 | 2.61 | −0.68 | 1.07 | −0.67 | 4.85*** | −0.67 | 2.64 |
| | MD vs. LD | SD | 0 | 0 | −1.04 | 1.52 | 0 | 5.6*** | 0 | 6.26*** | −0.67 | 4.78*** |
| | SD vs. MD | LD | −4.91*** | −4.68*** | −8.26*** | −2.94* | −9.73*** | −7.8*** | 3.69** | −5.05*** | −3.77** | −5.14*** |
| | SD vs. LD | LD | −0.80 | −0.85 | −3.57** | 2.03 | −4.28*** | −1.80 | −2.12 | −3.66** | 1.48 | 3.15* |
| | MD vs. LD | LD | 4.72*** | 4.2*** | 5.52*** | 4.9*** | 6.70*** | 6.43*** | 3.21** | 1.52 | 2.91* | 7.09*** |
| | SD | SD vs. LD | −1.63 | −2.12 | −3.47** | −7.99*** | −2.79* | −9.98*** | −0.67 | −5.75*** | −1.48 | −5.85*** |
| | MD | SD vs. LD | −3.28** | −3.30** | −4.27*** | −9.24*** | −6.41*** | −12.48*** | −3.69** | −8.74*** | −2.91* | −7.46*** |
| | LD | SD vs. LD | −1.96 | −2.32 | −5.12*** | −5.88*** | −4.8*** | −11.01*** | −2.12 | 12.42*** | 0.67 | −5.41*** |

than the other photoperiods after 3 or 5 d of hot stratification (Table 3). SD experienced during reproduction consistently impeded secondary dormancy induction. Thus in L*er*, seeds from maternal plants that experienced SD before and during reproduction had lower levels of secondary dormancy induction.

In summary, for both ecotypes, secondary dormancy induction was influenced by maternal photoperiods, and the effects of maternal photoperiod on progeny germination proportions persisted even after secondary dormancy induction.

## DISCUSSION

In this study, seed germination responded to maternal photoperiod more strongly than to the photoperiod experienced by seeds during imbibition, and this maternal effect on germination propensity was imposed both at the prereproductive and reproductive stages in the maternal life cycle. Moreover, maternal effects diminished over time with after-ripening, but those maternal effects reappeared with secondary dormancy induction. Thus, contrary to predictions based on maternal cues being less accurate predictors of progeny environmental conditions, and predictions that accuracy diminishes over time, the photoperiod experienced by parents even early in life can persist over time in seeds and influence their germination—even more strongly than the photoperiod experienced by seeds themselves.

***Effects of photoperiod persist and do not attenuate systematically with time***—Maternal effects on germination propensity are common in plants (Donohue, 2009; Baskin and Baskin, 2014), since primary dormancy is induced via maternal control during the late stages of seed maturation. In this study, seeds responded more strongly to maternal photoperiod than to their own photoperiod, and maternal photoperiod did not modify germination responses to seed photoperiod. This observation is contrary to the prediction that seeds would respond more strongly to their own environment than to the maternal environment because their own environment is a better predictor of progeny growth conditions. A recent study in *A. thaliana* also showed that germination responded to light cues (simulated vegetation canopy) experienced by maternal plants more strongly than those same cues experienced by imbibing seeds (Leverett et al., 2016). Those authors argued that the light quality experienced by maternal parents may actually predict future competitive conditions, stimulating seeds to germinate before future competitors that do not yet exist. In that example, it is possible that the maternal cue is a more accurate predictor of progeny competitive conditions than cues perceived by seeds themselves.

Maternal photoperiod experienced even before reproduction influenced progeny germination proportions. Therefore, the effect of prereproductive maternal photoperiod on germination proportions cannot be the result of responses of developing seeds to environmental conditions they experience themselves, but must be imposed by persistent effects of environmental conditions experienced by maternal parents. Comparable effects of prereproductive environmental effects on germination have been reported in *A. thaliana*; prereproductive rosette chilling increased germination propensity of progeny (G. Auge, unpublished data), and reduced ambient temperature at the prereproductive stage decreased germination propensity (Kendall et al., 2011; Kendall and Penfield, 2012; Chen et al., 2014). Some persistent effects of temperature have been

shown to be caused by stable histone or DNA methylation that occur at low temperature (Michaels et al., 2003; Sung and Amasino, 2004; Sheldon et al., 2008; reviewed by Ream et al., 2012), but the precise mechanism for these maternal effects is as yet unknown.

Maternal photoperiod effects on germination proportions diminished as seeds lost primary dormancy through after-ripening, but they reappeared in seeds that were induced into secondary dormancy. As seeds lost dormancy, effects of maternal photoperiod became smaller, but some maternal effects persisted, such that seeds from maternal plants grown in short days attained higher germination proportions sooner than seeds from plants grown in long days. Likewise, seeds from plants grown in short days were less strongly induced into secondary dormancy than seeds from plants grown in long days. These interactions between photoperiod and dormancy loss and induction therefore reflect both (a) that maternal photoperiod can influence how soon after dispersal primary dormancy is lost as well as the facility of induction of secondary dormancy, and (b) that maternal photoperiod effects persist over dormancy loss and reinduction.

Combined, these results indicate that environments experienced in the past—even in a different generation—can strongly influence phenotypes of the present. Moreover, the intensity of the influence of past environments is not related to the time since the environmental cue was perceived. Specifically, the photoperiod experienced before the initiation of reproduction influenced seed germination propensity just as strongly as the photoperiod experienced during reproduction under certain conditions (e.g., fresh Col seeds and 4-wk after-ripened L*er* seeds), and the photoperiod experienced at both of these times in the maternal generation influenced germination propensity more strongly than the photoperiod experienced by seeds. Furthermore, maternal photoperiod effects on secondary dormancy were sometimes just as strong as maternal effects on primary dormancy (especially in L*er*). Therefore, we did not find evidence of systematic attenuation of effects of maternal environmental cues as time passes.

***Combinations of photoperiods experienced across the life cycle influence germination propensity—***Environmental cues experienced at different stages in a life cycle can influence a phenotype independently of the time that elapses between the cue and the expression of that phenotype, as just discussed. Moreover, more recently experienced cues did not override the effects of cues experienced previously. Instead, cues experienced at different life stages can interact to determine a phenotype, such that cues experienced earlier in life may condition responses to more recent cues. It is therefore possible that certain combinations or sequences of environmental cues experienced throughout life may provide the most accurate information regarding future conditions.

In this study, we found no evidence that the maternal photoperiod altered responses to progeny photoperiod, since no effect of the photoperiod during seed imbibition was detected. Thus, seeds do not seem to be using sequential photoperiods between generations to regulate the seasonal timing of germination. In a previous study, maternal photoperiod altered germination responses to progeny cold treatment, such that *Arabidopsis* seeds matured under short days were more strongly stimulated to germinate in response to cold stratification (Munir et al., 2001). Thus maternal photoperiod can alter germination responses to certain cues experienced by progeny, even though it does not alter responses to progeny photoperiod itself.

In contrast, the photoperiods experienced before and during reproduction interacted to influence germination propensity. In some instances, the interaction could be explained by the observation that a short-day photoperiod before reproduction masked effects of photoperiod experienced during reproduction. This is because those seeds had already acquired minimum dormancy, such that even seeds that matured under long days were nondormant. In most instances, short days experienced either before or during reproduction decreased dormancy. That is, short days followed by either short or long days frequently produced comparable germination proportions as short or long days followed by short days. In Col, the effect of prereproductive photoperiod was sometimes stronger than the effect of photoperiod experienced during reproduction; in L*er*, the photoperiod experienced during reproduction was sometimes stronger.

The one exception to a short-day maternal photoperiod increasing germination propensity was that Col seeds from plants that experienced short days before reproduction but long days during reproduction had the highest germination proportions when they imbibed at 22°C. This pattern was also observed in Col seeds with secondary dormancy. It should be noted that seeds from plants that first experienced long days followed by short days did not exhibit comparably high levels of germination, indicating that the effects of photoperiod at these life stages are not additive, but instead the specific sequence of photoperiods experienced over time determines germination behavior.

Interpreted within a seasonal context, these effects of maternal photoperiod could influence the seasonal timing of germination. A typical winter-annual life cycle entails seed germination in the autumn, prereproductive plants overwintering under short days, seed maturation occurring under variable day lengths in spring, with later flowering leading to seed maturation under longer days and seed dispersal under warmer temperatures. Thus most winter annuals (short days before reproduction) are expected to produce seeds with an ability to germinate to high proportions under cool temperature (such as 10°C). If temperatures are warm (such as 22°C), those seeds would be less likely to germinate, especially if they matured late in the spring (short days followed by long days). Such seeds may have less risk of germinating just before summer drought. Spring or summer germinants (spring or summer annuals) would experience long days before reproduction, and if they survive to produce seeds in the long days of spring or summer, those seeds are likely to be more dormant, potentially delaying germination until autumn. In contrast, the specific combination of photoperiods that elicited germination in Col at 22°C—namely short days followed by long days—could result in fresh Col seeds that may actually germinate after maturing and being dispersed in late spring under warm conditions; if they do not germinate as fresh seeds, but experience dormancy induction by hot summer temperatures, these seeds would still be more likely to germinate under warm temperatures. This strategy would only be adaptive in locations that enable survival over the summer. Thus, the observed maternal photoperiod effects may influence seasonal germination phenology, and genetic variation in these maternal effects may be subject to variable natural selection according to specific seasonal contexts.

## CONCLUSIONS

Photoperiod has persistent effects on seed germination behavior. We found no evidence that the effect of photoperiod systematically

attenuates as time passes, but instead the photoperiod experienced at different stages influenced germination propensity independently of the time that elapsed between the cue and the timing of seed imbibition. Moreover, more recently experienced cues did not override the effects of cues experienced previously. Specific sequences of photoperiods experienced at the prereproductive and reproductive stages appear to influence germination behavior in ways that may alter seasonal germination timing. How they influence germination timing under natural conditions, and what the adaptive significance of these responses is in locations with different climates are likely to be fruitful targets for future study.

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