

## INHERITANCE OF RAPID CYCLING IN *BRASSICA RAPA* FAST PLANTS: DOMINANCE THAT INCREASES WITH PHOTOPERIOD

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*Premise of research.* The Wisconsin Fast Plants lines of *Brassica rapa* (RCBr) have been a useful model system for plant ecology, evolution, physiology, and development. However, inheritance of flowering time in the *B. rapa* Fast Plants has not been explored.

*Methodology.* I used quantitative genetics to explore additive, dominant, and epistatic genetic components of between-line variance in flowering time and for genotype × photoperiod interaction effects in crosses between RCBr and naturalized Californian populations.

*Pivotal results.* Strong directional dominance for rapid flowering was evident in the F<sub>1</sub> and F<sub>2</sub> generations. Evidence was equivocal for epistatic genetic variance between the RCBr and California parental types. The expression of additive genetic variance for flowering time in the naturalized California populations was not masked when combined with the RCBr genetic background. A strong genotype × photoperiod interaction was found; whereas flowering time for RCBr was unaffected by day length, flowering time was halved for California plants under a 24-h photoperiod compared with that under their natural photoperiod regime. Importantly, rapid cycling was completely dominant under constant light but partially dominant under shorter photoperiods.

*Conclusions.* Early flowering in the commonly used laboratory-derived lines of *B. rapa* compared with wild populations involves directional dominance that is intensified under long photoperiods. The dominance of accelerated flowering in this case contrasts with the different inheritance patterns seen in independently derived rapid cycling *B. rapa* lines. Generally, the apparent multiplicity of pathways available for flowering time evolution may contribute to the high incidence of local adaptation in this trait.

*Keywords:* Wisconsin Fast Plants, flowering time, genotype × environment interaction, directional dominance.

*Online enhancement:* appendix figures.

### Introduction

Rapid cycling *Brassica* lines are useful tools for research and teaching (Musgrave 2000). Their short generation times facilitate experiments in plant physiology and developmental genetics (Evans 1991; Edwards and Weinig 2011; Lou et al. 2011). In particular, rapid cycling *Brassica rapa* lines from the Wisconsin Fast Plants collection (Williams and Hill 1986) are informative models for studies on adaptive evolution in outcrossing populations (Miller and Schemske 1990; Stowe 1998; Kelly 2006; Waller et al. 2008), having sufficient genetic variation for response to artificial selection in a variety of traits (Ågren and Schemske 1992; Stowe 1998; Briggs and Goldman 2006; Tel-Zur and Goldman 2007; Stowe and Marquis 2011).

Although much continues to be learned using Fast Plants, the inheritance of their hallmark trait—accelerated flowering time—has received limited attention. Several investigations have used independently derived (not Wisconsin Fast Plants) rapid cycling varieties of *Brassica* crop species to probe inheritance of flowering time. For instance, crosses between rapid cycling *Brassica oleraceae* and standard crop varieties revealed quantitative trait loci for loss of the vernalization requirement (Okazaki 2007). Lou et al. (2007) found three quantitative trait loci for flowering time in a cross between a rapid cycling *B. rapa* strain and a Chinese cabbage cultivar. The F<sub>2</sub> generation of this cross had an intermediate flowering time, suggesting additive contributions by genes from the early and late parents. In another cross to create recombinant inbred lines between two rapid cycling crop varieties, one more rapid than the other, the mean flowering time of the advance generation hybrids indicated early flowering to be recessive (Bagheri et al. 2012).

However, genetic analysis is lacking for flowering time differences between Wisconsin Fast Plants and the natural populations they are used to model. Here I report on crosses be-

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tween rapid cycling *B. rapa* Fast Plants (hereafter referred to as RCB<sub>r</sub>) and accessions from three naturalized populations occurring along a steep cline in flowering time in southern California (Franke et al. 2006). These wild populations are outcrossing and harbor quantitative genetic variance for a number of traits (Poultin and Winn 2002; Franks et al. 2007). Both the RCB<sub>r</sub> lines and the California populations lack a vernalization requirement, so genetic differences in flowering time lie in the photoperiodic, gibberellin, or endogenous developmental pathways found in the Brassicaceae (Putterile et al. 2004; Amasino 2010). Whereas the RCB<sub>r</sub> lines were selected for accelerated flowering under a 24-h photoperiod (constant light), the California populations grow in the winter when photoperiods advance from ~9 h at germination (mid-November to early January) to ~11 h at flowering (late January to early March; Franke et al. 2006). California plants are also substantially larger at flowering than the rapid cyclers.

In light of the varied patterns of inheritance for rapid cycling in *B. rapa*, I addressed five questions about the flowering time differences between Fast Plants RCB<sub>r</sub> lines and the California wild populations. (1) Are the differences explained by additive genetic variance, directional dominance, epistasis or maternal effects? (2) If directional dominance is involved, can it be attributed to a single locus? (3) Is the additive genetic variance for flowering time that is known to exist in the California populations expressed when combined with the RCB<sub>r</sub> genetic background in F<sub>1</sub> hybrids? (4) Did selection for rapid cycling under 24 h of light alter flowering time sensitivity to photoperiod? (5) Are line differences in growth traits correlated with flowering time? Answers will provide a first step toward understanding the genetic differences between RCB<sub>r</sub> lines and the natural populations they model and add to our appreciation for the variety of genetic mechanisms underlying the evolution of flowering time.

### Material and Methods

Inheritance of flowering time was explored by crossing RCB<sub>r</sub> lines to accessions from one of three naturalized populations occurring along San Diego Creek in Orange County, California. These wild populations show clinal variation in flowering time (Franke et al. 2006). The central population occurs at the margin of the University of California–Irvine Arboretum (33°39'40"N, 117°51'00"W). The relatively early flowering Back Bay (BB) population is located 4 km to the west, and the later flowering Michelson/Carlson (M/C) population is 2 km to the east.

The first experiment crossed an RCB<sub>r</sub> line harboring an autosomal recessive allele for male sterility (RCB<sub>r</sub>-ms, kindly provided by Paul Williams, University of Wisconsin) to individuals from the Arboretum population. In the RCB<sub>r</sub>-ms line, individuals that are homozygous recessive for the sterility allele produce stunted, nonfunctional stamens, while stamens are fully functional in the homozygous dominant and heterozygote individuals.

I produced F<sub>1</sub> hybrids by crossing Arboretum plants as sires to RCB<sub>r</sub>-ms dams. Parents were grown in 10-cm pots in a 75:25 mixture of commercial potting soil (ProMix BX, Hummert, Our Earth City, MO) and coarse sand. To ensure simultaneous flowering between parents, the RCB<sub>r</sub>-ms dams were planted

1 wk after the Arboretum sires. Plants were watered daily, fertilized biweekly with 10:10:10 NPK liquid fertilizer, and censused daily for flowering. Parents were paired randomly and hand pollinated to produce 24 full-sib families.

Fifteen siblings per F<sub>1</sub> family were then grown in SuperCell Conetainers (Stuewe and Sons, Tangent, OR) in a randomized array under a 14-h photoperiod. Days to first flowering were recorded for each F<sub>1</sub> plant, which were all male fertile.

To produce the F<sub>2</sub> generation, the F<sub>1</sub>s were hand pollinated by feather twice weekly (Weis and Kossler 2004). The path taken through the randomized array was varied among pollination episodes. Self-incompatibility of *B. rapa* assured that the F<sub>2</sub>s were outcrossed.

To determine whether accelerated flowering by the RCB<sub>r</sub>-ms line was due to additive, dominant, or epistatic genetic effects, I simultaneously grew a random sample from the F<sub>1</sub> and F<sub>2</sub> hybrid generations along with the two parental types ( $N = 240$  for the F<sub>2</sub>s and 52–59 for the others). Plants were grown in Conetainers under natural light at a ~14-h photoperiod and watered and fertilized as above. Plants were censused daily for flowering. At maturity, the stem height, stem basal diameter, and number of nodes along the main stem were recorded.

A second experiment tested for genotype × photoperiod interaction effects on flowering time in RCB<sub>r</sub> × wild population crosses. I crossed plants of the standard purple-stemmed line RCB<sub>r</sub>-P (Carolina Biological Supply, Burlington, NC) to plants from the BB and M/C wild populations. Parental plants were grown in 10-cm pots under the same conditions as above. Seven crossing combinations were made: three reconstituted the parental types (RCB<sub>r</sub>-P × RCB<sub>r</sub>-P, BB × BB and M/C × M/C), and four produced reciprocal F<sub>1</sub> hybrids (RCB<sub>r</sub>-P × BB, BB × RCB<sub>r</sub>-P, RCB<sub>r</sub>-P × M/C, and M/C × RCB<sub>r</sub>-P). (Note that no BB × M/C crosses were made, but other work [Weis 2015] established an additive genetic basis for flowering time differences among the California populations.) Thirty plants from each parental type were designated as dams and then crossed to one of 30 sires from each of the appropriate sire types; for example, each BB dam was crossed to unique BB and RCB<sub>r</sub>-P sires. Pollinated flowers were marked according to pollen source. The resulting seeds from within each cross type were pooled, with each dam contributing 8–12 seeds.

The resulting progeny were grown in Conetainer pots, as above, at one of three photoperiods. These included the natural photoperiod regime of the California populations (increasing from ~9 to 11 h over the course of the experiment), the 24-h photoperiod under which the RCB<sub>r</sub> line was developed, and an intermediate 14-h period. For each of the seven crossing combinations, 120 pots were planted, with two seeds per pot (840 pots total), on December 23 in the greenhouse. These were then distributed evenly among six greenhouse rooms, where the photoperiod treatment was applied, with two rooms per treatment level. In the natural photoperiod rooms, plants were grown under natural light only. Photoperiods were extended to 14 and 24 h in the other rooms by paired mercury and sodium vapor lights, which were turned on ~30 min before sunset. In the 24-h rooms, they were turned off ~30 min after sunrise. Plants were re-randomized across bench positions weekly. Pots were censused for emergence on December 31, at which time they were thinned to one seedling per pot. In a few pots, emer-

gence (and thinning) occurred later. Accordingly, seedling emergence was scored as early or late. Plants were censused daily, and flowering time (days since planting) was recorded, along with basal stem diameter.

### Statistical Analysis

In the first experiment (RCBr-ms  $\times$  Arboretum), flowering times of the parental types and their  $F_1$  and  $F_2$  offspring were analyzed through failure time analysis (Fox 2001), implemented in the LIFEREG procedure (SAS Institute 1985). This experiment was terminated before the final 17 plants (of 480) had flowered. Failure time analysis accommodates censored data of this type (Fox 2001) and produces a Wald  $\chi^2$  as its test statistic. Directional dominance was tested by planned contrasts of the generation coefficients. As an additional test for dominance, I performed  $z$ -tests that compared observed hybrid means with the midpoint of the parental strains.

For the first experiment, I also performed a nonparametric test to determine whether the differences between parental lines could be explained by a single dominant locus (Lynch and Walsh 1998). This test compared the distribution of the  $F_2$ s to an expected distribution constructed from the distributions of the parental and  $F_1$  generations, giving double weighting to the  $F_1$ s. Observed and expected distributions were compared by the Komolgorov-Smirnov test.

Further, epistatic components for population differences in flowering time in the RCBr-ms  $\times$  Arboretum cross were explored with the  $\Delta$ -test (Lynch and Walsh 1998, p. 215). This compares the  $F_2$  mean phenotype to the weighted average of the  $F_1$  and parental generations; a nonzero difference between observed and expected indicates that interacting loci segregate in the  $F_2$  generation. The test statistic is the ratio of the deviation from the expected mean,  $\Delta$ , to the square root of its sampling variance (the standard error of the  $F_2$ s plus a weighted average of the parental and  $F_1$  standard errors), which is asymptotically normally distributed.

Additive genetic variance for flowering time is known to exist in the Arboretum wild population ( $b^2 = 0.46$ , 95% confidence level = 0.23–0.68; Franks et al. 2007). As detailed below, the two line cross experiments revealed that the earlier flowering of the RCBr line was genetically dominant to the later flowering of the California populations. I considered the possibility that the effects of the dominant flowering time alleles from the RCBr-ms line, expressed early in development, could mask expression of later-acting additive genetic variance known for the wild population—a form of dominant  $\times$  additive epistasis. To test this, I applied parent-offspring regression to the parents and offspring of the individual crosses made to generate the  $F_1$  generation for the first experiment. Mean flowering times for 21  $F_1$  sibships (each with >12 offspring) were regressed over flowering times of both parents. To avoid scaling of the variance to the mean, dam (RCBr-ms), sire (Arboretum), and  $F_1$  phenotypic scores were separately standardized.

Genetic and photoperiod effects on flowering times of offspring in the second experiment were tested by ANOVA (GLM; SAS Institute 1985). The crosses of the single RCBr-P population to the two California populations (BB and M/C) were analyzed separately. In each analysis, a significant effect of paternal pop-

ulation indicates an additive genetic component to the variance between populations. A population-level maternal effect would be evidenced by a maternal population effect that is greater than the paternal effect (Lynch and Walsh 1998). A significant paternal  $\times$  maternal term would indicate that population differences are due to a dominance component of genetic variance. A significant term for photoperiod indicates a plastic response of flowering time to day length, while significant interactions between photoperiod and the paternal population indicates genetic differences in phenotypic plasticity. Importantly, a significant three-way interaction (photoperiod  $\times$  paternal  $\times$  maternal) would indicate that the strength of directional dominance changes with day length. Germination group (early vs. late) was treated as a covariate. Block effects (greenhouse rooms) were not significant in preliminary analyses and so were dropped. Note that because seeds were drawn from a pooled sample, genetic variation between parental populations was estimated, but not within-population variance.

Stem size (diameter, height, flowering node) in the first line cross experiment was analyzed by ANOVA, with directional dominance tested by SNK contrasts. Correlations between stem dimensions and flowering time were calculated separately for parents and hybrids. Correlations between flowering time and stem diameter were also calculated for each photoperiod treatment in the second experiment; the reciprocal hybrids were pooled for this analysis.

## Results

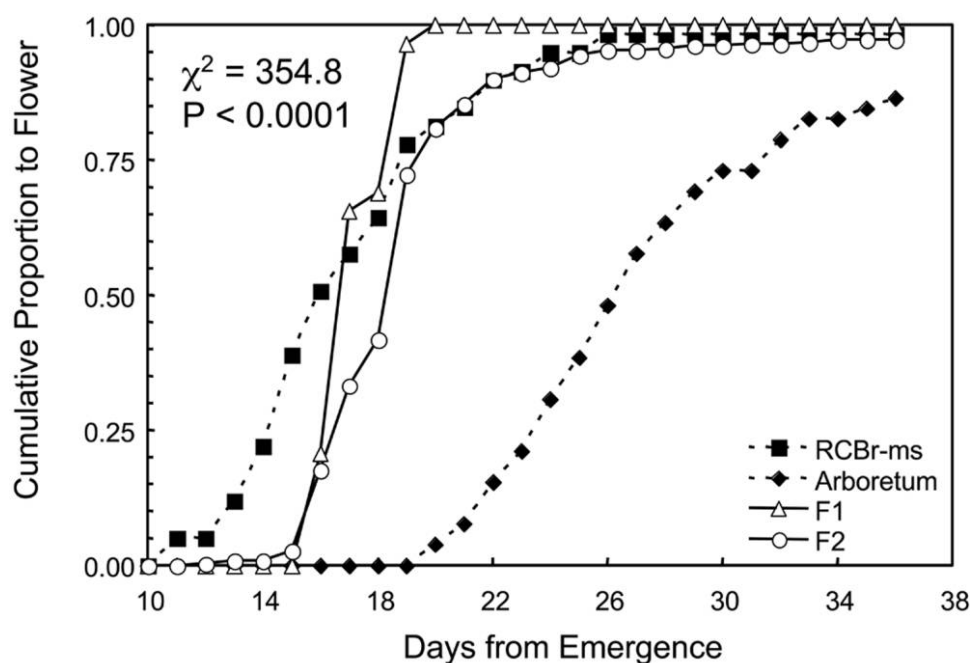
### Directional Dominance and Epistasis

Accelerated flowering showed directional dominance (fig. 1). In the first experiment, the median flowering times of the  $F_1$  and  $F_2$  hybrids were 1 and 2 d later than the RCBr-ms line, respectively. However, they were 9 and 8 d earlier than the Arboretum population. Both hybrid generations flowered significantly earlier than the midparent mean ( $F_1$ :  $z = -38.43$ ,  $P < 0.0001$ ;  $F_2$ :  $z = -4.98$ ,  $P < 0.001$ ), that is, earlier than expected if population differences were caused by additive genetic variance alone. Accelerated flowering in the RCBr-ms strain could not be attributed to a dominant allele at a single locus; the observed distribution of flowering times within the  $F_2$  generation is more restricted than predicted from distributions of the parents and the  $F_1$ s (fig. 2; Komolgorov-Smirnov test,  $P < 0.01$ ).

Evidence for a general epistatic basis to population differences is equivocal. The  $\Delta$ -test, which compares the observed  $F_2$  mean to that predicted from the weighted parental and  $F_1$  means, was marginally significant ( $\Delta = -0.86$ ,  $z = -1.77$ ,  $P = 0.076$ ).

### Expression of Additive Genetic Variance in the $F_1$ Generation

The directional dominance toward rapid cycling did not mask the additive genetic variance in flowering time known to exist in the Arboretum population (fig. 3). Regression of  $F_1$  flowering time over the Arboretum wild parent (sire) phenotype was highly significant (slope = 0.42,  $F_{1,19} = 20.5$ ,  $P = 0.0002$ ). In contrast, regression over the RCBr-ms (dam) phenotype was not significantly different from 0 (slope = 0.08,



**Fig. 1** Cumulative distributions of flowering times for the parental,  $F_1$ , and  $F_2$  generations of the cross between the Arboretum California *Brassica rapa* population and the male sterile RCBBr strain. The significant Wald  $\chi^2$  indicates heterogeneity of the group coefficients in a failure time analysis.

$F_{1,19} = 0.41$ ), indicating a lack of additive genetic variance for that trait in the maternal line.

#### Acceleration of Flowering Time under Longer Photoperiods

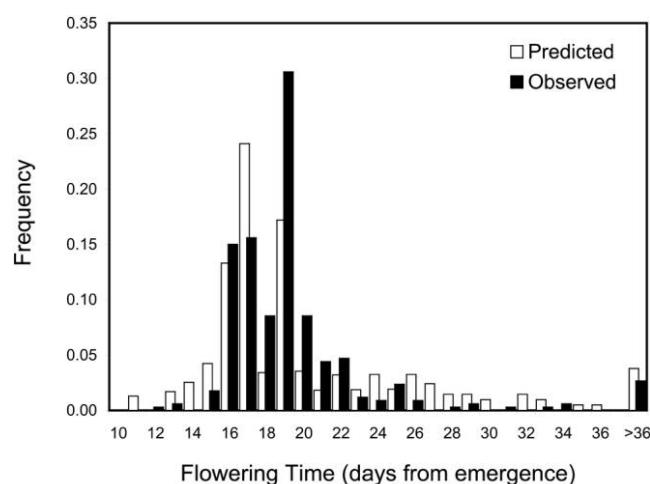
The second experiment showed that flowering time in the RCBBr-P line was insensitive to day length, with a median flowering time of 14 d at all photoperiods. In contrast, increasing day length accelerated flowering in the California populations (fig. 4). Under a natural photoperiod regime (9 → 11 h), the BB plants flowered 37.5 d later than under constant light, while the M/C plants flowered 50 d later.

This experiment not only confirmed directional dominance in flowering time but also demonstrated an intensification of dominance under longer photoperiods. Within each photoperiod regime, the median flowering time for the  $F_1$  hybrids was more similar to the RCBBr-P parent than the wild-population parent (fig. 4), and there is a highly significant paternal × maternal population effect for both crosses (table 1). However, resemblance of the hybrids to the RCBBr-P parent was strongest at the 24-h day length (fig. 4A), where they too had a median flowering time of 14 d. At the shorter photoperiods, the median flowering times for hybrids ranged from 17 to 25 d (fig. 4B, 4C). The significant photoperiod × paternal × maternal population interaction (table 1) indicates that these differences in dominance are greater than expected by random.

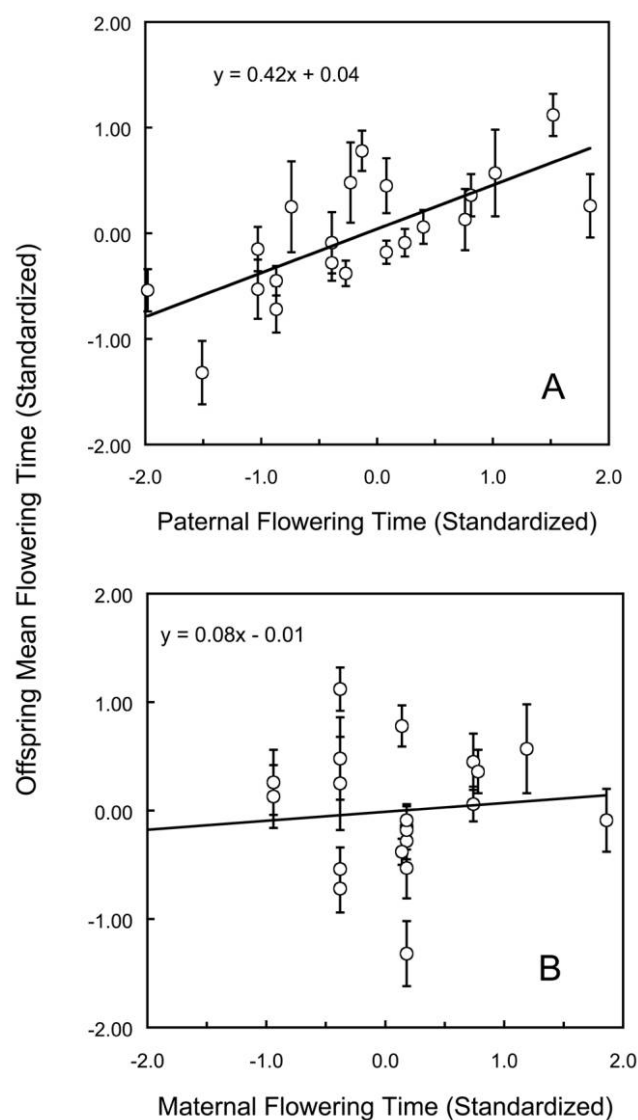
Finally, evidence for a maternal effect on flowering time was lacking in this experiment, since the interquartile ranges for the reciprocal hybrids strongly overlapped (fig. 4). Further, the  $F$  ratios of the mean squares for the maternal population over that for the paternal population were 0.98 and 1.41 for the BB and the M/C populations, respectively (table 1).

#### Population Differences in Stature

Rapid cycling plants were smaller than those from California, while hybrids were generally intermediate. In the first line cross experiment (RCBBr-ms × Arboretum), stem diameter at flowering was significantly different among generations ( $F_{3,458} = 47.1$ ,  $P < 0.0001$ ); the SNK test showed that the two hybrid generations did not differ from one another and were interme-



**Fig. 2** Observed distribution of flowering times in the  $F_2$  generation of the cross between the Arboretum California *Brassica rapa* population and the male sterile RCBBr strain compared with the distribution expected under inheritance due to a single dominant/recessive locus.



**Fig. 3** Parent-offspring regressions for the cross between the Arboretum California *Brassica rapa* population and the male sterile RCB-r strain.

diate to the two parental lines (fig. 5A). Generations also differed for stem heights ( $F_{3,459} = 46.4, P < 0.0001$ ). Heights of the two hybrid generations were intermediate (fig. 5B), although the  $F_1$  more closely resembled the Arboretum parent and the  $F_2$  more closely resembled the RCB-rs parent. With respect to the number of stem nodes at flowering, there were significant differences among generations ( $F_{3,459} = 41.9, P < 0.0001$ ). The SNK contrasts revealed that the Arboretum plants flowered at approximately the eighth node, while the RCB-rs,  $F_1$ , and  $F_2$  types flowered at approximately the sixth node (fig. 5C). This suggests directional dominance for flowering node, as with flowering time.

The positive relationship between size and flowering time that was evident at the among-population/line level was not consistently found within populations/lines. In the first exper-

iment, the RCB-rs group showed significant correlations of flowering time to stem diameter, height, and number of nodes (table 2; fig. A1; figs. A1, A2 available online), but correlations were sporadic or nonexistent within the California population and the hybrids. In the second experiment, the correlation between flowering time and stem diameter was negative within the RCB-r-P line in two of three cases (table 3; fig. A2). The flowering time–diameter correlation tended to be no different from 0 or positive in the other crossing combinations, with no particular pattern other than that they tended to be strongest at the 14 h photoperiod (table 3; fig. A2).

## Discussion

This study explored inheritance of accelerated flowering in widely used lines of rapid cycling *Brassica rapa* by crossing them to well-studied natural populations. I discuss results in relation to the five questions raised in the introduction, compare inheritance patterns of accelerated flowering in the Wisconsin Fast Plants with those observed in other lines of *Brassica* and related species, and conclude with remarks on mechanisms and evolutionary implications.

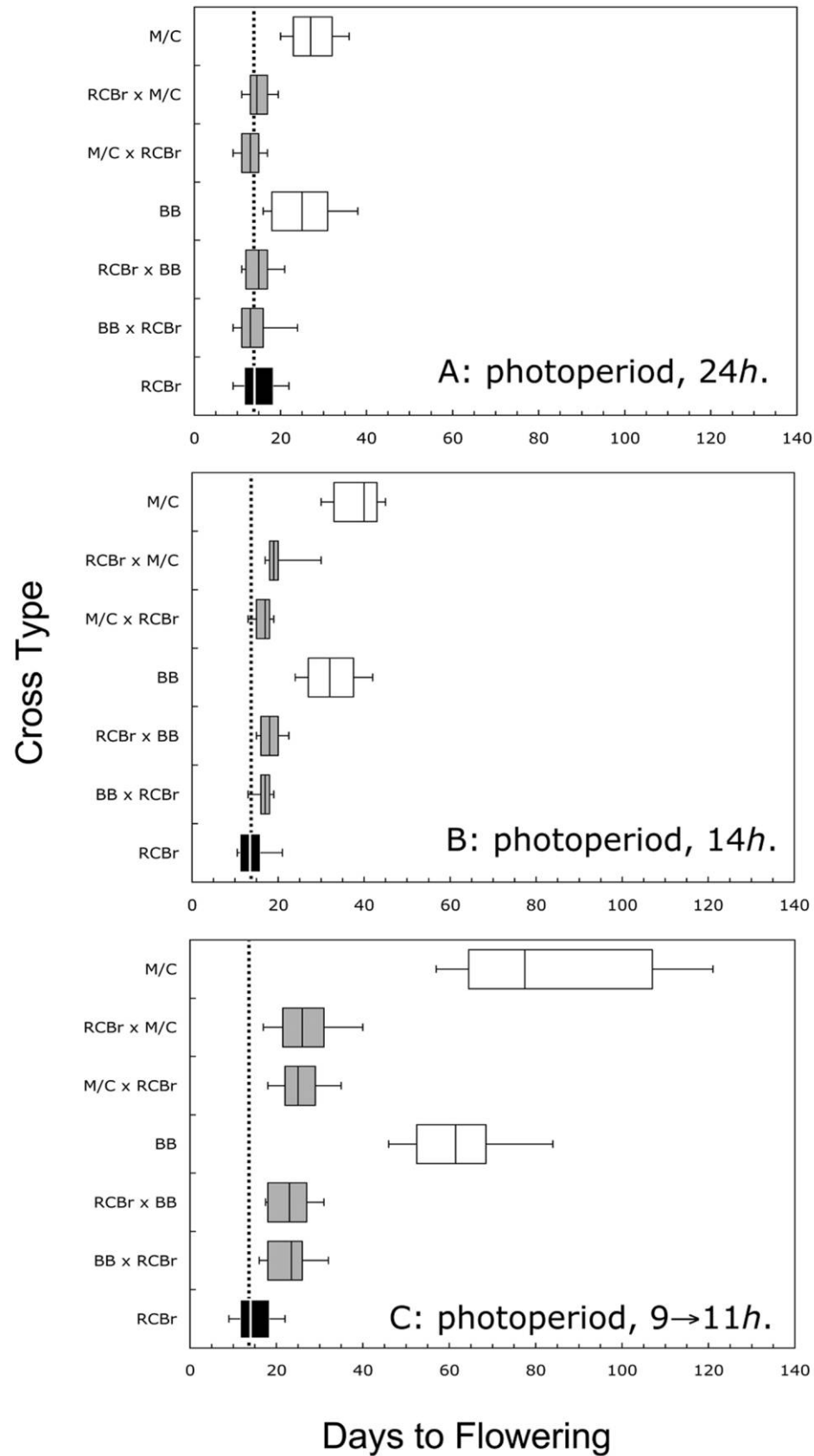
### *Components of Genetic Variance for Flowering Time Differences*

The main question was whether genetic variance in flowering time between the RCB-r lines and the Californian wild populations had additive, dominant, epistatic, and/or maternal effect components. Both experiments demonstrated strong directional dominance of accelerated flowering when *B. rapa* Fast Plants are crossed to the Californian populations (figs. 1, 4). Evidence for additional epistatic genetic variance between populations, as determined by the  $\Delta$ -test, was inconclusive. There was no evidence for a maternal effect on flowering time.

As for the second question—could the differences be explained by segregation at a single locus of a dominant early allele?—the distribution of flowering times in the  $F_2$  generation did not match expectations under that simple scenario (fig. 2). As noted in the introduction, rapid cycling lines of *B. rapa* have been developed from cultivars on at least two other occasions. In one of these, accelerated flowering was recessive to late (Bagheri et al. 2012), the opposite of what was observed in these crosses. In another independent line, early and late genes acted additively (Lou et al. 2007). These varied dominance relationships indicate multiple genetic pathways to the rapid cycling phenotype.

### *Expression of Additive Genetic Variance Contributed by the California Parents*

Third, I asked whether dominant genetic contributions to flowering time inherited from the RCB-r parent masks the additive genetic variance in flowering time that is known to occur in the Californian populations. Recalling that several developmental-genetic pathways can control flowering time in *Brassica* and related species, it seemed possible that a dominant factor, expressed at an early plant age in one pathway, could preempt the variation in flowering signals arriving later through other pathways.



**Fig. 4** Distributions of flowering time, represented by box plots, for crosses between the BB and M/C California *Brassica rapa* populations with the purple-stemmed RCB r under three photoperiod treatments. Dotted lines indicate the median flowering time of the RCB r line at the indicated photoperiod.

**Table 1**  
ANOVA for Date of First Flowering under Different Photoperiods among Offspring from Rapid Cycling *Brassica rapa*, Two Naturalized California *B. rapa* Populations, and Their F<sub>1</sub> Hybrids

Factor	BB:RCBr			M/C:RCBr		
	df	MS	F	df	MS	F
Photoperiod	2	5845	149***	2	18,233	157***
Paternal population	1	15,172	364***	1	26,552	229***
Maternal population	1	14,865	356***	1	37,304	322***
Maternal × paternal	1	3976	95***	1	9345	80***
Photoperiod × paternal	2	2611	62***	2	5131	44***
Photoperiod × maternal	2	2029	48***	2	5175	44***
Photoperiod × paternal × maternal	2	1224	29***	2	1975	17***
Germination date	1	6822	163***	1	15,549	134***
Residual	394	42		428	116	

\*\*\*  $P < 0.001$ .

The first line cross revealed a strong covariance between the flowering times of F<sub>1</sub> hybrids and their wild (Arboretum) parents but not their RCBBr-ms parents (fig. 3). The most parsimonious explanation is a lack of additive genetic variance in the male sterile line. Studies using other Fast Plants lines have found that flowering phenology responds to selection (Ågren and Schemske 1992; Stowe 1998). Thus, not every RCBBr Fast Plants line lacks additive genetic variance for this trait. Perhaps the male sterile line went through a population bottleneck during its breeding history, leading to a loss of genetic variance (Briggs and Goldman 2006).

The standardized regression slope of F<sub>1</sub> flowering time onto the Arboretum parent was 0.42 (fig. 3). This showed that genes from the RCBBr-ms strain do not mask expression of the additive genetic variance known to exist in the California populations. Franks et al. (2007) reported a heritability of  $h^2 = 0.46$  for the Arboretum population (obtained from regression of offspring over midparent) in a greenhouse environment. Since the regression slope of offspring onto the sire is expected to be only half the heritability—that is,  $1/2h^2 = 0.23$  for the Arboretum population—one might erroneously conclude from the steeper observed slope (0.42) that genetic variance in the California population was inflated by epistatic interactions with the RCBBr genes. However, the 1/2 numeric relationship between the paternal regression slope and heritability applies to randomly breeding populations. In the present case, a genetically variable natural population was crossed to a genetically uniform population. This reduces the additive genetic component of the phenotypic variance in the offspring, since dams are genetically invariant. The genetic variance that is expressed in the offspring comes only from the sires. Thus, the similarity of the offspring-sire regression to the heritability of the sire's populations reflects a lack of genetic variance in the dam's RCBBr population and not variance inflation.

#### Effects of Photoperiod

The *B. rapa* Fast Plants lines were selected under a 24-h photoperiod. The fourth question explored the possibility that the intensity and inheritance of rapid cycling changes when more natural photoperiods are imposed. The second experiment showed that the RCBBr-P line is insensitive to photoperiod,

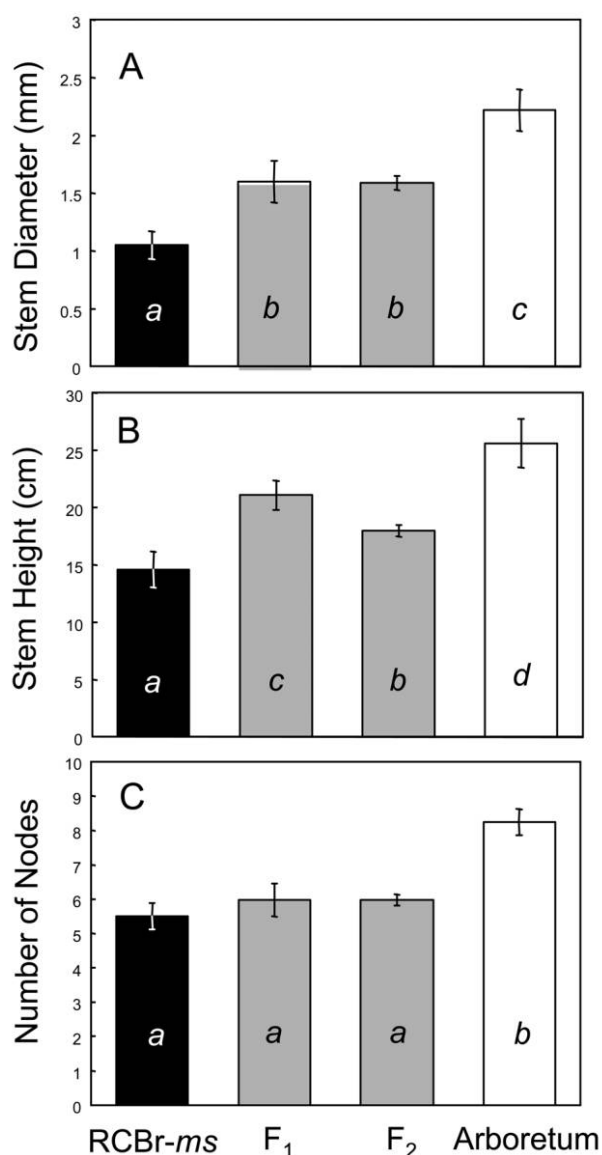
flowering at ~14 d, regardless of day length. In contrast, longer days strongly accelerated flowering for the BB and M/C California populations. At its natural photoperiod regime (9 → 11 h; Franke et al. 2006), the BB population required ~61 d to flower but only ~26 d under constant light. The corresponding reduction for M/C was even stronger—from 77 to 27 d.

Flowering times in the F<sub>1</sub> hybrids showed weaker plasticity. Under the 9 → 11 h treatment, hybrids formed by crossing to the M/C and BB population required 25 and 24 d, respectively, to flower, which is ~10 d longer than the RCBBr-P line but still 1 mo less than the California parents. Under continuous light, in contrast, hybrid and RCBBr flowering times were virtually identical (fig. 4). Thus, the dominance of accelerated flowering increased from partial under shorter day lengths to complete under the 24-h photoperiod in which rapid cycling was selected. This dominance × photoperiod interaction is demonstrated by the significant photoperiod × paternal × maternal ANOVA terms (table 1).

#### Potential Developmental Pathways Underlying Divergence in Flowering Time

This last section evaluates data on the final question—the relation of flowering time to size differences between the RCBBr and California plants—to explore possible mechanisms for the dominant inheritance of rapid cycling in *B. rapa* Fast Plants.

The genetic control mechanisms for flowering time in *Arabidopsis thaliana* (Putterile et al. 2004; Amasino 2010) provide a framework for interpreting the results presented here. In that species, dozens of loci are known to affect flowering time, acting through the vernalization, photoperiodic, gibberellin, and endogenous pathways. Some of these loci were identified through mutagenesis, while others were recovered from wild ecotypes. Alleles at many of these loci show dominance, and epistatic interactions among loci are prominent (Putterill et al. 2004). Many of these genes ultimately act by suppressing expression of *FLOWERING LOCUS C (FLC)*, which itself suppresses expression of the meristem identity genes that ultimately initiate floral development. It is reasonable to think that *A. thaliana* and *B. rapa* have similar genetic control mechanisms over flowering time (Osborne et al. 1997).



**Fig. 5** Means for stem traits in the  $F_1$  and  $F_2$  generations of the cross between the Arboretum California *Brassica rapa* population and the male sterile RCBr strain. Bars with the same letter are not significantly different from one another. A, Stem diameter at flowering. B, Stem height at flowering. C, Number of nodes below the inflorescence on the main stem.

The naturalized Californian populations were an appropriate choice to probe the inheritance of rapid cycling in the *B. rapa* Fast Plants. Several developmental pathways that prolong the preflowering vegetative growth phase of the life cycle are inoperative in both RCBr and the California populations. Specifically, RCBr has no vernalization requirement (Williams and Hill 1986) and neither do the California populations, which readily go from emergence to flowering at any time of year, given sufficient soil moisture for germination and growth (Franke et al. 2006). Further, differences between the laboratory and wild populations are not due to genetic differences in a critical photoperiodic threshold requirement. The RCBr was selected

for accelerated flowering under a 24-h photoperiod, but the second experiment showed scant photoperiod sensitivity in this population. The California populations germinate during November or December and can flower as early as the first week of January (Franke et al. 2006). Thus, maturation occurs during the shortest days of the year, when day length has its lowest rate of change, making photoperiod weakly informative about changing growing conditions.

Nevertheless, longer days accelerated flowering in the natural California populations (Franke et al. 2006), and this plasticity could operate through the photoperiodic pathway (Putterill et al. 2004). Alternatively, the extra hours of light may have allowed greater gross photosynthesis per day, which in turn could accelerate flowering in the California plants through the endogenous pathway. Even if there is a critical photoperiod threshold requirement for flowering in the California populations, it is shorter than the shortest day they are exposed to in the environments where they have recently evolved.

The factors conferring rapid cycling to the RCBr Fast Plants lines might suppress expression of *FLC* early in plant development. RCBr does not appear to conform to either of the two genetic mechanisms known to lead to a summer annual life history in *A. thaliana* (described as rapid cycling, with winter annual as the ancestral state). Nonfunctional alleles at the *FRI* locus, a promoter of *FLC*, relieve the suppression of meristem identity genes in some *A. thaliana* populations (He and Amasino 2005). Alternatively, weekly functioning alleles of *FLC* have been recovered from some summer annual populations (Michaels et al. 2003). In both instances, early flowering is recessive. Three homologs of the *A. thaliana FLC* locus have been identified in *B. rapa* (Schranz et al. 2002). In Chinese cabbage cultivars of *B. rapa*, flowering soon follows their downregulation, once vernalization has occurred (Kim et al. 2007).

*FLC* expression can be suppressed by a number of loci within the autonomous pathway (He and Amasino 2005), and so the dominance of early flowering time in the RCBr  $\times$  California crosses could be explained if one or more of these *FLC* suppressors are expressed early in the rapid cycling strain but later in the wild population. If heterozygotes express a sufficient dose of such suppressors, rapid cycling would show the observed directional dominance. Note that in addition to flowering earlier, the RCBr population and the two hybrid generations initiated flowering at a lower stem node than the California population (fig. 5). However, stem diameter and height were intermediate in hybrids. This could suggest that loci conferring rapid

**Table 2**

**Phenotypic Correlation between Flowering Time and Three Stem Traits in the Cross between the RCBr Male Sterile Strain and the Arboretum Population of *Brassica rapa***

Generation	Diameter	Height	Node
RCBr-ms	.55***	.62***	.40***
$F_1$	-.42*	.31 <sup>†</sup>	-.25 (ns)
$F_2$	.10 <sup>†</sup>	-.10 <sup>†</sup>	.46***
Arboretum	.45**	.12	.22 (ns)

<sup>†</sup>  $P < 0.10$ .

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .



Table 3

Phenotypic Correlations of Flowering Time and Stem Diameter under Different Photoperiods (h) among Offspring from Rapid Cycling *Brassica rapa*, Two Naturalized California *B. rapa* Populations, and Their F<sub>1</sub> Hybrids

Cross type	9 → 11	14	24
RCBr-P × RCBr-P	.06 (ns)	−.38*	−.21 (ns)
BB × BB	−.02 (ns)	.61***	.04 (ns)
BB × RCBr-P	.08 (ns)	.43***	−.14 (ns)
M/C × M/C	.30†	.29†	.46***
M/C × RCBr-P	.48***	.35**	.23*

†  $P < 0.10$ .

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

cycling operate through a switch triggered by developmental rate as opposed to growth rate per se, although specific mechanisms are not immediately obvious.

The discovery here of another genetic path to accelerated flowering time in *B. rapa*, a well-studied species, relates to the evolutionary lability of this trait. A literature review by Mazer and LeBuhn (1999) found that flowering time differed among conspecific populations more frequently than other life-history traits. Local differentiation in flowering time is often, if not usually, adaptive (Ellis et al. 2006; Griffith and Watson 2006; Hall and Willis 2006; Coulatti et al. 2010; Ågren and Schemske 2012; Samis et al. 2012), and recent studies have found that

this trait can evolve over very short timescales (Franks et al. 2007; Coulatti et al. 2010). Assortative mating by flowering time may contribute to its swift evolution by inflating its heritable variation (Weis et al. 2014) and by constricting maladaptive gene flow (Weis 2015). In addition, the multiple developmental-genetic pathways controlling this trait offer a multiplicity of targets for beneficial mutation. For example, *A. thaliana* was introduced from Europe to North America 150–200 yr ago and, in that brief time, has re-evolved the longitudinal cline in flowering time seen in its native range. Remarkably, Samis et al. (2012) found that the new and ancestral clines have different genetic bases. The varied paths to rapid cycling in *B. rapa* thus underscore the multiplicity of opportunities for genetic variance to arise in flowering time, which may contribute to frequent and swift adaptive change in this key trait.

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