Changing Responses to Changing Seasons: Natural Variation in the Plasticity of Flowering Time[1[OPEN]]

Benjamin K. Blackman*

Department of Plant and Microbial Biology, University of California, Berkeley, California 94720

ORCID ID: 0000-0003-4936-6153 (B.K.B.).

For plants that live in seasonally changing environments, timing is everything. Matching developmental transitions with the best times of year for growth and reproduction is necessary to maintain high fitness. Consequently, plants employ many mechanisms to sense and integrate multiple predictive seasonal cues to regulate their major developmental shifts. As the annual timing with which the growing season starts and ends changes across the landscape, natural selection has led to the evolution of the mechanisms that regulate the developmental plasticity of flowering among populations or varieties of species and crop plants that inhabit broad geographic ranges. There has been significant recent progress in describing the diversity of this variation in flowering time plasticity and in identifying the specific genetic changes responsible. Such work is an essential step toward understanding the processes that have shaped current and past adaptation, managing genetic diversity and improving crops in the face of climate change, and forecasting how populations may respond plastically and evolutionarily to future environmental challenges. In this Update, I review the findings of recent studies of natural variation in the plasticity of flowering to photoperiod, vernalization, and ambient temperature, and the implications and open questions raised by this work are considered.

A fundamental adaptation of plants inhabiting seasonal environments is their ability to match the annual timing of major life history transitions to the local growing season. Most species achieve this synchrony through developmental plasticity. In other words, individuals sense how environmental cues like daylength and temperature change from winter to spring to summer to fall. The information gleaned from these cycles is then integrated molecularly so that germination, flowering, and other key transitions occur during periods favorable for growth, reproduction, and seed set. However, as the climate changes over the 21st century and the relative timing of annual cycles in temperature and precipitation shifts, once adaptive responses will no longer effectively predict the best calendar dates to initiate these essential developmental events (Nicotra et al., 2010; Wilczek et al., 2010). Because natural populations and cultivated landraces of many taxa have evolved to thrive in geographically diverse habitats and climates as their ranges have expanded, they harbor natural variants that may prove instructive in breeding crops and conserving native plant diversity in the face of future environmental challenges. Thus, a critical objective in plant biology is

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- Surveys of natural variation in how flowering responds to various environmental cues have revealed that adaptation is complex and can occur through alterations in the type of response, its magnitude, or the thresholds that govern the expression of the response.
- Variants that affect how day length regulates flowering are found throughout the photoperiod pathway, including in sensors or integrators of light reception and circadian signaling, as well as in downstream floral inducers or repressors acting in the leaf and shoot apex.
- In contrast, allelic series in a few genes explain most of the known variation in vernalization response in the Brassicaceae and cultivated grasses.
- The diversity and the mechanisms of how flowering responds to ambient temperature are receiving increased attention in *Arabidopsis thaliana*.

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* Address correspondence to bkblackman@berkeley.edu.

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not only to understand the mechanisms by which plants interpret seasonal cues to guide development but also to learn how evolution has historically altered the underlying molecular bases of these responses as plants have adapted to cope with environmental heterogeneity across the landscape.

Flowering time has emerged as a model trait for investigating the evolutionary genetics of developmental plasticity. A deep history of physiological studies in crop and wild taxa has demonstrated that several seasonal cues, photoperiod, ambient temperature, and exposure to prolonged cold (vernalization), among others, regulate the floral transition in many species (Allard and Garner, 1940; Chouard, 1960; Lang, 1965; Thomas and Vince-Prue, 1997; Kim et al., 2009; Amasino, 2010). Moreover, these early studies revealed important aspects of these responses, including that leaves produce a mobile inductive signal in response to inductive photoperiods (Knott, 1934; Zeevaart, 1962) and that the perception of vernalization directly in the shoot apical meristem leads to a mitotically stable memory of winter (Chouard, 1960; Lang, 1965). Building on this history of investigation, many genetic studies performed over the past two decades in the genetic model plants Arabidopsis (thale cress, *Arabidopsis thaliana*) and rice (*Oryza sativa*) as well as an increasingly broad array of additional organisms have produced abundant insight into the underlying molecular basis of these responses. As reviewed elsewhere, including in this issue, environmental cues modulate the timing of flowering through diverse transcriptional and posttranscriptional regulatory mechanisms, including DNA methylation, chromatin modification, small and long noncoding RNA activity, protein degradation, and protein transport (Andrés and Coupland, 2012). As we continue to learn more, it is hard not to be impressed by how the complex, interacting mechanisms involved achieve a high level of specificity in the type and duration of cues required to trigger floral initiation as well as the degree of reversibility and the quality of the induced response.

And yet, despite their critical functions, the pathways that regulate the plasticity of flowering to environmental signals are not all that highly conserved compared with other developmental networks. These mechanisms differ extensively between model taxa, indicating that the flowering time gene regulatory network has the flexibility to evolve in myriad ways. For instance, although homologs of the core photoperiod pathway genes all regulate floral induction by daylength cues in Arabidopsis and rice, by long days and short days, respectively, their regulatory relationships have been rewired (Hayama et al., 2003). Moreover, additional pathways by which photoperiod cues regulate flowering exist in monocots that have no clear counterpart in dicots (Itoh et al., 2010), and whether homologs of the key integrator gene *CONSTANS* contribute to flowering regulation has been questioned in several dicot species (Hsu et al., 2012; Wong et al., 2014; Simon et al., 2015). Likewise, the pathways by which the memory of winter is retained in Arabidopsis and grasses share few details in common (Ream et al., 2012).

However, such macroevolutionary differences likely reflect long-term convergence and/or developmental system drift (True and Haag, 2001) and may bear little resemblance to the types of genetic alterations by which populations have incrementally adapted to environmental gradients across space or will adapt to climate change through time. Thus, particularly as more genetic and genomic tools have been adopted in new systems, many investigators have begun concentrating on how wild and crop plants have adapted to thrive in diverse habitats that vary in the timing and predictability of the growing season across both broad and fine geographic scales. In doing so, they have found abundant variation in the plasticity of flowering, ranging from subtle tweaks in particular parameters to abolition or full reversal of the direction of responses. Understanding the genetic architecture of this diversity, whether divergence occurs rapidly through allelic substitutions of major effect at few loci or through the accumulation of changes at many minor loci, can inform our outlook on whether populations may be able to adapt at a rate that can keep pace with a changing climate (Chevin et al., 2010). Likewise, if developmental plasticity often evolves through selection on alleles already segregating as standing genetic variation in populations rather than awaiting new adaptive de novo mutations to arise, the prognosis for species to adapt in the face of a changing climate may be more optimistic. Finally, characterizing how the causal variants impact seasonal phenology and fitness across field locations and years as well as assessing whether they have pleiotropic effects on other traits is essential (Wilczek et al., 2009, 2010; Anderson et al., 2012). Only by doing so can we gain a comprehensive view of the adaptive value of this variation and model how its impacts on whole-plant biology may constrain or enhance responses to selection imposed by future, potentially novel climates.

Here, my goal is to review how the ever-growing recent literature on the ecological genetics of natural variation in the developmental plasticity of flowering has begun to shed light on these questions. Focusing on the three cues that have received the greatest attention, photoperiod, vernalization, and ambient temperature, I will explore how surveys of natural populations and landraces are revealing that perception and responses to these cues change along diverse axes of variation, often structured along environmental gradients. I will also consider in turn what genetic studies have shown to be the genetic architecture and molecular basis of this natural variation in plasticity. Finally, I will consider how these findings that have come from dissecting this variation under controlled conditions can be extended to move us toward understanding the evolution of seasonal phenology in past, current, or future field conditions.

EVOLUTION OF THE PHOTOPERIODIC REGULATION OF FLOWERING

Response Type Diversity

Photoperiod responses can be facultative or obligate, and they generally fall into one of two categories. Short-day plants flower earlier (or only) in short days, and long-day plants flower earlier (or only) in long days (Allard and Garner, 1940). Facultative responses are approximately sigmoidal with two threshold photoperiods. Below one threshold, noninductive conditions fail to further delay flowering; above the other threshold, inductive conditions fail to further accelerate flowering. A final major category is day-neutral flowering; these plants do not exhibit photoperiod-sensitive flowering. More complex, but rarer, types also occur (Thomas and Vince-Prue, 1997). For instance, ambiphotoperiodic plants flower earlier in long and short days relative to intermediate conditions. Other species require a succession of long days before short days (or vice versa) to flower.

In both long-day and short-day species, intraspecific transitions to day neutrality are observed frequently. These transitions are generally quantitative and clinal, resulting from incremental reductions in the magnitude of the response, rather than abrupt losses, and thus are covered separately below. Transitions from long-day to short-day flowering, or vice versa, are far less common on the microevolutionary scale (Thomas and Vince-Prue, 1997). A few cases have recently received attention. Notably, they all implicate alterations to the control of floral induction in the shoot apex rather than divergence in the measurement of photoperiod in the leaf. First, long-day flowering evolved from short-day flowering as wild populations of the common sunflower (*Helianthus annuus*) expanded the species range into southern Texas (Blackman et al., 2011). This reversal is associated with differences in the photoperiod sensitivity of shoot apical but not foliar expression of homologs of the floral inducer *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*). Second, some cultivated varieties of the perennial plants rose (*Rosa chinensis*) and woodland strawberry (*Fragaria vesca*) have been selected for perpetual flowering relative to a short-day ancestral state. Loss-of-function mutations in homologs of the floral repressor *TERMINAL FLOWER1* remove the seasonal block to flowering, leading to perpetual flowering (Iwata et al., 2012; Koskela et al., 2012). However, as this mechanism is more akin to day-neutral flowering and the long-day response in *F. vesca* depends on an interaction with ambient temperature (Sønsteby and Heide, 2008), switches to perpetual flowering serve as less straightforward examples of reversal in plasticity as the change in sunflower.

Variation in Magnitude of Photoperiodic Response

In species with facultative responses to photoperiod, the difference between flowering time under inductive and noninductive photoperiods often evolves along geographic transects. For instance, among wild sunflower populations, the difference in flowering time between inductive short days and noninductive long days decreases with increasing latitude, such that extreme northern populations are day neutral, allowing rapid flowering in short northern growing seasons (Blackman et al., 2011). Similar clines are observed in long-day plants, likely because extending the preflowering growth period later into the milder springs and summers found at higher latitudes increases seed yield (e.g. Turner et al., 2005). Association and quantitative trait locus (QTL) mapping studies of photoperiod response have implicated anywhere from a handful to as many as 14 loci contributing to intraspecific variation (e.g. Coles et al., 2010; Anderson et al., 2011; Hung et al., 2012; Henry et al., 2014; Jiang et al., 2014). Some of these loci represent allelic series, and different subsets of the loci may be combined to produce equivalent day-neutral phenotypes (e.g. Xu et al., 2013; Jiang et al., 2014).

Alleles influencing the magnitude of photoperiod response occur throughout the flowering network, affecting upstream regulation by circadian clock and light signaling or the expression and function of downstream floral integrators or inducers. In two long-day legumes (pea [*Pisum sativum*] and lentil [*Lens culinaris*]), loss-of-function variants in homologs of *EARLY FLOWERING3* (*ELF3*), a modulator of the clock and its outputs, promote earlier flowering under noninductive short-day conditions, are widespread in cultivated germplasm, and may have facilitated expansion into northern Europe (Weller et al., 2012). Spontaneous and induced mutations in the barley (*Hordeum vulgare*) *ELF3* homolog also have contributed to the production of barley breeds cultivated in the short growing seasons of northern regions (Faure et al., 2012; Zakhrabekova et al., 2012). In cultivated soybean (*Glycine max*), coding polymorphisms at four loci, including variants leading to premature stops in two paralogs homologous to phytochrome A and a homolog of *GIGANTEA*, combine to build the latitudinal cline such that photoperiod sensitivity decreases with increasing latitude (Zhang et al., 2008; Xu et al., 2013; Jiang et al., 2014). These same alleles do not explain a similar cline in wild soybean, however.

Allelic variation in several pseudo-response regulator genes that function at the intersection of clock and light signaling have been implicated in the evolution of reduced photoperiod responses following the expansion of cultivation to temperate environments in barley (Turner et al., 2005; Jones et al., 2008; Lister et al., 2009), rice (Xue et al., 2008; Koo et al., 2013; Yan et al., 2013), and maize (*Zea mays*; Hung et al., 2012; Yang et al., 2013). In the final case, a transposable element disrupts the promoter sequence and reduces photoperiod response by attenuating the expression of this floral repressor in noninductive long days (Yang et al., 2013). The insertion likely arose after domestication and experienced a strong selection in temperate maize.

Evolutionary changes affecting homologs of the floral inducer *FLOWERING LOCUS T* (*FT*) also have contributed to changes in photoperiod sensitivity during domestication or the subsequent diversification of crop plants. A frameshift mutation in a sunflower *FT* paralog segregating at low frequency in wild populations experienced a selective sweep during domestication, and heterologous transformation studies in Arabidopsis indicate that this variant causes a photoperiod-specific delay in flowering through dominant-negative interference with the function of another *FT* paralog (Blackman et al., 2010; Blackman, 2013). Recent work also has associated cis-regulatory variants in *FT* homologs of soybean (Zhao et al., 2016) and sorghum (*Sorghum bicolor*; Cuevas et al., 2016) with the evolution of day-neutral varieties. Finally, *FT* has been implicated in the reduced photoperiod sensitivity of one Arabidopsis accession (Strange et al., 2011).

Shifting Daylength Thresholds

In many species, the floral transition is obligately dependent on a critical photoperiod. Below a minimum threshold daylength or above a maximum threshold daylength, plants do not flower (Thomas and Vince-Prue, 1997). Recent studies of the common monkeyflower *Mimulus guttatus*, an obligate long-day wildflower species distributed throughout western North America with both annual and perennial ecotypes, have begun to explore patterns of phenotypic and genetic variation in this parameter. Among populations of the annual ecotype, critical photoperiod increases with the latitude and elevation, tracking variation in the annual start of the growing season (Kooyers et al., 2015). Notably, time to flower in inductive greenhouse conditions evolves instead along clines in growing season duration. That these two aspects of seasonal phenology locally adapt following distinct environmental parameters suggests that the allelic variation affecting each trait occurs in largely independent sets of loci.

Critical photoperiod also largely distinguishes the annual and perennial *M. guttatus* ecotypes; perennial populations require at least 14-h days to flower, whereas only annual populations at high elevation and latitude require such daylengths (Friedman and Willis, 2013). In contrast, *Mimulus nasutus*, a largely selfing and often sympatric congener, has evolved a lower critical photoperiod (11 h), allowing earlier seasonal flowering than most *M. guttatus* (Fishman et al., 2014). QTL mapping experiments in a few controlled crosses indicate that the differences in critical photoperiod among ecotypes or species are accounted for by a few loci of major effect (Friedman and Willis, 2013; Fishman et al., 2014), but the functions and evolutionary histories of the underlying alleles remain unknown.

The diversity and genetics of natural variation in threshold parameters have received less attention in obligate short-day species and species with facultative responses. Interestingly, it is the critical night length that varies between two cultivars of the obligate short-day plant *Pharbitis nil*, and this difference is associated with a shift in the diurnal phase of expression for two *FT* paralogs (Hayama et al., 2007). In facultative long-day Arabidopsis, the ceiling photoperiod below which shorter days no longer delay flowering is typically 10 h, and the critical photoperiod above which longer days no longer accelerate flowering is typically 14 h. Differences between individual accessions in these parameters map to a few major QTLs (Giakountis et al., 2010).

EVOLUTION OF FLOWERING REGULATION BY VERNALIZATION

Chilling Out Promotes Flowering in Some Genotypes But Not Others

Many species germinate in the summer or fall and overwinter before the onset of flowering in the spring. Vernalization response, or the acceleration or permission of flowering by prolonged exposure to cold, is often an adaptation that prevents premature flowering in species with such seasonal phenologies. In nonvernalized Arabidopsis plants, the *FRIGIDA* (*FRI*) gene up-regulates the MADS box transcription factor *FLOWERING LOCUS C* (*FLC*), which represses the expression of *FT* in the leaf and *SOC1* in the shoot apex (Michaels and Amasino, 1999; Sheldon et al., 1999; Johanson et al., 2000). Vernalization represses this repressor to activate flowering. Relief of a repressor of *FT* homolog expression and flowering by vernalization is also observed in cereal crops and wild grasses in the core Pooideae. However, the repressor is instead the pseudo-response regulator *VERNALIZATION2* (*VRN2*), and a MADS box transcription factor (*VRN1*) not orthologous to *FLC* and up-regulated by prolonged cold is responsible for its repression (Levy et al., 2002; Yan et al., 2004; Woods et al., 2016).

Loss of vernalization response within species is frequently attributable to a loss-of-function mutation at a single major locus. For instance, in Arabidopsis, spring annual accessions that are able to germinate and set seed in a single growing season have evolved as many as 20 times independently through unique loss-of-function mutations in *FRI* (Johanson et al., 2000; Shindo et al., 2005; Strange et al., 2011). In contrast, the loss of vernalization response in the perennial *Arabis alpina*, also in the Brassicaceae, has evolved through multiple independent inactivating coding or regulatory mutations in its *FLC* homolog (i.e. in the floral repressor rather than its activator; Albani et al., 2012). Conversions of winter annual to spring annual varieties by loss of vernalization response in cultivated wheat (*Triticum aestivum*) and barley have evolved analogously, through allelic variation in the floral repressor *VRN2* (Yan et al., 2004). Multiple *VRN2* loss-of-function alleles segregate among spring diploid wheat varieties, including a charge-changing nonsynonymous substitution, a deletion in the promoter region, and a deletion of the full coding region. Likewise, the *VRN2* gene is absent from spring barleys (Yan et al., 2004).

Diversity in How Long Winter Needs To Be Experienced

Plants with vernalization responses may vary in how long winter chilling must be experienced to accelerate flowering or in the temperature profiles that promote vernalization (e.g., Yan et al., 2004; Lempe et al., 2005; Werner et al., 2005; Friedman and Willis, 2013; Ream et al., 2014). Quantitative variation in the former parameter has been best explored in the Brassicaceae, where it largely involves major-effect changes at a few loci (Anderson et al., 2011; Salomé et al., 2011; Strange et al., 2011; Grillo et al., 2013).

Although variation in *FRI* functionality is the major cause of the presence/absence of a vernalization requirement in Arabidopsis, *FLC* appears to be the most frequent source of variation in the strength of the vernalization response (e.g. Michaels et al., 2003; Sánchez-Bermejo et al., 2012). For instance, European Arabidopsis accessions segregate for five major *FLC* haplotypes, two associated with rapid vernalization and three associated with slow vernalization (Coustham et al., 2012; Li et al., 2014, 2015). For one of these haplotypes, a set of noncoding polymorphisms impacts how quickly prolonged cold promotes the accumulation of silencing chromatin marks on *FLC*'s cis-regulatory sequences and gene body (Coustham et al., 2012; Li et al., 2014). A single single-nucleotide polymorphism in another haplotype impacts the splicing of the long noncoding RNA *COOLAIR* expressed from the *FLC* locus, leading to higher *FLC* expression and, consequently, an increased vernalization requirement (Li et al., 2015).

Association and QTL mapping studies have also associated variation in flowering time or vernalization response with polymorphisms in the *FLC* homologs of *Brassica oleracea*, *Brassica rapa*, and *Brassica napus* (Zhao et al., 2010; Hou et al., 2012; Wu et al., 2012; Irwin et al., 2016). For instance, one of two major *FLC* haplotypes in *B. oleracea* is transcriptionally repressed by cold exposure more slowly than the other (Irwin et al., 2016). In the selfing species *Capsella rubella*, a rare variant introduces a new splice acceptor site, resulting in a frameshift and truncation of the *FLC* open reading frame and thus reducing but not abolishing vernalization response (Guo et al., 2012). Notably, a splice donor variant in an *FLC* homolog of tetraploid *Capsella grandiflora* is found in multiple geographic regions and also is associated with flowering time variation (Slotte et al., 2009), but its direct impact on vernalization response is not described.

Diversity in Optimal and Critical Thresholds for Vernalization

The temperature threshold parameters below which plants express vernalization or that result in the greatest response to vernalization may also vary among genotypes (Rawson et al., 1998; Wollenberg and Amasino, 2012; Ream et al., 2014; Duncan et al., 2015). For instance, some *Brachypodium distachyon* accessions can be successfully vernalized by temperatures as high as 16°C, while others require lower temperatures (Ream et al., 2014). In Arabidopsis, the magnitude of the vernalization response is greater at cooler temperatures, although the response is less pronounced below 2°C for some accessions and below 8°C for others. In the case of one Swedish accession, the higher optimum is due to differences in the efficacy of cold in mediating epigenetic silencing of *FLC* (Duncan et al., 2015). The upper temperature threshold permissive to a vernalization response of any magnitude in Arabidopsis varies from 10°C to 19°C (Wollenberg and Amasino, 2012).

Interacting Photoperiod and Vernalization Responses

In many species, both daylength and prolonged exposure to cold can act to accelerate flowering, and the magnitude of response to one cue may be accentuated or diminished depending on whether a plant experiences inductive or noninductive conditions with respect to the other cue. For example, vernalization accelerates flowering by fewer days under long days than under short days in Arabidopsis. However, in some species, any expression of a vernalization or photoperiod response or requirement can depend directly on the other cue. For instance, all populations of *M. guttatus* flower rapidly when grown under long days, but when most perennial populations of *M. guttatus* are first raised in short days, they then require vernalization in order for long days to induce flowering (Friedman and Willis, 2013). Differences in the regulation of homologs of the MADS box transcription factor and *FT* repressor *SHORT VEGETATIVE PHASE* (*SVP*) appear to explain whether populations exhibit this photoperiod-dependent vernalization requirement or not (Preston et al., 2016). In contrast, biennial accessions of both domesticated beet (*Beta vulgaris*) and its wild progenitor *B. vulgaris* ssp. *maritima* differ from annual accessions in that the expression of photoperiodic flowering requires vernalization (Pin et al., 2010). Allelic variation in a single gene, *BOLTING CONTROL1*, explains this difference between annuals and biennials, and notably, this gene is lineage-specific duplicate in the same clade as the pseudo-response regulators that alter photoperiodic flowering variation in maize, barley, and rice (Pin et al., 2012).

EVOLUTION OF FLOWERING REGULATION BY AMBIENT TEMPERATURE

Ambient temperature, as measured in accumulated growing degree days or other metrics, is commonly used to build developmental models that predict flowering time in crop and wild species (Donohue et al., 2015). However, patterns of adaptive natural variation in this response and the questions of whether and how the thermoregulation of flowering has been altered by domestication have received limited attention. For instance, although multiple studies have found that natural variation in the response is abundant in Arabidopsis (Lempe et al., 2005; Balasubramanian et al., 2006; Sasaki et al., 2015), few have made observations in more than two temperature treatments or demonstrated that the distribution of developmental plasticity bears any relationship to the geographic distribution of environmental factors (Hoffmann et al., 2005). Yet, ample potential exists for future surveys to uncover more and more complex natural variation within and between closely related species in this response. For any given species, a threshold may exist such that temperatures on one side of that threshold promote earlier flowering and temperatures on the other side of the threshold delay flowering. Moreover, expression of these responses may depend on whether temperature is constant or fluctuating (e.g. Burghardt et al., 2016), they may be photoperiod dependent (e.g. Hemming et al., 2008), and at some temperatures, ambient temperature and vernalization responses may both be active (Wollenberg and Amasino, 2012).

The genetics of natural variation in the ambient temperature response also has received limited attention, in part because the contributing underlying mechanisms have been described only recently and even then only in Arabidopsis. As reviewed elsewhere (Verhage et al., 2014; Capovilla et al., 2015), these studies have implicated temperature-dependent splice variation in *FLOWERING LOCUS M* (*FLM*) and other *MADS AFFECTING FLOWERING* genes as well as temperature-dependent degradation of *SVP* in the plasticity of flowering to ambient temperature (Lee et al., 2007; Kumar et al., 2012; Gu et al., 2013; Posé et al., 2013; Rosloski et al., 2013; Hwan Lee et al., 2014; Airoldi et al., 2015). Several QTL and association mapping studies in Arabidopsis have highlighted *FLM*, *FRI*, *FLC*, *FT*, and *VERNALIZATION INSENSITIVE3* as candidate genes harboring natural variants that affect the ambient temperature response (Schwartz et al., 2009; Lutz et al., 2015; Sasaki et al., 2015; Sanchez-Bermejo and Balasubramanian, 2016).

Of these examples, the only causal variant that has been characterized rigorously from both molecular and evolutionary perspectives is a 5.7-kb LINE retrotransposon insertion allele in the first intron of *FLM* (Lutz et al., 2015). The allele is still functional and temperature sensitive, but the insertion is necessary and sufficient to accelerate flowering, especially under cool temperatures, by reducing overall *FLM* transcript abundance and altering isoform splicing. The insertion allele arose once and appears in 10 largely unrelated northern European accessions, but field experiments demonstrating that the variant is adaptive remain to be performed.

MAJOR PATTERNS, ALTERNATE EXPLANATIONS, AND FUTURE DIRECTIONS

Phenotypic Diversity

As reviewed above, the plasticity of flowering to environmental cues can vary greatly among wild populations and cultivated varieties. This diversity involves multiple differentiable parameters (i.e. type, magnitude, threshold, and interdependence), and alterations may be dramatic or subtle. Because the spectrum of variation segregating within or among natural populations is filtered by natural selection, understanding the genetic underpinnings of this variation may yield mechanistic insights into the flowering time network not accessible through standard mutant screens. It also provides the most relevant path to identifying the types of adaptive variation relevant for crop improvement and conservation, and for further improving ecophysiological and gene network models of seasonal phenology to forecast how populations will respond to future, potentially no-analog, climates (e.g. Satake et al., 2013; Donohue et al., 2015; Burghardt et al., 2016).

Although this Update has focused on natural variation in plasticity to photoperiod, vernalization, and ambient temperature, studies of diverse taxa have revealed that the timing of flowering may respond to a host of additional environmental parameters, including ambient CO_2 concentration, water availability, soil nutrient availability, and soil microbiota, among others (e.g. Springer and Ward, 2007; Riboni et al., 2013; Wagner et al., 2014). Broad and fine-scale comparisons hint that diversity in certain responses may provide a fruitful tool to explore how plants adjust their life cycles to cope with environmental heterogeneity. For instance, limited water availability treatments accelerate flowering in Arabidopsis but delay flowering in rice (Galbiati et al., 2016). Moreover, alterations to pathways by which drought or nitrogen availability regulate the expression of floral inducers have likely been critical for the evolution of mass flowering and masting in many perennial species (Kobayashi et al., 2013; Miyazaki et al., 2014). But overall, limited work has described the physiological parameters that characterize these responses, the genetic mechanisms that transduce the cue to alter flowering behavior, or whether these responses vary within or among closely related species in patterns that indicate that the variability has been shaped by past selection.

Because flowering time and its plasticity often vary along latitudinal or altitudinal gradients, investigators primarily test for and find that variation is most likely to be an adaptive response to pressures imposed by the seasonal timing of climatic factors like snowmelt and drought. However, biotic interactions deserve consideration as well. The abundances of competitors, herbivores, seed predators, and pollinators vary seasonally and geographically (e.g. Pilson, 2000), and their cycles are changing with the changing climate as well. Thus, to

Table I. *Catalog of genes that segregate for allelic variation affecting flowering time plasticity*

For brevity, when orthologous relationships are clear, the name of the homologous gene in Arabidopsis is provided instead of species-specific nomenclature. Note that the pseudo-response regulator gene that causes the presence/absence of a vernalization requirement in beet is more closely related to those that affect photoperiod response than to those that affect the vernalization response in grasses.

Cue	Form of Variation	Gene or Gene Class	Species
Photoperiod	Response type	*TERMINAL FLOWER1*	Rose, strawberry
	Response magnitude	Phytochrome A	Soybean
		AP2-like transcription factor	Soybean
		GIGANTEA	Soybean
		EARLY FLOWERING3	Pea, lentil, barley
		Pseudo-response regulator	Barley, rice, maize
		FLOWERING LOCUS T	Sunflower, soybean, sorghum, Arabidopsis
Vernalization	Presence/absence	*FRIGIDA*	Arabidopsis
		FLOWERING LOCUS C	*Arabis alpina*
		Pseudo-response regulator	Wheat, barley, beet
	Duration of chilling necessary or response magnitude	*FLOWERING LOCUS C*	Arabidopsis, *Arabis alpina,* multiple *Brassica* spp., *Capsella rubella*
Temperature	Response magnitude	*FLOWERING LOCUS M*	Arabidopsis
		FRIGIDA	Arabidopsis
		FLOWERING LOCUS T	Arabidopsis

the extent that these cycles can be predicted by seasonally cycling abiotic factors, such biotic patterns also may maintain polymorphism or promote divergence in flowering time plasticity.

Genetic Underpinnings

Several notable trends emerge from the genetic findings reviewed above (Tables I and II). Most prominently, the genetic architecture of natural variation in flowering time plasticity may differ greatly depending on the parameter examined. Loss and modulation of vernalization response show a strong substitution bias, evolving frequently by repeated substitutions of independent alleles that impact just a small core set of regulators (e.g., *FRI* and *FLC* homologs). In contrast, range-wide or species-wide modulation of the photoperiod response often involves several to many loci, and these variants affect a broader gene set occurring throughout relevant portions of the flowering network. Given that many genes affect the vernalization response when mutated (Andrés and Coupland, 2012), these different patterns likely reflect a stronger fixation bias for the vernalization response; variants in few genes have large enough impacts on plasticity and fitness with sufficiently limited deleterious pleiotropic consequences (Streisfeld and Rausher, 2011).

It is also noteworthy that variants altering or deleting the coding sequences of genes more commonly contribute to the evolution of flowering time plasticity than regulatory mutations, and most nonnull mutations attenuate rather than augment gene function (Table II). This trend may in part reflect a mutation bias. Many more possible mutations eliminate gene function than selectively reduce, modulate, or add new function. Consequently, when loss of function does confer a favorable phenotype, the adaptive mutation rate is higher, and convergent evolution through allelic series like those seen for *FRI* in Arabidopsis (Shindo et al., 2005), pseudo-response regulators in grasses (Yan et al., 2004; Xue et al., 2008; Koo et al., 2013), or soybean phytochromes (Xu et al., 2013; Jiang et al., 2014) are more likely.

Table II. *Counts of alleles involved in the evolution of photoperiod, vernalization, or ambient temperature responses in flowering, broken down by cultivation status, type of DNA region, and effect type*

When multiple alleles that alter these responses relative to the hypothesized ancestral state segregate in a single gene, all are included the total variant count.

Parameter	Classification	Photoperiod	Vernalization	Temperature
Polymorphic group	Cultivated	29	9	0
	Wild	1	32	3
Variant location	Coding	25	30	1
	Regulatory	5	10	2
Variant effect	Null	20	29	1
	Attenuated function	9	9	2
	Gain of function	1	2	0

OUTSTANDING QUESTIONS

- What mechanisms regulate the plasticity of flowering to additional cues—e.g., ambient $[CO_2]$, water availability, and microbiota—and how do these responses evolve across the landscape?
- How do reversals in environmental regulation (e.g. shifts from long-day to short-day flowering) evolve?
- Does the plasticity of metabolism necessary to support flowering coordinately evolve with the plasticity of developmental induction?
- Why do we see repeated evolution through similar genes for some parameters or systems and a broad array of loci contributing to variation in others?
- How do adaptation to local climates and adaptation to seasonal and local variation in pollinator, herbivore, and competitor communities interact to mold the environmental regulation of flowering?
- How will the plasticity of flowering evolve as novel combinations of cues and selective agents arise in the future?

Although these conclusions are based on an increasingly large set of variants, there are strong ascertainment biases. For instance, approximately 78% of the vernalization alleles surveyed are drawn from a few taxa in the Brassicaceae. Moreover, nearly all variants implicated in the evolution of photoperiodic flowering have been discovered within cultivated taxa (Table I). Responses to selection in cultivated systems may not be representative of responses to similar pressures in wild populations. Water and nutrient inputs can differ greatly between agricultural and natural habitats, and the strength and consistency of selection on a target phenotype may often be greater during domestication and improvement. These differences may offset tradeoffs due to negative pleiotropic impacts of individual variants (Otto, 2004). Similar biases may be expected for local adaptation in wild populations with small effective population sizes, like those of Arabidopsis, particularly if adaptive variants with more specific effects arise by mutation or are introduced by gene flow less frequently (Stern and Orgogozo, 2008).

Nonetheless, these variants that have been genetically examined give us a fruitful starting point to ask why these substitution biases are found (or not) and what these biases indicate about how best to breed crops or manage populations for future climates. Moving beyond the growth chamber to understanding how these variants function in whole organisms and in field environments is truly essential to this goal. Manipulating individual cues in isolation may unmask phenotypes not seen under the natural diurnal and seasonal cycles of many environmental factors. For instance, it has frequently been observed that flowering time QTLs detected in controlled conditions have no detectable effect on flowering in field conditions (e.g., Weinig et al., 2002; Anderson et al., 2011).

Variants affecting flowering time plasticity may often impact other traits as well. For instance, the photoperiod and vernalization pathways can also impact germination traits (Chiang et al., 2009; Chen et al., 2014), inflorescence architecture (Krieger et al., 2010), leaf size and shape (Cartolano et al., 2015; Digel et al., 2016), and carbohydrate metabolism at flowering (Ortiz-Marchena et al., 2014). Such pleiotropic effects may mediate tradeoffs that mitigate the selective benefit on seasonal phenology. For instance, nonfunctional *FRI* alleles do not always have higher fitness relative to functional *FRI* alleles because they also alter shoot architecture (Scarcelli et al., 2007) and have a strong tradeoff with drought-avoidance traits (Lovell et al., 2013).

Finally, if we consider the many natural variants at candidate flowering loci shown by population genetic studies to vary clinally with environmental factors or to show signatures of selective sweeps (e.g., Burgarella et al., 2016; Mattila et al., 2016), an even richer set of variants to pursue emerges. I have not reviewed these here, however, as the multiple functions of many genes that regulate flowering time plasticity frustrate the interpretation of clinal patterns in the absence of phenotypic information. Indeed, a recent study even found that clinally distributed sequence variation in the promoter of *FT* (i.e. the florigen molecule) affected its photoperiod-specific expression pattern and fitness but not its flowering time in field conditions (Liu et al., 2014). Nonetheless, these studies highlight alleles of evolutionary importance, and deeper investigation into their molecular and phenotypic consequences will be of great value.

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