The Molecular Evolutionary Ecology of Plant Development: Flowering Time in *Arabidopsis thaliana*

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ABSTRACT

Flowering time is a major fitness determinant of plants in seasonal habitats. In *Arabidopsis thaliana* flowering time is largely determined by photoperiod, vernalization, and ambient temperature, although foliar shade, water availability, and herbivory can also have an effect. There is selection on flowering time via both mortality and fruit production, and typically selection favors flowering time plasticity. Much of the variation in flowering time can be attributed to molecular variation in the genes that are responsible for sensing light and temperature, and many of these genes owe their discovery to these effects as determined by quantitative trait locus (QTL) mapping. Not surprisingly, many flowering time QTLs are environment-dependent. Molecular analyses of the genes underlying the response of flowering time to the environment provide further evidence that these genes have been repeated targets of natural selection.

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I. INTRODUCTION

The onset of flowering, that is, the change from vegetative to reproductive development, is a major life history transition in flowering plants and is sensitive to various seasonal climatic signals (Koornneef *et al.*, 2004). Flowering phenology is critically tied to the reproductive ecology of flowering plants, and is a central feature in the evolutionary trajectory of many angiosperm species (Murfet, 1977). Moreover, the shift to flowering represents a major developmental transition that can reshape the architecture of the plant, and change its interaction with the biotic and abiotic environment. The study of flowering time provides an opportunity to investigate the diversification of a key developmental process in both molecular genetic and ecological contexts, and results in a synthetic view that encompasses the molecular evolutionary ecology of development.

II. EVOLUTIONARY ECOLOGY OF FLOWERING TIME

Seasonal habitats exhibit regular annual fluctuations in precipitation, temperature, day length, length of growing season, and potential disturbance due to storm activity or flooding. Some habitats may experience variation in more than one of these environmental parameters (e.g., a cool, dry season and a warm, wet season), and in some cases fluctuations can be extreme. Dry conditions can lead to desiccation whereas flooding can lead to anoxia, fungal growth, or can disrupt seed dispersal and seedling recruitment. Changes in daylength can reduce the amount and quality of photosynthetically active radiation available, therefore affecting growth rate. Plants in seasonal environments may also experience season-dependent fluctuations in predators and competitors.

Most plants living in seasonal environments have adapted to these changes in one of two ways: they have constitutive mechanisms that increase their range of environmental tolerances or they have plastic responses that are timed with seasonal changes (Alpert and Simms, 2002; Murfet, 1977). Plastic responses may be induced by direct exposure to the environmental condition (e.g., shade, predators) or may be timed to other cues that reliably predict seasonal shifts. In temperate environments, there are four major cues for anticipating seasonality: temperature, day length, change in temperature, and change in daylength. In other environments, water availability may also be an important seasonal cue.

Flowering time is an important determinant of fitness in a variable environment, and represents a discrete developmental transition in response to

ecological cues. In outcrossing species, it is critical that flowering be timed such that both pollinators and other flowering individuals are present (Rathcke and Lacey, 1985). Even in selfing species, if the timing of flowering is correlated with the timing of seed set, timing of flowering can determine the conditions under which seeds will be dispersed and germinate. Not surprisingly, flowering time is a complex trait controlled by both internal states (developmental and physiologic status of the plant) as well as external conditions.

Arabidopsis thaliana (L.) Heynh. (family Brassicaceae) is a weedy annual plant, most often found in disturbed habitats such as the margins of agricultural fields. It has become a model system for the study of the molecular evolutionary ecology and genetics of plant adaptation (Mitchell-Olds, 2001; Pigliucci, 1998; Shimizu and Purugganan, 2005). *A. thaliana* is characterized by small size and rapid growth, able to complete its life cycle in less than six weeks depending on strain and conditions, and a low outcrossing rate, ~1% (compiled by Hoffmann *et al.*, 2003). *A. thaliana* is estimated to have diverged from other *Arabidopsis* species 5–6 million years ago (Hoffmann, 2002). Its native range extends across Eurasia and Northern Africa, although its naturalized range is much more extensive, including North America and Japan (Hoffmann, 2002). Genetic analysis performed by Sharbel *et al.* (2000) indicates that two major post-Pleistocene expansions in the species range occurred from glacial refugia in the Iberian Peninsula and Asia ~17,000 years ago. However, studies (Nordborg *et al.*, 2005; Schmid *et al.*, 2005) suggest that the post-Pleistocene expansion of *A. thaliana* may be more complex.

In *A. thaliana*, the early stage of the life cycle is a vegetative phase in which the shoot apical meristem produces rosette leaves. As the life cycle proceeds, external ecological cues or internal signals trigger the reproductive developmental transition, and the shoot apical meristem begins to produce the inflorescence, with the associated elongation or bolting of the main shoot. Life histories of *A. thaliana* ecotypes can be classified into three main flowering strategies: winter annual, summer annual, and rapid cycling. The winter annual strategy is prevalent, particularly at southern latitudes (Donohue, 2002; Weinig and Schmitt, 2004). Winter annuals germinate in the fall and overwinter as rosettes, where they experience both cold temperatures and short day lengths. Flowering is delayed until early spring. Fruits ripen and the plants die prior to the onset of the summer heat. The summer annual plants germinate in spring and grow quickly to maturity, dropping seeds in the fall of that same season. Rapid cycling plants germinate in fall, quickly mature, and drop their seeds prior to the onset of winter. Which life history a given ecotype will adopt, the timing of flowering within that life history, and

therefore the reproductive success of that ecotype, depend on the interactions of photoperiod and temperature and, to a lesser extent, water availability with the genetic background of the population under study.

Both summer annual and rapid-cycling life histories have been observed in northern latitudes or at high altitudes (Donohue, 2002; Griffith *et al.*, 2004; Nordborg and Bergelson, 1999). Donohue (2002) has shown that when plants adopt an inappropriate strategy, mortality prior to initiation of flowering was 100% under natural conditions. In a similar study, Griffith *et al.* (2004) showed very poor survivorship and fruit production in spring germinating plants at a Kentucky field site. In fall-germinated plants, they showed selection both for earlier bolting and for bolting at a larger size. Winter annual plants often flower at a later developmental stage (Diggle, 1999; Weinig and Schmitt, 2004), that is, greater leaf number and typically larger size, leading to increased fruit production (Engelmann, K., and Purugganan, M., unpublished data; Ungerer *et al.*, 2003).

Flowering time variation is a classic quantitative trait, with phenotypes among individuals continuously distributed rather than qualitatively differentiated. The range of variation in flowering time can be large. Nordborg and Bergelson (1999) reported means ranging from 35 to 251 days, with some individuals not flowering at all under their experimental conditions. The broad sense heritability for bolting time among a set of recombinant inbred lines of *A. thaliana* ecotypes has been estimated to range from 0.07 to 0.744 (Ungerer *et al.*, 2002; Van Berloo and Stam, 1999). Rosette leaf number at bolting, widely regarded as a developmental surrogate for bolting time, is also highly variable, with means ranging from 4.2 to 70.9 leaves depending on conditions and strain (Karlsson *et al.*, 1993). Ungerer *et al.* (2002) also estimated the heritability for this trait in two sets of recombinant inbred lines and results ranged from 0.396 to 0.534.

Photoperiod or daylength is a major seasonal cue for flowering in *A. thaliana* and many studies have compared the fitnesses of plants grown under short vs long days. Studies in controlled environment growth chambers often show an increase in size and fruit production under short day lengths (Engelmann, K., and Purugganan, M., unpublished data; Ungerer *et al.*, 2003). Although it is important to bear in mind that in most field studies the effects of photoperiod are confounded with those of temperature, many studies have also showed increased fitness in winter annual plants. Donohue (2002) found that locally adapted strains of *A. thaliana* have increased survivorship and fruit production when grown in winter field conditions vs spring conditions. Weinig *et al.* (2003b) planted 98 *Ler* × Col recombinant inbred lines at field sites in Rhode Island and North Carolina and found spring germinants attained higher fitness at the northern Rhode

Island site, while winter annuals had higher fitness at the southern North Carolina site, perhaps due to the hotter, drier spring conditions at the southern field site. Studies done under 24 hr light are somewhat more difficult to interpret, as "long day" conditions can occur in both spring and fall. Nevertheless, Ungerer and Rieseberg (2003) showed there is selection for early flowering under long (24 hr) days. Collectively, these findings are consistent with the notion that a winter annual strategy is adaptive for *Arabidopsis* at a range of latitudes.

While selection for earlier bolting is common under most of the environments that have been studied, both photoperiod and light quality can affect optimal flowering time in a given environment. Callahan and Pigliucci (2002) found selection for earlier flowering in greenhouse studies done on local strains in Tennessee; however these seeds were planted in late January, making it difficult to compare with winter or summer annual life histories. They also found selection for earlier bolting, based on mortality, in two field plots, both planted in November. Selection for early bolting was more consistent in the shaded field site. Dorn *et al.* (2000) found that earlier reproduction at a smaller size under foliar shade (and long days) was consistent with selection under these conditions whereas later reproduction in full sun, where there are presumably fewer competitors, was associated with increased fitness.

Many plants in temperate habitats require a period of cold exposure in order to flower, a mechanism known as vernalization, to ensure that flowering takes place after winter has passed. In *A. thaliana*, vernalization is not required for flowering, but in many ecotypes vernalization substantially shortens the time to initiation of flowering. Stratification, that is, the exposure of seeds to cold period, has a similar effect on flowering time in many but not all ecotypes. Nordborg and Bergelson (1999) found that both treatments induced earlier flowering, both required about 30 days for a significant effect and they noted that combining seed and rosette cold treatment was not additive. Furthermore, all plants that responded to cold treatment as rosettes also responded to cold treatment as seeds, although the reverse was not always true.

Pigliucci and Marlow (2001) and Callahan *et al.* (2004) showed that under long days vernalization confers earlier flowering and a correlated increase in fitness, measured as fruit production. Callahan *et al.* (2004) also showed that nonplastic genotypes had a similar time to flowering and fitness as the plastic genotypes when fully vernalized.

Flowering time also responds plastically to ambient temperature, although the fitness consequences of this flexibility are not always clear. Westerman and Lawrence (1970) tested 33 inbred lines under long days and found that days to

flowering and rosette leaf number at flowering decrease as ambient temperature increases. Stinchcombe *et al.* (2004a) reanalyzed the data of Westerman and Lawrence (1970) and showed that there was selection against flowering time plasticity, but only from 20 °C to 25 °C, which may only be representative of the extreme southern range of the distribution of *A. thaliana*. There was no such evidence of selection against plasticity from 15 °C to 20 °C.

In *Arabidopsis*, plants receiving limited water flower earlier than plants receiving liberal amounts of water (Engelmann and Schlichting, 2005), however, this response in water-limited plants did depend on the consistency of the water regime. Plants that received limited overall water in infrequent, large pulses bolted at the same time as plants receiving generous amounts of water, but these plants also suffered very high mortality and therefore a large decrement in fitness. The earlier flowering plants, those that received limited but consistent amounts of water, showed survivorship comparable to the generously watered plants.

It has also been noted that across genotypes later flowering strains tend to have higher water use efficiencies (Juenger *et al.*, 2005a; McKay *et al.*, 2003). These authors conducted a suite of studies to explore this correlation and found several lines of evidence suggesting likely pleiotropic effects of flowering time genes on water use efficiency, that is, amount of carbon fixed relative to amount of water transpired, as measured by carbon isotope ratios. They found that several known strains of flowering time mutants also showed changes in water use efficiencies, and several water use mutants, though not all, showed changes in flowering time. In a QTL study (Juenger *et al.*, 2005a), they found several loci that independently determined both flowering time and water use efficiency, but they also found a single locus on chromosome III that clearly regulated both, although the effects of this locus on each trait were negatively correlated.

The effect of nutrients on flowering time has not been extensively examined in *A. thaliana*. Pigliucci and Schlichting (1998) have addressed this question and found that the effect of nutrients on flowering varies greatly among genotypes and families. More generally, however, they have shown that differences in nutrient availability lead to differences in correlations between traits.

III. THE GENETIC BASIS OF ENVIRONMENTAL PERCEPTION IN FLOWERING TIME SIGNALING

Plants sense light via three known classes of photoreceptors: cryptochromes, phytochromes, and phototropins (Casal, 2002). Of these photoreceptors, both cryptochromes and phytochromes play important roles in plant development,

including timing of flowering. Cryptochromes detect blue light and ultraviolet A radiation (UV-A), while the phytochromes detect red and far-red light. Numerous authors (Borevitz *et al.*, 2002; Casal and Smith, 1989a,b; Chory and Li, 1997; Johnson *et al.*, 1994; Pigliucci and Schmitt, 2004; Reed *et al.*, 1994; Schmitt *et al.*, 1999) have shown that these receptors mediate plant growth via gibberellin and brassinosteroid hormones, resulting in adaptively appropriate phenotypes for a given light quality. The ratio of red to far-red light, for example, is known to indicate the presence of both shade and twilight, and prolonged exposure to light with low red: far red ratios induces hypocotyl elongation, a mechanism that allows plants to overgrow the neighbors before initiating leaf expansion. It has been shown (Borevitz *et al.*, 2002; Maloof *et al.*, 2000, 2001; Stenoien *et al.*, 2002) that there is extensive variation to light sensitivity that is mediated by the cryptochrome and phytochrome pathways in natural accessions of *A. thaliana*.

Phytochromes are also thought to regulate the onset of flowering through a complex pathway that also involves cryptochrome photoreception, which defines the first steps in a photoperiod or daylength-dependent flowering time pathway (Schultz and Kay, 2003; Searle and Coupland, 2004; Valverde *et al.*, 2004). The mRNA of the gene *CONSTANS* (*CO*), a flowering time gene downstream of the phytochrome and cryptochrome loci, is expressed in a circadian pattern. Peak expression corresponds to what is early evening under short days, but what is late afternoon under long days. During dark periods, the CO protein cannot accumulate because it is degraded by the proteasome. However in the presence of both red and blue light, proteasome degradation of CO is inhibited by two of the phytochrome and cryptochrome proteins, PHYA and CRY2, respectively. Therefore under long days in full spectrum light, CO protein, a potent activator of the gene *FLOWERING TIME* (*FT*), can accumulate and flowering is induced. Under conditions of foliar shade, that is low red: far-red light ratios, PHYB activates expression of another gene, *PHYTOCHROME AND FLOWERING TIME 1* (*PFT1*), which upregulates *FT* expression (Cerdan and Chory, 2003).

The timing of flowering has also been shown to be sensitive to ambient growth temperature. The autonomous pathway, a series of photoperiod-independent genes and gene products necessary for flowering, may define a temperature-regulated flowering pathway. In wild-type plants, flowering occurs earlier at 23 °C than at 16 °C. This effect is mediated by two autonomous pathway genes, *FVE* and *FCA* (Blazquez *et al.*, 2003; Kim *et al.*, 2004). Furthermore this difference in flowering time is also enhanced in *CRY1* and *CRY2* mutants, suggesting there may be some interaction between light exposure and ambient temperature. Thingnase *et al.* (2003) did show that while increased ambient night temperatures and increased mean daily temperature

both decrease the number leaves at bolting, their effects are statistically independent. Furthermore, they showed that increased ambient night temperature decreased the days to bolting whereas mean daily temperature did not.

One of the more dramatic temperature-regulated pathways in flowering is the vernalization pathway, which promotes flowering in response to a prolonged cold treatment such as that observed in winter conditions. Many plants have a vernalization requirement and in the Brassicaceae, there are two unique vernalization genes, *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) (Koornneef *et al.*, 1994), and these are quite well characterized in *Arabidopsis*. The *FRI* gene upregulates *FLC* which expresses a MADS-box transcription factor that inhibits flowering in unvernalized plants. Vernalization, via *VERNALIZATION 1* (*VRN1*), *VRN2*, and *VERNALIZATION INSENSITIVE 3* (*VIN3*), irreversibly alters histone methylation at the *FLC* locus, permanently repressing *FLC* expression enabling photoperiod to induce flowering substantially earlier than in unvernalized plants (Sung and Amasino, 2005). *VIN3* is also required for *FLC*-independent vernalization via its action as a promoter of *LUMINIDEPENDENS* (*LD*) in the autonomous pathway (Sung and Amasino, 2004).

IV. QUANTITATIVE TRAIT LOCUS MAPPING OF FLOWERING TIME VARIATION

Molecular developmental genetic studies have elucidated many of the key pathways that plants utilize to sense seasonal cues, and allowed investigators to examine the molecular genetic basis of flowering time in an ecological context. Understanding the evolution and ecology of flowering time, however, requires us to understand not only what genes regulate this trait but also which specific genes are responsible for natural variation in flowering time and the role selection plays in defining this variation. The quantitative nature of flowering time variation in *A. thaliana* allows us to employ modern quantitative trait locus (QTL) mapping approaches in dissecting the genetic architecture of this trait. Flowering time has been the subject of the most intensive effort in QTL mapping in this species, with at least 18 QTL mapping studies published in the last 10 years (Bandaranayake *et al.*, 2004; Clarke *et al.*, 1995; El-Assal *et al.*, 2001; El-Lithy *et al.*, 2004; Jansen *et al.*, 1995; Juenger *et al.*, 2005a,b; Kuittinen *et al.*, 1997; Maloof, 2003; Mitchell-Olds, 1996; Remington and Purugganan, 2003; Stratton, 1998; Ungerer *et al.*, 2002, 2003; Weinig and Schmitt, 2004; Weinig *et al.*, 2002, 2003a; Werner *et al.*, 2005b). QTL mapping studies of flowering time have defined

at least 28 loci that affect natural variation in flowering time among individual accessions of this species under different conditions (Weinig *et al.*, 2002). The effects of these individual QTL follow a long-tailed distribution, with one to three loci of moderate to large effect (>10% of variation explained) and a larger number of loci with smaller effects (Juenger *et al.*, 2005a,b; Kuittinen *et al.*, 1997; Ungerer *et al.*, 2002; Weinig *et al.*, 2003a). Epistatic effects among QTLs have also been observed, indicating that phenotypic variation could be explained in part by nonadditive multilocus interactions within genomes.

As discussed above, two ecological cues, daylength and exposure to a cold period associated with winter conditions (vernalization), have been extensively studied as environmental cues to flowering in *A. thaliana*. In two sets of recombinant inbred line mapping populations of *A. thaliana* (the Col × *Ler* and Cvi × *Ler* mapping populations), QTL × environment interactions, that is, the environment-dependent detection of loci, have been documented for three of the five identified loci between long and short day conditions (Ungerer *et al.*, 2003). Although still at the level of QTL identification, these studies have begun to address the genetic basis for differential response of *A. thaliana* accessions to ecological cues.

Studies on the genetic architecture of flowering time variation have largely been undertaken in controlled environmental conditions, and few studies have explored the extent to which the genetic basis for phenotypic variation in this trait differs under field settings. Studies by Weinig *et al.* (2002), however, have explored the genetic architecture of flowering time in the field under ecologically relevant conditions. A study of the Col × *Ler* recombinant inbred line mapping population at two field locations (Rhode Island and North Carolina) over winter and spring seasons revealed, not surprisingly, field- and season-dependent QTLs, suggesting strong genotype-by-environment interactions for this trait in natural conditions. The number and identity of QTLs differed in significant ways between controlled growth chamber and field conditions, indicating that our view of relevant genes underlying flowering time behavior may be skewed by reliance on controlled conditions in studying flowering time loci (Weinig *et al.*, 2002).

Intriguingly, and perhaps not surprisingly, variation in flowering time is genetically correlated with other developmental and physiological phenotypes in *A. thaliana*. One clear example is between bolting time, a life history trait, and rosette leaf number, a morphological trait. The genetic correlation, r_G, between these two traits is 0.94 in a collection of 21 accessions (Ungerer *et al.*, 2002). Other genetic correlations have been observed between bolting time and various features of shoot architecture, including lateral branch number and fruit production. Joint QTL analyses of flowering time and

shoot architectural traits suggests that they share common QTLs, indicating the presence of trait suites underpinned by common loci (Engelmann, K., and Purugganan, M., unpublished data; Ungerer *et al.*, 2002, 2003).

One interesting correlation observed in an ecological context is that observed between flowering time and herbivory. Weinig *et al.* (2003a,c) examined resistance to herbivory and found that this depends significantly on flowering time. Early bolting plants are more susceptible to herbivores, in this study rabbits, but this susceptibility does not necessarily result in a fitness decrement. In fact, on average *Arabidopsis* tends to overcompensate for apical meristem damage such that herbivory can lead to an increase in fruit production via proliferation of basal branches. They also showed that three QTLs associated with flowering time are also associated with herbivore resistance, although not all flowering time QTLs showed this association.

Whether these correlations arise from pleiotropy or close linkage among specific genes must await fine mapping analyses and possibly isolation of relevant QTL genes. One approach has been to examine the mutational covariance associated with mutant alleles of known flowering time genes. A study of genetic correlations suggests a relationship between flowering time as a drought-escape mechanism and dehydration-avoidance mechanisms as measured by δ^{13} C isotope ratio. Near-isogenic lines of *FRI* and *FLC* flowering time alleles also affect δ^{13} C ratios, providing strong evidence for possible pleiotropic effects of these flowering time genes or very tight linkage to dehydration avoidance loci (Juenger *et al.*, 2005a; McKay *et al.*, 2003).

V. ISOLATION OF GENES UNDERLYING FLOWERING TIME VARIATION

Quantitative trait locus mapping studies have defined the genomic regions that harbor genetic polymorphisms associated with flowering time variation and have elucidated the genetic architecture of this trait. Further progress in examining the evolutionary ecology of flowering time, however, requires us to identify and isolate the specific genes that underlie natural variation in flowering time in this species. Genes underlying quantitative variation in flowering time have been isolated in recent years. Two approaches to isolating QTL genes have been pursued: (1) fine mapping and positional cloning of QTL genes and (2) candidate gene association studies.

One of the first genes demonstrated to underlie a flowering time QTL is *EARLY DAYLENGTH INSENSITIVE* (*EDI*), which was first identified in a QTL mapping study using the recombinant inbred Cvi × *Ler* mapping

population (El-Assal *et al.*, 2001). Positional cloning of this QTL demonstrated that *EDI* is equivalent to the *CRY2* gene. The Cvi accession of *A. thaliana* has a *CRY2* allele with two amino acid changes that result in altered *CRY2* protein levels during the circadian cycle, and results in early flowering of plants under short day conditions. Although the *EDI* QTL is a large-effect allele, its cloning remains a landmark feat in the identification of the genetic basis for quantitative variation in *A. thaliana* (El-Assal *et al.*, 2001).

New genomics technologies have also advanced the ability to fine-map and isolate genes underlying QTL, including flowering time loci. A study identified flowering time QTLs in a recombinant inbred mapping population between the Nd and Col accessions (Werner *et al.*, 2005b). Microarray hybridization with genomic DNA identified an Nd-specific deletion of the *FLOWERING LOCUS M* (*FLM*) gene, which encodes a MADS-box transcription factor and is a duplicate of *FLC*. Like *FLC*, this gene appears to repress flowering and the effect of this gene deletion on flowering time was confirmed by transgenic complementation.

Candidate gene studies have also been useful in associating flowering time variation with particular *A. thaliana* haplotypes. Positional QTL cloning identified the *CRY2* Cvi allele as a flowering time QTL, but this allele has been observed only in the Cape Verde Islands (El-Assal *et al.*, 2001). It is thus unclear whether it represents an adaptation to local conditions or a rare, possibly slightly deleterious mutation maintained by relaxed selection in this inbreeding species. A study of nucleotide variation at this gene, undertaken as a candidate gene approach to identifying flowering time QTLs, revealed other major, moderate-frequency haplotypes that exist within the species range of *A. thaliana* (Olsen *et al.*, 2004). Two major *CRY2* haplotype groups exist within this species, one of which includes several alleles that feature a nonsynonymous glutamine (Q) substitution in an otherwise conserved serine (S). Candidate gene association studies reveal that these different haplotypes/haplotype groups are significantly associated with flowering time differences in the species (Olsen *et al.*, 2004).

An important determinant of standing genetic variation in flowering time in *A. thaliana* is the *FRI* gene (Johanson *et al.*, 2000). As noted above, the *FRI* gene appears to act by upregulating expression of another flowering time gene, *FLC*, which encodes a MADS-box transcriptional activator. Molecular analysis reveals that *FRI* harbors several large deletions that lead to loss-of-function alleles, at least two of which are found at moderate frequency (Hagenblad and Nordborg, 2002; Hagenblad *et al.*, 2004; Johanson *et al.*, 2000; Le Corre *et al.*, 2002; Stinchcombe *et al.*, 2004b).

A latitudinal cline in flowering time, measured in days to flowering, has been shown to be dependent on *FRI* genotypes in *A. thaliana* accessions.

This cline is observed only in accessions that do not carry any of the *FRI* deletion alleles, and when flowering time is assayed under field conditions where plants are vernalized by exposure to winter conditions or cold treatment (Lempe *et al*., 2005; Stinchcombe *et al.,* 2004b). Shindo *et al.* (2005), however, did not find a statistically significant cline when several North American accessions were included.

The latitudinal cline observed in *A. thaliana* may be driven in part by an epistatic effect of *FRI* with *FLC* (Caicedo *et al.*, 2004; Lempe *et al*., 2005; Michaels and Amasino, 2001). In the latter gene, two major haplotype groups have been detected in *A. thaliana* accessions, and there is significant flowering time variation associated with *FRI FLC* two-locus genotypes. *FLC* haplotypes also show a significant latitudinal distribution, but only in putatively functional *FRI* genotypic backgrounds. Finally, *FRI* and *FLC* show significant intergenic linkage disequilibrium, even though the two genes are found in two different *A. thaliana* chromosomes. Together, these results suggest that epistatic selection may underlie flowering time variation in winter annuals of this species (Caicedo *et al.*, 2004; Stinchcombe *et al.*, 2004b). Other *FLC* and *FRI* alleles associated with low levels of *FLC* expression have been found (Lempe *et al*., 2005; Shindo *et al.*, 2005; Werner *et al*., 2005a).

VI. MICROEVOLUTION OF FLOWERING TIME LOCI

QTL mapping studies and the subsequent isolation of genes provide crucial insights into the molecular genetic basis of natural flowering time variation. This, in turn, is complemented by molecular evolutionary studies, which determine the levels and patterns of nucleotide variation to infer evolutionary forces that have acted at specific loci. Molecular population genetic studies of six flowering time genes have been reported, including the *FRI* (Hagenblad and Nordborg, 2002; Le Corre *et al.*, 2002) and *FLC* (Caicedo *et al.*, 2004) genes associated with the vernalization response, the photoreceptor gene *CRY2* (Olsen *et al.*, 2004) in the photoperiod pathway, and the *LFY, AP1*, and *TFL1* (Olsen *et al.*, 2002) loci, which are either flowering time integrators and/or floral meristem identity genes. These genes show different patterns of nucleotide variation consistent with differing evolutionary forces than those acting on known flowering time genes.

From the viewpoint of molecular population genetics, the *FRI* gene is the most intensively studied flowering time gene in *A. thaliana*. Studies of this gene indicate the presence of several large independent deletions that remove portions of the coding region and presumably result in nonfunctional *FRI* alleles. At least three major deletions have been identified, each of which has

TABLE I
Levels and Patterns of Mean Nucleotide Variation for A. thaliana *Flowering Time Genes*

Gene	n[a]	π[b]	Tajima's *D* value
FRI	26	0.003	−1.225
CRY2	31	0.013	+0.348
FLC	14	0.004	+0.858
LFY	15	0.002	−1.572
AP1	15	0.005	−2.102[c]
TFL1 (coding region)	14	0.001	−2.032[c]
TFL1 (promoter/5′ UTR)	14	0.019	+1.532[c]

[a]Sample size.
[b]Nucleotide diversity per silent site.
[c]Significant at the $p < 0.05$ in a coalescent simulation of the neutral-equilibrium model under no recombination.
UTR, untranslated region.

arisen independently and two of which are present in moderate frequencies in *A. thaliana*. The level of silent site polymorphism in this gene, π, is 0.003 and is lower than the genomic average of $\pi = 0.009$ (Schmid *et al.*, 2005) (Table I), and this low value is due in part to the very low variation of the two major deletion alleles at this locus. Studies of the genomic region around *FRI* also appear to be consistent with positive selection, with linkage disequilibrium extending to ~250 kb and with the *FRI* deletion haplotypes extending unbroken across large genomic distances (Hagenblad *et al.*, 2004). Other nonsynonymous and premature stop codon mutations have been reported at this gene that may also have functional consequences, although these mutations are present at low frequency (Le Corre *et al.*, 2002; Shindo *et al.*, 2005). The results on *FRI* suggest recent and strong directional selection for the evolution of nonfunctional *FRI* alleles in this species.

The evolution of *FRI* appears closely linked to that of its downstream target gene *FLC* (see earlier discussion). A study indicates that *FLC* alleles are found in two major haplotype clades, and that epistatic interaction between *FRI* and these two *FLC* haplotypes may be associated with a latitudinal cline in flowering time (Caicedo *et al.*, 2004). Despite the presence of two differentiated allele groups at *FLC*, levels of variation are lower than the genomic average (Table I). The presence of these two haplotype groups, however, results in a positive Tajima's *D* value, which is associated with the presence of these two differentiated allele classes at moderate frequency.

Like *FLC, CRY2* also appears to have two major haplotype groups, and variation between and within these groups appears to be associated

with flowering time variation (Olsen *et al.*, 2004). The level of variation at this gene is higher than the genomic mean (Table I) and Tajima's *D* value is also positive as a result of these differentiated haplotype groups, but these values are not significantly different from neutral-equilibrium expectations.

Among the three downstream genes of the flowering time pathway that have been explored, only the inflorescence developmental gene *TFL1* has a striking pattern of molecular variation. The silent site nucleotide diversity of the *TFL1*-coding region is the lowest of the six genes that have been studied at that time, and this variation is significantly reduced when compared to other inflorescence developmental genes (Olsen *et al.*, 2004). The value of Tajima's *D* value is also significantly negative for this gene. Both these results suggest that the coding region has been subjected to a recent bout of directional selection, and the reduced variation in the coding region is the result of a selective sweep. The promoter and 5′ untranslated region of *TFL1*, however, shows a contrasting pattern from the coding region. The level of silent site nucleotide diversity for the *TFL1* promoter is relatively high, and the Tajima's *D* value is significantly positive with respect to neutral-equilibrium expectations (Table I). Both the high level of nucleotide diversity and significantly positive Tajima's *D* value is associated with the presence of two moderate-frequency haplotype groups at the *TFL1* promoter, in contrast to the near absence of variation at the *TFL1* coding region. The variation at the *TFL1* promoter, with the observed differentiation into two distinct haplotype groups, is reminiscent to that observed for *FLC* and *CRY2*.

The two other floral integrator/meristem identity genes, *LFY* and *AP1*, do not appear exceptional with regard to their evolutionary dynamics (Olsen *et al.*, 2004). The levels of variation at these loci are both lower than the genomic mean for this species, and *AP1* has a significantly negative Tajima's *D* value with respect to the neutral-equilibrium model. However, the levels and patterns of nucleotide variation at these two loci do not show strong evidence of any nonneutral evolution in their recent history.

These studies provide important insights into the divergent types of evolutionary forces that act at specific flowering time loci. A comprehensive assessment of the microevolution of these genes in the context of the flowering time genetic network may permit us to draw conclusions on the diversification of regulatory gene networks in an explicitly evolutionary ecological context. By assessing the impact of evolutionary forces that have shaped variation at these loci, we may be able to determine to what extent network structures channel and constrain evolutionary trajectories of phenotypes.

VII. SUMMARY

The studies on the molecular, ecological, quantitative, and population genetics of flowering time in *A. thaliana* serve as a model for studying the evolution of development, particularly at the stage of microevolutionary changes that can ultimately lead to changes in fitness. By examining flowering time variation at various hierarchical levels, from the molecular to the organismal to the ecological, we can take a broad view of the evolution of a key developmental transition. Flowering time is a particularly appropriate trait in this regard, as it is a developmental transition that is sensitive to ecological cues to allow adaptive response to seasonal and other environmental variations. One of the features that sets plant development apart from animal development is the greater sensitivity of plant developmental processes to environmental cues. This is rooted in the sessile nature of plants, which relies in part on changing developmental patterns to react to environmental changes to optimize survival and reproductive success.

The study of the evolution of development in recent years has focused almost exclusively on understanding the molecular genetic mechanisms underlying developmental diversification at a macroevolutionary level. There have been few attempts to examine developmental diversification at microevolutionary levels, an approach that also allows us to examine the ecological context of the evolutionary process. A microevolutionary perspective on developmental diversification allows us to catch the origins of the evolutionary process which we can then strive to integrate with its observed endpoint, providing a fuller understanding of the nature of evolutionary change.

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