The effect of the floral repressor FLC on the timing and progression of vegetative phase change in Arabidopsis

Matthew R. Willmann and R. Scott Poethig*

SUMMARY
Plants undergo two major post-embryonic developmental transitions – the juvenile-to-adult vegetative transition (vegetative phase change) and the adult-to-reproductive transition (flowering). In woody plants, these transitions can be separated by years, but in herbaceous species they are often very close together, making it difficult to differentiate the effects of vegetative phase change and floral induction on vegetative development. To distinguish between these factors, we have compared the vegetative morphology of plants highly expressing the floral repressor FLC (FRI;FLC) with plants mutant for this gene (FRI;flc-3) under both photoinductive (long day, LD and night interruption, NI) and non-photoinductive (short day, SD) conditions. We show that the onset of abaxial trichome production is insensitive to floral induction, but the distribution and overall number of abaxial trichomes, as well as several other leaf traits associated with vegetative change, strongly influence flowering. Most of the differences in leaf morphology between FRI;FLC and FRI;flc-3 plants grown in LD can be attributed to the early flowering phenotype of FRI;flc-3, because these differences are not apparent in plants grown in SD. These include differences in leaf size, hydathode number and the distribution of abaxial trichomes along the length of the leaf. Leaf shape and the total number of abaxial trichomes are affected by FLC independently of its effect on flowering. Our results demonstrate that the onset and the progression of vegetative phase change are regulated by different combinations of endogenous and environmental factors, and reveal a role for FLC in vegetative development.

KEY WORDS: FLC, Flowering, Shoot development, Vegetative phase change

INTRODUCTION
The sexual life cycle of both plants and animals involves several changes in state during which the organism is transformed from a somatically immature, sexually incompetent individual, to a somatically mature, sexually reproductive adult. In flowering plants, post-embryonic life is characterized by at least three distinct phases of shoot development – a reproductively incompetent juvenile vegetative phase, a reproductively competent adult vegetative phase and a reproductive phase (Poethig, 2003). Each of these stages produces different types of leaves, buds and internodes. In angiosperms, the adult-to-reproductive transition (floral induction) is marked by a change in the architecture of the shoot and the appearance of flowers. The juvenile-to-adult transition (vegetative phase change) usually involves changes in a variety of species-specific traits, which can include leaf shape, the presence of trichomes and thorns, the production of phytochemicals, leaf retention, internode length, and disease and pest resistance (Doorenbos, 1965; Hackett, 1985; Kerstetter and Poethig, 1998; Schaffalitzky de Muckadell, 1954). In addition to these phase-specific characteristics, there are gradual changes that are related to the physiological aging of the shoot, but which are sometimes influenced by the juvenile-to-adult transition (Bond, 2000; Wareing, 1959).

Maize and Arabidopsis have proven to be particularly useful genetic models for studying the regulation of vegetative phase change. In maize, the juvenile and adult phases differ in wax and trichome production, cuticle thickness, cell wall biochemistry, internode length and axillary bud identity (Poethig, 2003). In Arabidopsis, juvenile leaves are flat and round to orbicular in shape, with a smooth margin, trichomes restricted to the adaxial surface and a long petiole. Adult leaves are curled, spatulate and serrated, with trichomes on both the adaxial and abaxial surfaces and a shorter petiole (Chien and Sussex, 1996; Röbbelen, 1957; Telfer et al., 1997). The presence of trichomes on the abaxial surface of the leaf is often used as a marker for the adult phase in Arabidopsis because it is a qualitative trait that is easy to score. Traits such as the overall number of serrations (Röbbelen, 1957), trichomes (Martinez-Zapater et al., 1995) and hydathodes (Takaya et al., 2000), the size of the petiole and leaf blade, the length-to-width ratio of the leaf blade, vascular complexity (Cookson et al., 2007; Steynen et al., 2001) and cell size (Cookson et al., 2007; Usami et al., 2009) vary continuously, but have also been used to study vegetative phase change.

The relationship between vegetative phase change and flowering in Arabidopsis is still unresolved. This issue is of fundamental interest, and also has important practical consequences. There is considerable evidence that reproductive competence is associated with the adult vegetative phase of the shoot in woody plants (Zimmerman et al., 1985), and this also appears to be the case in Arabidopsis (Telfer et al., 1997; Weigel and Nilsson, 1995). This observation raises the issue of whether genes involved in floral induction play a role in vegetative phase change, as has previously been proposed (Schultz and Haughn, 1993; Steynen et al., 2001). From a practical standpoint, the fact that most of the accessions used in Arabidopsis genetics are extremely early flowering suggests that some of the traits used to study vegetative phase change in this species may be influenced by floral induction, and, therefore, reflect the reproductive state of the shoot, not its vegetative phase.

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It has been known for some time that factors that affect flowering time can also affect the expression of phase-specific vegetative traits. This is well known in the case of gibberellin, which delays or accelerates both vegetative phase change and flowering in a number of species (Evans and Poethig, 1995; Zimmerman et al., 1996; Telfer et al., 1997). Photoperiod also has a significant effect on both of these transitions (Chien and Sussex, 1996; Steynen et al., 2001; Telfer et al., 1997). Chien and Sussex observed that the sensitivity of abaxial trichome production and flowering to photoperiod was age dependent and occurred only within a specific window of time (Chien and Sussex, 1996). Interestingly, the window for the photoperiodic induction of abaxial trichomes was 3 days earlier than the window for flowering, suggesting that, although these transitions may be regulated by common factors, their timing is separable (Chien and Sussex, 1996). This is also evident from the phenotypes of three classes of mutations – those affecting the timing of vegetative phase change but not flowering (Berardini et al., 2001; Hunter et al., 2003), those affecting the timing of flowering but not vegetative phase change (Gomez-Mena et al. 2001; Michaels et al., 2003a; Soppe et al., 1999), and those affecting both transitions (Chien and Sussex, 1996; Martinez-Zapater et al., 1995; Telfer et al. 1997). This latter class includes loss-of-function mutations in members of the autonomous (FPA, FCA, FVE), photoperiodic (CO, GI) and GA pathways (Chien and Sussex, 1996; Martinez-Zapater et al., 1995; Telfer et al., 1997). In addition, it was recently discovered that miR156 and its targets – which are important regulators of flowering because it has a loss-of-function mutation in FRI and much of the natural variation in flowering time in Arabidopsis is attributable to polymorphisms within the genes encoding these proteins (reviewed in Amasino, 2010). Late-flowering accessions often have functional alleles of both genes, whereas early flowering ones typically possess loss-of-function alleles of either FRI or FLC (Gazzani et al., 2003; Johanson et al., 2000; Lempe et al., 2005; Michaels et al., 2003b; Shindo et al., 2005). For example, Columbia (Col) has a wild-type allele of FLC, but is early flowering because it has a loss-of-function mutation in FRI (i.e. its genotype is fri;FLC). FRI;FLC plants can be induced to flower early by prolonged exposure to cold temperature, which leads to epigenetic modifications that block its transcription (reviewed by Sung and Amasino, 2005). In addition to their late flowering phenotype, plants with high levels of FLC have been reported to have a prolonged juvenile phase (Lee et al., 2000; Martinez-Zapater et al., 1995; Telfer et al., 1997) and to display enhanced germination at cool, but not warm temperatures (Chiang et al., 2009).

Here, we present a detailed morphological analysis of the growth and development of FRI-Sf-2;FLC (hereafter FRI;FLC) and a mutant derivative of this line, FRI-Sf-2;fie-3 (FRI;fie-3) (Lee et al., 1993; Michaels and Amasino, 2001), demonstrating the wide-ranging effects of flowering and FLC on vegetative development in Arabidopsis. In addition to the effect of floral induction on the final size and shape of pre-existing leaves, this event has a significant effect on trichome production – a key marker of vegetative phase change in Arabidopsis. We show that FLC has both flowering-dependent, as well as flowering-independent effects on vegetative phase change and leaf morphology, and that some of its effects are dependent on light intensity. These results demonstrate some of the complex interactions that influence the vegetative morphology of Arabidopsis, and reveal a new function for FLC in vegetative development.

**MATERIALS AND METHODS**

**Plant materials**

All seed stocks were in the Columbia (Col) accession. Seeds of FRI;FLC (Col with an introgressed FRI allele from Sf-2; Lee et al., 1993), FRI;flc-3, 35S::FLC (Michaels and Amasino, 1999), fpa-7;FLC and fpa-7;fie-3 (Michaels and Amasino, 2001) were provided by Rick Amasino (University of Wisconsin, Madison, WI) and Scott Michaels (Indiana University, Bloomington, IN); ft-1 (Kardailsky et al., 1999; Kobayashi et al., 1999; Moon et al., 2005), socl-2 (Lee et al., 2000), ft-L;soc-1-2 and ft-1;soc-1-2;fie-3 (Moon et al., 2005) were obtained from Ilha Lee (Seoul University, Seoul, South Korea); LFY::GUS (Blázquez et al., 1997) was obtained from Detlef Weigel (Max-Planck Institute, Germany); and ft-10 (GabriKati-290E08) (Yoo et al., 2005) was obtained from the Nottingham Arabidopsis Stock Centre (NASC). The hydathode GFP marker E325 (http://enhancertraps.bio.upenn.edu) and LFY::GUS were introduced into FRI;FLC and FRI;fie-3 by crossing.

**Plant growth conditions**

Plants were sown in Fafard #2 soil in 96-well flats. The flats were placed at 4°C for 2 days before being put in a Conviron growth chamber at a constant 23°C under a one-to-one ratio of T8 Sylvania Octron 4100K Ecologic and GroLite WS fluorescent lamps (Interlectric). Plant age was measured from the date flats were transferred to the growth chamber. The photoperiods and light intensities used for the various experiments were as follows:

- **Long days**: 16 hours light (125 µmol m⁻² s⁻¹):8 hour dark; short days: 8 hours light (250 µmol m⁻² s⁻¹):16 hours dark; night interruption=6 hours light (250 µmol m⁻² s⁻¹):8 hours dark:2 hours light (250 µmol m⁻² s⁻¹):8 hours dark; low light intensity=16 hours light (75 µmol m⁻² s⁻¹):8 hours dark.
- **Low-light conditions**: created by using multiple layers of Miracloth to shield the light bank; measurements taken with a LabSpec VNIR 512 spectroradiometer (Analytical Spectral Devices, Boulder, CO) revealed that this treatment had no effect on the light spectrum.

**Phenotypic analyses**

The onset of abaxial trichome production was measured as the presence of at least one trichome on the abaxial leaf surface; typically, this trichome was located at the base of the midrib. The juvenile-to-adult transition was considered complete when there was at least one trichome within 2 mm of the distal end of the leaf blade. Juvenile leaves were defined as rosette leaves without abaxial trichomes, transition leaves as rosette leaves with abaxial trichomes that did not fully span the proximodistal axis, and adult leaves as rosette leaves with abaxial trichomes to the distal tip. To minimize the effect of stochastic variation in these traits, at least one of the two subsequent leaves on the shoot had to meet the same criterion to be recorded. Hydathodes were observed using the GFP enhancer trap line E325. Leaf shape was recorded by attaching leaves to paper with double-sided tape, flattening them with strips of single-sided tape, and then photocopying the sheet; photocopies were scanned into a computer for subsequent image analysis. Leaf primordia were observed using plants expressing LFY::GUS and stained according to Donnelly et al. (Donnelly et al., 1999). To determine the number of visible leaves, only the leaves that could be viewed without the aid of a microscope were counted. To determine the point of full expansion, leaves were measured daily until the average daily increase in total leaf length was less than 1 mm on each of two consecutive days, with the second day called the point of full expansion. The length-to-width ratio of the leaf blade is the ratio of the distance from the blade-petiole junction to the distal leaf tip and the width of the blade at the midpoint of this line. Leaf length is the length of the blade plus the petiole. To determine palisade cell area, the outlines of at
least 60 cells in six water-infiltrated leaf samples were traced using a camera lucida and scanned into the computer. The cross-sectional cell area was measured using Photoshop.

RESULTS
The progression but not the onset of vegetative phase change is delayed by FLC under long-day conditions
Non-vernalized FRI;FLC plants grown under long day conditions (LD) are very late flowering (Lee et al., 1993) (Fig. 1A). Previous studies have reported that FRI;FLC and other genotypes with elevated FLC expression also have delayed vegetative phase change, as indicated by the onset of abaxial trichome production (Lee et al., 2000; Martinez-Zapater et al., 1995; Telfer et al., 1997). However, we observed no difference in the timing of abaxial trichome production between FRI;FLC and FRI;flc-3 plants (Fig. 1A).

During vegetative phase change, Arabidopsis produces a series of transition leaves in which abaxial trichomes do not completely span the proximodistal axis of the leaf but are found in progressively more distal regions. We noticed that, although the onset of abaxial trichome production was identical in FRI;FLC and FRI;flc-3, their distal progression seemed to be slower in FRI;FLC. To characterize this phenotype, we recorded the position of the first 60 cells in six water-infiltrated leaf samples were traced using a camera lucida and scanned into the computer. The cross-sectional cell area was measured using Photoshop.

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leaf that was completely covered with abaxial trichomes, i.e. the first fully adult leaf, allowing us to calculate the number of transition and adult leaves. We found that FRI;FLC makes significantly more transition leaves than FRI;flc-3 (P<0.0001, Student’s t-test) (Fig. 1A), suggesting that FLC delays the progression of the juvenile-to-adult transition.

Abaxial trichome distribution is only one of several traits that change during vegetative development in Arabidopsis. To determine whether FLC has a global effect on vegetative phase change, we examined several of these traits in FRI;FLC and FRI;flc-3 plants. The spread of abaxial trichomes towards the distal end of the leaf is associated with an increase in their total number (Chien and Sussex, 1996). The rate of this increase was much lower in FRI;FLC than in FRI;flc-3 (Fig. 1B). The number of hydathodes also increases in successive leaves within the rosette (Hunter et al., 2003; Tsukaya et al., 2000). Hydathode number increased at approximately the same rate in FRI;FLC and FRI;flc-3; however, FRI;FLC leaves had slightly fewer hydathodes than FRI;flc-3 leaves (Fig. 1C), even though they were larger overall (Fig. 1D,H). Cell size has long been known to decrease in successively higher leaves on the shoot (Ashby, 1948), and this is also true in early flowering genotypes of Arabidopsis (Usami et al., 2009). Palisade cell size declined gradually with leaf position in both FRI;FLC and FRI;flc-3, but – with the exception of leaf 1 – cell size was consistently larger in FRI;FLC than FRI;flc-3 (Fig. 1E). Vegetative phase change is also associated with a change in leaf shape (Fig. 1H), which can be measured by the length-to-width ratio of the leaf blade (Steynen et al., 2001). Although young rosettes of FRI;flc-3 and FRI;FLC look grossly similar to one another (Fig. 1I), the length/width ratio of rosette leaves revealed that all but the first two leaves of FRI;FLC are significantly rounder than those of FRI;flc-3, and this ratio increases more slowly in FRI;FLC than in FRI;flc-3 (Fig. 1F,H).

We have previously hypothesized that transition leaves arise from the imposition of a new cell fate on incompletely determined organs present on the shoot apex when phase change occurs (Orkwiszewski and Poethig, 2000). According to this hypothesis, one way in which FRI;FLC could extend the transition zone is by increasing the number of incompletely determined leaf primordia at the shoot apex. This would occur if FRI;FLC had a faster rate of leaf initiation than FRI;flc-3 or if leaf development was prolonged in FRI;FLC relative to FRI;flc-3. To address this issue, we measured the rate of leaf initiation and the duration of leaf expansion at six different positions on the shoot in plants grown in LD. Leaves 1 and 2 were examined together because they are initiated at the same time in the embryo. We determined when each of these leaves was initiated by crossing the LFY::GUS transcriptional reporter into both genotypes (Blázquez et al., 1997). LFY::GUS is expressed in very young leaf primordia, disappearing around the time a primordium is 1.0 mm long (Blázquez et al., 1997) (data not shown). By counting leaf primordia with, and without, GUS activity in rosettes harvested at different times, we obtained a rough estimate of the growth rate of these young primordia (Fig. 1G). We also recorded the date at which leaves were 1.0 mm in length, and the date at which they reached full expansion. As shown in Fig. 1G, we found that the rate of leaf initiation and the rate of primordium expansion was the same in both genotypes for all leaf positions considered. Thus, the difference in the length of the juvenile-to-adult transition in FRI;flc-3 and FRI;FLC is not related to the rate of leaf initiation or the growth rate of the primordia in these genotypes.

To further explore the effects of FLC on vegetative development, we examined the phenotypes of the late flowering genotype fpa-7;FLC, which has elevated levels of FLC (Rouse et al., 2002), and Col plants expressing FLC under the regulation of the constitutive CaMV 35S promoter (35S::FLC). The phenotype of fpa-7;FLC was compared with fpa-7;flc-3 to control for any effects of fpa-7 that are independent of FLC. There was no significant difference in the onset of abaxial trichome production between these high FLC genotypes and the low FLC controls (Fig. 2A); however, like FRI;FLC, both genotypes had an increased number of transition leaves (Fig. 2A), a slower increase in abaxial trichome abundance (Fig. 2B), and rounder leaves (Fig. 2C) than plants with low levels of FLC. These differences were not attributable to differences in the rate of leaf initiation between genotypes (Fig. 2D). Thus, FLC has a role in vegetative development in addition to its well-known effect on flowering time.

**Flowering-dependent and flowering-independent effects of FLC on vegetative development**

As mentioned above, FRI;FLC plants flowered after making over 70 leaves, nearly two months after planting. By contrast, FRI;flc-3 plants were florally induced by 11 or 12 days after planting, as
evident from a change in the morphology of the shoot apical meristem and the fact that they produced no additional leaves after this time (Fig. 1G). At this stage, leaves 7 and 9 (the last rosette leaf) were less than 1.0 mm in length, and none of the other rosette leaves were fully expanded. Thus, all of the leaves of this early flowering genotype completed their development after the shoot had been induced to flower. This observation suggests that many of the differences in the vegetative morphology of FRI;FLC and FLC;flc-3 might arise from the effect of floral induction on leaf development.

To test this hypothesis, we compared the phenotypes of FRI;FLC and FRI;flc-3 plants grown under short days (SD), and SD with a 2-hour night interruption (NI); this relatively brief light exposure induces flowering in FRI;flc-3 (albeit less strongly: 24.5 rosette leaves in NI versus 9.4 in LD), without affecting the total amount of light received by plants in these treatments. The phenotypic differences between FRI;FLC and FRI;flc-3 in the NI treatment reflect the direct effects of FLC on vegetative development, as well as the effect of the difference in the flowering time of these genotypes. Differences attributable to flowering should disappear in SD because FRI;FLC and FRI;flc-3 are both late flowering under these conditions; differences that remain under SD are controlled by FLC independent of its effect on flowering.

The rate of leaf initiation was identical in FRI;FLC and FRI;flc-3 under both SD and NI conditions (Fig. 3A), indicating that all of the differences observed between these genotypes reflect
differences in the timing of these processes. Consistent with the results obtained in LD (Fig. 1A), FRI;FLC and FRI;flc-3 plants grown in NI produced abaxial trichomes at the same leaf position, but the length of the transition zone was significantly longer in FRI;FLC than FRI;flc-3 (Fig. 3B). The effect of FLC on leaf shape is probably independent of FT and SOC1 because the blade length-to-width ratios of FRI;flc-3 and ft-1;soc1-2 are similar. Results are mean ± s.d.

Fig. 4. FT and SOC1 influence total abaxial trichome numbers but not leaf roundness. (A) FT and SOC1 have additive effects on the total number of abaxial trichomes under long days; ft-1;soc1-2, ft-1;soc1-2;flc-3 and FRI;FLC have similar numbers of abaxial trichomes. (B) The effect of FLC on leaf shape is probably independent of FT and SOC1 because the blade length-to-width ratios of FRI;flc-3 and ft-1;soc1-2 are similar. Results are mean ± s.d.

the ft-1;soc1-2 double mutant and the ft-1;soc1-2;flc-3 triple produced even fewer abaxial trichomes and were indistinguishable from FRI;FLC for this trait (Fig. 4A). This result suggests that the effect of FLC on abaxial trichome number is mediated by these transcription factors. By contrast, the shape of the leaf blade in ft-1;soc1-2 was significantly different from FRI;FLC, but not significantly different from FRI;flc-3 (Fig. 4B), implying that other targets of FLC control this phenotype.

Light intensity modulates vegetative phase change in FRI;FLC but not FRI;flc-3

In contrast to the results presented here, previous studies have shown that genotypes with increased FLC activity, including FRI;FLC, fpa;FLC and fve;FLC, have delayed trichome production (Lee et al., 2000; Martinez-Zapater et al., 1995; Telfer et al., 1997). We noticed that the timing of abaxial production in FRI;FLC varied with the age of the fluorescent bulbs in our growth chambers, suggesting that light intensity might be responsible for the difference between our results and these earlier studies. Consistent with this idea, Lee and colleagues (Lee et al., 2000) reported that the light intensity in their growth chambers was 100 μmol m⁻² s⁻¹, which is lower than the light intensity (125 μmol m⁻² s⁻¹) in our chambers. Our original studies in which we observed an effect of FLC on abaxial trichome production (Telfer et al., 1997) were performed in growth chambers illuminated with VHO fluorescent light bulbs, the light output of which declined from 140 μmol m⁻² s⁻¹ to less than 100 μmol m⁻² s⁻¹ within a few weeks. Although we did not measure the light intensity in these original experiments, it is reasonable to assume that it was closer to 100 μmol m⁻² s⁻¹ than to 140 μmol m⁻² s⁻¹.

To test the effect of light intensity on abaxial trichome production, we compared the onset of abaxial trichome production under light intensities of 125 μmol m⁻² s⁻¹ (high light) and 75 μmol m⁻² s⁻¹ (low light) in plants growing in LD. Low-light conditions were produced by shielding the fluorescent light bank with Miracloth, which did not produce a change in light spectrum. Low-light conditions did not affect the onset of abaxial trichomes in FRI;flc-3, but produced a significant delay in abaxial trichome production in FRI;FLC (Fig. 5A). Other genotypes with low FLC levels (Col and fpa;flc-3) were also unaffected by low light, whereas genotypes with high FLC (fpa;FLC and 35S::FLC) produced abaxial trichomes later under low-light conditions than under high light (Fig. 5A). Leaf initiation was slowed equally in all genotypes by the lower light levels (Fig. 5B). Thus, in the presence of FLC, the onset of abaxial trichome production is uniquely sensitive to light intensity. This sensitivity is probably due to the lower FT expression in these genotypes because ft-1 and ft-10 were also sensitive to light intensity, whereas soc1-2 was insensitive to this condition (Fig. 5A).

DISCUSSION

The identity of lateral organs of the shoot of flowering plants is determined by the timing of two key postembryonic developmental transitions – vegetative phase change and flowering. In late-flowering plants, floral induction occurs long after vegetative phase change so that it is relatively easy to distinguish changes that are specific to vegetative phase change from those that are the result of floral induction. However, in early flowering plants, flowering occurs shortly after the juvenile-to-adult transition. This complicates analyses of vegetative phase change because it raises the possibility that changes induced by flowering might overlap with, and potentially modify, the expression of traits associated
with the juvenile-to-adult transition. To distinguish traits that are components of vegetative phase change from features that are affected by floral induction, we compared the phenotype of the late-flowering genotype FRI;FLC with an early-flowering mutant derivative of this line, FRI;flc-3. Our results are consistent with the results of Cookson et al. (Cookson et al., 2007), and indicate that floral induction affects some features of leaf morphology and not others, and that most rosette leaves are modified by this event. Importantly, flowering had no effect on the onset of abaxial trichome production, a key marker of vegetative phase change. We also found that FLC has effects on leaf morphology that are independent of its effect on flowering time, revealing a novel function for this gene in vegetative development.

**FLC, light intensity, and the onset of vegetative phase change**

Many traits vary as a function of leaf position in Arabidopsis. We focused on abaxial trichome production because this trait has been widely used as a marker of vegetative phase change. Previous studies have shown that increasing the expression of FLC, blocking gibberellin synthesis or signaling, and growing plants under non-inductive photoperiods, delay the onset of abaxial trichome production and reduce the number of adaxial trichomes (Chien and Sussex, 1996; Lee et al., 2000; Martinez-Zapater et al., 1995; Telfer et al., 1997). Because all of these conditions affect flowering time, it was not clear whether their effect on trichome production is an indirect result of their effect on flowering time, or a consequence of their involvement in vegetative phase change.

We found that the onset of abaxial trichome production is insensitive to floral induction, indicating that the presence versus absence of abaxial trichomes is a good marker of the vegetative phase of the shoot. Unexpectedly, we also discovered that the onset of abaxial trichome production is sensitive to light intensity, but only in plants with high levels of FLC. This phenotype probably reflects the effect of FLC on the expression of FT. FLC directly represses FT and SOC1, and most of its effects have been attributed to one or both of these genes (reviewed in Amasino, 2010). Whereas soc1-2 has no effect on abaxial trichome production under either high or low-light conditions, ft mutants are as sensitive to light intensity as FLC, and this phenotype is epistatic to the light-insensitive phenotype of flc-3; in other words, FT is required for the light-insensitive phenotype of flc-3. The observation that abaxial trichome production is delayed in the absence of FT under low-light conditions (i.e. in FRI;FLC and in ft mutants), but not in the presence of FT (i.e. in FRI;flc-3) indicates that FT overrides the effect of light intensity on this trait and, more importantly, demonstrates that FT directly or indirectly regulates vegetative development.

Loss-of-function mutations of the FT ortholog SFT have been reported to affect leaf morphology in tomato (Shalit et al., 2009), but this is the first evidence that FT regulates vegetative development in Arabidopsis. FT is transcribed in cotyledons and leaf primordia and is expressed at a low level in the shoot apex of 6- to 7-day-old seedlings in LD (Kardailsky et al., 1999; Kobayashi et al., 1999; Yamaguchi et al., 2005). In addition, FT protein is translocated from leaves to the shoot apex during floral induction (Corbesier et al., 2007; Jaeger and Wigge, 2007). Floral induction occurs between 9 and 11 days after planting in LD, at which time the first transition leaves (leaves 5 or 6) are 1 mm or less in length (Fig. 1G). It is unclear whether the effect of FT on leaf development is mediated by the FT protein produced in leaf primordia or FT protein imported into leaf primordia upon floral induction but, in either case, it is present in the shoot apex at the correct time to affect the expression of phase-specific leaf traits. How might it regulate these traits? The mRNA levels of SPL3, SPL9 and several other SPL genes are reduced in the shoot apex of ft mutants (Schmid et al., 2003), whereas SPL3 mRNA is elevated in plants over expressing FT (Schmid et al., 2003; Wang et al., 2009; Yamaguchi et al., 2009), demonstrating that SPL genes are directly or indirectly regulated by FT. SPL genes regulate various aspects of vegetative phase change, including abaxial trichome production (Schwarz et al., 2008; Usami et al., 2009; Wu et al., 2009; Wu and Poethig, 2006). Consequently, it is reasonable to propose that FT regulates abaxial trichome production via its effect on SPL gene expression. The observation that light intensity affects abaxial trichome production in the absence of FT (i.e. in FRI;FLC and ft mutants) implies that light acts downstream or in parallel to FT. One possibility is that light intensity acts by affecting the abundance of miR156, a negative regulator of SPL gene expression, such that miR156 is expressed at higher levels...
under low light intensity. In this scenario, FT promotes the transcription of SPL genes in leaf primordia, thereby overcoming the increased miR156-mediated repression of these genes under low light conditions. In the absence of FT, SPL gene expression would become acutely sensitive to light-dependent changes in miR156 expression.

**Flowering speeds the juvenile-to-adult transition and has other effects on leaf development**

Although flowering does not affect the onset of vegetative phase change, it has a significant effect on the subsequent progression of this process. FRI;FLC plants grown under LD, NI or SD, and FRI;flc-3 plants grown under SD to prevent flowering, produce significantly more transition leaves than FRI;flc-3 plants grown in LD or NI. Vegetative phase change is mediated by an increase in the expression of miR156-regulated members of the SPL gene family, which occurs in response to a decrease in the expression of miR156 (Chuck et al., 2007; Wu et al., 2009; Wu and Poethig, 2006). In LD, but not in SD, some of these miR156-regulated SPL genes undergo a further increase in expression during the adult phase (Cardon et al., 1999; Chuck et al., 2007; Schmid et al., 2003). We suggest that the onset of vegetative phase change is regulated by the increase in SPL gene expression that results from a decrease in miR156 levels, and that the rate of this transition is dependent on the level of SPL gene transcription, which is influenced by a variety factors. Our observation that the length of the transition zone is identical in FRI; FLC plants grown in LD and SD, and in FRI;FLC and FRI;flc-3 plants grown in SD, suggests that the difference in the expression of SPL genes in SD and LD (Cardon et al., 1999) is attributable to floral induction, not photoperiod per se.

In addition to influencing vegetative phase change, flowering has other effects on leaf development. Leaf length, the shape of the leaf blade, the rate of leaf expansion, cell size, cell number and hydathode number all differ in early and late-flowering plants, and these differences are apparent as early as leaf 3 (Cookson et al., 2007). In early-flowering varieties of Arabidopsis, floral induction occurs while all of the leaves are still expanding, so it is perhaps not surprising that most rosette leaves are affected by this event. The effect of flowering on leaf development should be taken into account when studying the effects of early flowering genotypes on leaf development and vegetative phase change, to avoid confusing the direct and indirect effects of these genes.

**Flowering-independent effects of FLC on leaf development**

Although most of the effects of FLC on leaf morphology are an indirect result of its effect on flowering, FLC also has a more direct role in leaf development. This is apparent from the observation that FRI;FLC has significantly rounder leaves and fewer abaxial trichomes than FRI;flc-3 under SD conditions, when both genotypes are late flowering. Along with the evidence that FLC regulates seed germination (Chiang et al., 2009), these results indicate that FLC has additional functions outside its well-described role in flowering. This might be expected from the fact that FRI;FLC plants flower only under certain environmental conditions, usually after a long exposure to cold temperatures (Amasino, 2010). FRI;FLC genotypes that fail to flower before the onset of winter must be adapted to cold, and many other biotic and abiotic stresses. Selection for a winter annual flowering habit would necessarily have been accompanied by selection for traits that allow plants to survive winter, and it may be that FLC has both of these functions.

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**Competing interests statement**

The authors declare no competing financial interests.

**Supplementary material**

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