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Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*

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SUMMARY

Higher plants pass through several phases of shoot growth during which they may produce morphologically distinct vegetative structures. In *Arabidopsis thaliana* this phenomenon is apparent in the distribution of trichomes on the leaf surface. Leaves produced early in rosette development lack trichomes on their abaxial (lower) surface, leaves produced later have trichomes on both surfaces, and leaves in the inflorescence (bracts) may have few or no trichomes on their adaxial (upper) surface. Here we describe some of the factors that regulate this distribution pattern. We found that the timing of abaxial trichome production and the extent to which bracts lack adaxial trichomes varies in different ecotypes. The production of abaxial trichomes appears to be regulated by the age, rather than the size of the plant. This conclusion is based on the observation that mutations that affect either the rate (*altered meristem programming1*) or onset (*paused*) of leaf initiation respectively increase or decrease the number of leaves that lack abaxial trichomes, but have only a minor effect on the time at which the first leaf with abaxial trichomes is produced. The production of abaxial trichomes is coordinated with the reproductive development of the shoot as this trait is delayed by photoperiodic conditions and some mutations that delay flowering. The loss of adaxial trichomes is likely to be a consequence of floral induction, and is accelerated by *terminal flower1-10*, a mutation that accelerates inflorescence development. We demonstrate that gibberellins promote trichome production in *Arabidopsis* and present evidence indicating that abaxial trichome production is regulated by both the level of a trichome inducer and the competence of the abaxial epidermis to respond to this inducer.

Key words: phase change, trichomes, *paused*, *Arabidopsis*, shoot development

INTRODUCTION

All higher plants undergo a process of shoot maturation during which they develop reproductive competence (see reviews by Hackett, 1985; Zimmerman et al., 1985; Poethig, 1990; Hackett and Murray, 1993; Greenwood, 1995; Lawson and Poethig, 1995). In many plants, the change in reproductive competence is preceded by changes in the anatomy, morphology and physiology of vegetative parts of the shoot, with the result that vegetative structures produced during the juvenile and adult phases may be strikingly different. Little is known about how these changes in the vegetative character of the shoot (known as *vegetative phase change*) are regulated, nor has the nature of the relationship between vegetative phase change and reproductive aspects of shoot maturation been defined (Longman, 1976; Schwabe, 1976; Zimmerman et al., 1985; Hackett, 1985).

The present study represents the first step in our genetic and physiological analysis of vegetative phase change in *Arabidopsis thaliana*. In order to conduct a thorough analysis of phase change it is necessary to have unambiguous markers for the different phases in shoot development. In many plants, the most obvious marker in this respect is leaf shape (Allsopp, 1967). *Arabidopsis* leaves do vary in shape during shoot growth (Röbbelen, 1957; Medford et al., 1992; Martínez-Zapater et al., 1995), but the variation is gradual and it is difficult to identify factors that affect vegetative phase change simply by their effects on this trait. Another trait that distinguishes juvenile and adult leaves in some species is the density and/or distribution of trichomes (Schaffalitzky de Muckadell, 1954; Wareing and Frydman, 1976; Brand and Lineberger, 1992; Lawson and Poethig, 1995). This trait is obvious and easily quantifiable. In maize, for example, trichomes are confined to the margin of juvenile leaves, but are present on both the leaf margin and on the adaxial (upper) surface of adult leaves (Freeling and Lane, 1994; Lawson and Poethig, 1995). Trichome production in maize is a useful marker of phase change because it is correlated with the expression of many other phase-specific traits, both in wild-type plants and in mutants in which phase change is accelerated or delayed.

In *Arabidopsis*, the total number of trichomes on the adaxial leaf surface changes with leaf position (Martínez-Zapater et al., 1995), although like leaf shape, the variation in this trait is gradual, limiting its utility as a phase marker. A better marker may be found in the changing spatial distribution of trichomes on the abaxial and adaxial leaf surfaces (Telfer and Poethig, 1994; Chien and Sussex, 1996). Here we describe variation in the expression of this trait in different ecotypes, and investigate how it is affected by factors such as gibberellins and flowering time mutations that are known to affect vegetative phase change and/or reproductive development in other species. We demonstrate that abaxial trichome development is

independent of the growth rate of the shoot. We also describe the failure of bracts to develop adaxial trichomes and discuss the relationship between this trait and inflorescence development.

MATERIALS AND METHODS

Genetic stocks and growth conditions

Mutant seed stocks were generously provided by the following individuals: *amp1-1*, A. Chaudhury; *amp1(cop2)*, A. Lehman and J. Ecker; *tfl1-10*, R. Meeks-Wagner; *spy-4*, S. Jacobsen. *spy-3*, *ga1-3*, *ga4*, *ga5*, *gai*, and the late flowering mutant strains were obtained from the Arabidopsis Biological Resource Center at Ohio State University (Columbus, Ohio). The *paused*(*psd*) mutation was isolated by K. Barton in our laboratory in a screen of EMS-mutagenized Landsberg *erecta* plants.

Except where noted otherwise, seeds were sown on Metromix 200 (Scotts) in 4 inch pots (5-10 seeds per pot) and placed at 4°C for 2 days before transfer to growth chambers (Conviron) maintained at 22°C. High humidity was maintained during germination and early growth by placing pots in covered flats. The lids were removed after about 2 weeks. In each experiment, pots containing the strains to be compared were interspersed in the same flats to compensate for microclimate differences that could affect growth rate or trichome development. Light-dark regimens and the number of plants examined are noted for each experiment. Short day conditions represent cycles of 8 hours of light and 16 hours of darkness. The late flowering mutants and their Landsberg *erecta* control population were grown as above but without cold treatment. The requirement of *ga1-3* seeds for exogenous GA for germination was circumvented by dissecting the seeds to remove the seed coats. Dry *ga1-3* and control seeds were imbibed overnight on water-soaked filter paper at 4°C in the dark. The seed coats were removed by scraping the testa with dissecting needles and applying gentle pressure to pop out the embryos. Dissected embryos were transferred to soil and grown as above, but without further cold treatment.

GA_3 treatments

Wild-type Columbia plants grown in soil under 16 hour days at 22°C were divided into developmentally staged sub-populations and treated with 10^{-5} mM GA_3 (Sigma) dissolved in 0.05% ethanol. Seedlings treated beginning at germination had partially emerged radicles. Plants treated beginning later in development were staged according to the number of leaves and leaf primordia visible under a dissecting microscope. 100 μl of GA_3 solution was applied to the soil at the base of the hypocotyl every 2 or 3 days. The amount of GA_3 solution applied was increased to 300 μl when six leaves and leaf primordia were visible. Control plants were treated with 0.05% ethanol from the time of germination.

To measure the response of *ga1-3* mutants to different levels of GA_3, seeds were imbibed in water, embryos were dissected as described above, and transferred to Petri dishes containing filter paper moistened with GA_3 solution or water. The Petri dishes were sealed with parafilm and placed in a growth chamber on 16 hour days, 22°C. After 2 days undamaged seedlings with green cotyledons were transferred to soil and grown under constant illumination at 22°C without further GA treatment.

Growth rate analysis

The rate of leaf initiation was determined by counting leaves and leaf primordia visible under a dissecting microscope at various times after planting (i.e. when cold-treated seed was transferred to the growth chamber). Therefore, these rates do not actually reflect when leaves are produced by the shoot meristem, but rather when they become visible in the apex. Populations to be compared consisted of plants grown from seed that had been sown, cold-treated, and transferred to the growth chamber at the same time and had germinated on the same day. The exception to this was the comparison of *cop2*, *amp1* and their Columbia control population. These mutants uniformly germinated a day earlier than wild-type sown on the same day.

amp1-1 and *amp1(cop2)* mutant plants often produce abnormal rosettes in which multiple leaves appear to be initiated simultaneously (Chaudhury et al., 1993). Because the aim of this analysis was to estimate when a particular leaf was initiated, we only examined individuals that produced leaves individually and formed normal rosettes. In the case of *psd*, leaf initiation rates were determined using plants that had at least one primordium visible by 4 days after germination and which went on to produce additional leaves individually, in a rosette (64% of the population).

Histological analysis

Seedlings were fixed overnight in 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8), and postfixed for 2 hours in 2% OsO_4. They were then dehydrated in ethanol, transferred to acetone and embedded in Spurr's resin. 1-2 μm sections were stained with 0.5% methylene blue in 0.5% $Na_2B_4O_7$ and mounted in immersion oil. Sections were photographed with Kodak technical pan film 2415.

RESULTS

The distribution of trichomes on the adaxial and abaxial leaf surfaces defines two phases of vegetative development

Arabidopsis thaliana produces leaves in a compact rosette during the vegetative phase of shoot development. These leaves exhibit differences in the distribution of trichomes on their abaxial leaf surfaces (Fig. 1). The first few leaves in the rosette possess trichomes on their adaxial surface, but do not develop abaxial trichomes. Leaves produced later in rosette development have both adaxial and abaxial trichomes. Abaxial trichomes usually appear first on the midrib or at the base of the leaf, and are initially few in number. The distribution of abaxial trichomes gradually becomes more extensive on successive leaves, until the entire abaxial surface is uniformly covered with trichomes. Based on this variation in trichome production, we have subdivided vegetative development into two phases: a juvenile phase marked by the production of leaves without abaxial trichomes, and an adult phase defined by the production of leaves that have trichomes on both upper and lower surfaces.

Abaxial trichome production varies in different ecotypes

The Columbia (Col), Landsberg *erecta* (Ler) and Wassilewskja (Ws) ecotypes produce abaxial trichomes at different leaf positions. When plants were grown in constant light (CL), the first abaxial trichomes were produced on leaf three in Ws, on leaf three or four in most Ler plants, and on leaf five or six in most Col plants (Fig. 2A, Table 1 section a). Thus, Ws had the fewest number of leaves without abaxial trichomes (two), and Col has the most (four or five). The first leaf with abaxial trichomes varies somewhat in different experiments (see Table 1), presumably due to slight differences in growth conditions. However, the relative numbers of leaves that lack abaxial trichomes in these three ecotypes does not change in different growth conditions, nor have trichomes been observed under the first or second leaf of these ecotypes.

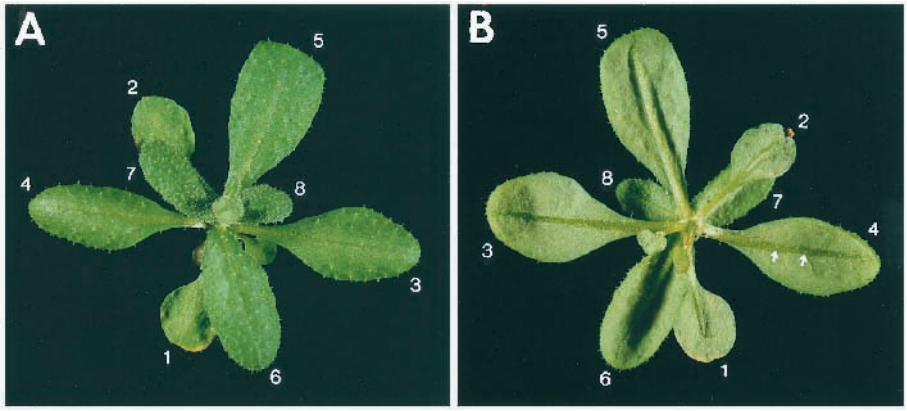

Fig. 1. Trichome distribution on developing rosette leaves. (A) Landsberg *erecta* photographed from above to show adaxial trichomes. Numbers refer to the positions of leaves as determined by their order of appearance. (B) Abaxial view of the same plant. Trichomes first appear on the midrib of leaf 3 and 4 (arrow), but are not produced in large numbers until leaf 7.

Variation in the number of leaves lacking abaxial trichomes could reflect differences in the temporal duration of the juvenile phase in different ecotypes. It could also arise if the ecotypes have juvenile phases of the same duration but initiate leaves at different rates. To distinguish between these possibilities, the timecourse of leaf initiation in each ecotype was determined (Fig. 2B). All three ecotypes were found to initiate leaves at approximately the same rate under CL. Thus, the difference in abaxial trichome production in these ecotypes is likely to reflect differences in the temporal duration of the juvenile phase. The initiation of the first leaf with abaxial trichomes was estimated to occur 6-7 days after planting in Ws, 7-8 days after planting in Ler, and 9-10 days after planting in Col.

The development of abaxial trichomes may be temporally regulated

In many plants, developmental changes in morphology are thought to be associated with changes in the size of the shoot (Allsopp, 1967; Hackett, 1985). To determine if shoot size affects trichome distribution in *Arabidopsis*, we examined the phenotype of mutations that affect the onset or rate of leaf initiation. Plants homozygous for a new recessive mutation *paused* (*psd*) germinate normally, but initiation of the first leaves is delayed relative to wild type. Whereas enlarged leaf primordia are readily seen in longitudinal sections of the shoot meristems of germinating Ler seedlings made 3 days after planting, *psd* seedlings have no detectable primordia at this stage, presumably as a result of the death of cells in the central region of the shoot apical meristem (Fig. 3A,B). Most *psd* plants 'recover' after several days and begin to initiate leaves. The remainder of the plants, which were not analyzed further, produce no leaves initially, or produce only a single leaf or leaf-like structure, but then initiate multiple leaves simultaneously after a delay of 1-2 weeks. Longitudinal sections of the shoot meristem of 8-day old wild-type and recovered *psd* seedlings reveal that the layered structure of the *psd* meristem is relatively disorganized (Fig. 3C,D), but it appears to be otherwise normal. Despite their delayed initiation, the first leaf primoridia develop in the proper location in *psd* plants. The first two leaves of recovered *psd* plants differ from the first leaves of wild-type plants in that they have an oval shape more typical of leaves produced during the adult phase of development and may not be initiated simultaneously (Fig. 3E). Furthermore, over 70% of these plants produced abaxial trichomes on leaf one or two, whereas wild-type Ler plants produce at least two leaves without abaxial trichomes (Table 1, section b). Thus, *psd* plants make few, if any, leaves lacking abaxial trichomes. We determined the timecourse of leaf production in *psd* and wild-type plants and estimated the time of appearance of the first leaf with abaxial trichomes (Fig. 4). The two populations produced leaves with abaxial trichomes at approximately the same time, about 7 days after planting.

In contrast to *psd* mutants, plants homozygous for *altered meristem programming1* (*amp1*) mutant alleles initiate leaves at an accelerated rate (Chaudhury et al., 1993). Both *amp1-1* and *cop2* (Hou et al., 1993) – which we have shown to be allelic (data not shown) – cause plants to develop more than twice as many leaves without abaxial trichomes as wild-type plants

Table 1. The position of the first leaf with abaxial trichomes in different ecotypes and mutants of *Arabidopsis thaliana*

Table section	Strain	Day length	First leaf with abaxial trichomes*	*n*
a	Ws	CL	3.0±0.0	20
	Ler	CL	3.7±0.3	22
	Col	CL	5.4±0.2	29
b	Wild type (Ler)	CL	3.2±0.1	45
	psd	CL	1.9±0.3	41
c	Wild type (Col)	CL	5.3±0.4	11
	amp1(cop2)	CL	12.5±0.7	36
	amp1-1	CL	12.7±0.3	23
d	Ws	SD	5.2±0.3	21
	Ler	SD	6.0±0.4	25
	Col	SD	7.1±0.3	19
e	Wild type (Col)	CL	6.4±0.6	12
	tfl1-10	CL	6.5±0.3†	19
f	Wild type (Ws)	CL	3.0±0.0	13
	spy-4	CL	3.0±0.0	10
g	Wild type (Ler)	CL	3.9±0.2	33
	ga4	CL	5.7±0.2	39
	ga5	CL	4.4±0.5	18
	gai	CL	6.1±0.5	8
h	Wild type (Ler)	CL	4.3±0.5	12
	ga1-3	CL	none observed	13

*Each value represents the average ± 2 × s.e.m. Unless otherwise indicated, plants within each group are significantly different from each other (sections a and e) or from the wild-type control ($P<0.05$, Student's *t*-test).

†Not significantly different from wild type ($P>0.05$, Student's *t*-test).

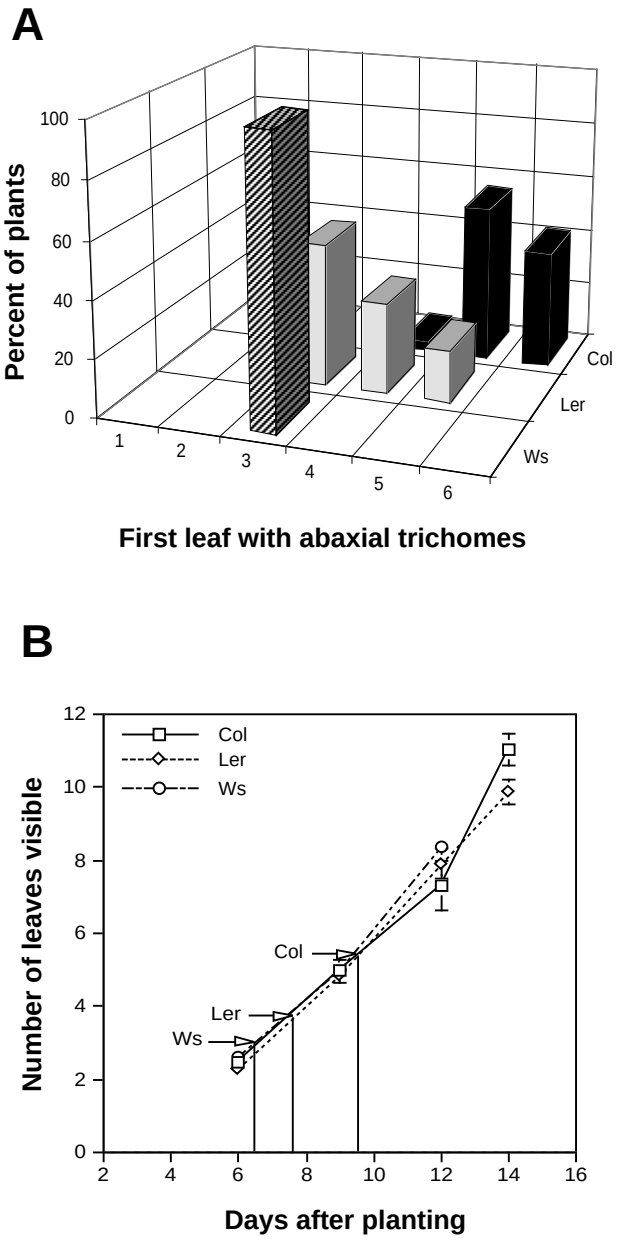

Fig. 2. Comparison of the rate of leaf initiation, and the leaf position and time at which abaxial trichomes are first produced in the Col, Ler and Ws ecotypes. (A) Frequency distributions for the position of the first leaf with abaxial trichomes. The percentage of each population (n=20-30) that first developed abaxial trichomes at each leaf position is indicated in the graph. Plants were grown in CL. (B) The rate of leaf initiation of the plants in A. The arrows indicate the average position of the first leaf with abaxial trichomes in each ecotype (see Table 1). From this point, the average time at which abaxial trichome development was initiated was estimated (as indicated by the vertical lines). Error bars indicate 2× s.e.m.

(Fig. 5, Table 1, section c). However, as in the case of *psd*, these mutations have very little effect on the timing of abaxial trichome production. *amp1-1* and *amp1(cop2)* plants produced leaves with abaxial trichomes approximately 10 days after planting, 1 day later than the Col controls.

Thus, *psd*, *amp1-1* and *amp1(cop2)* plants produce leaves

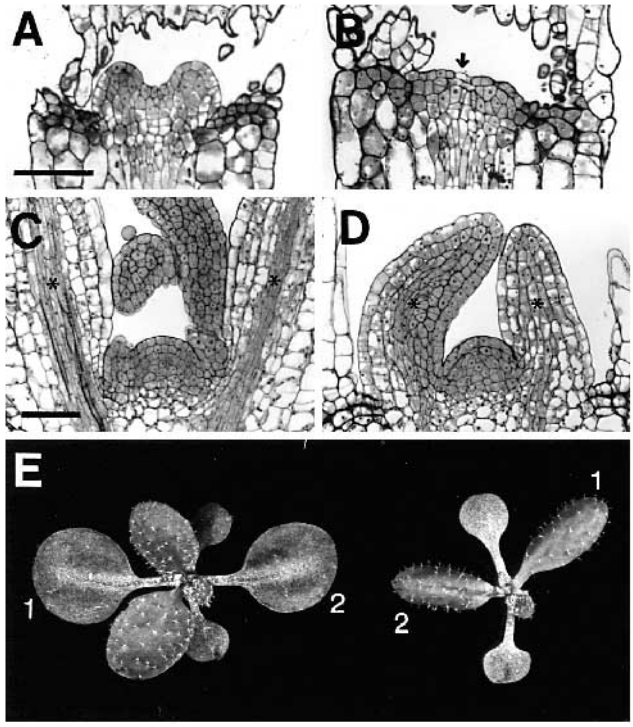

Fig. 3. Comparison of *psd* and wild-type Ler plants. (A,B) Longitudinal section through the shoot apical meristem of Ler (A) and *psd* (B) seedlings 3 days after planting. The Ler seedling has 2 leaf primordia, whereas no leaf primoridia are obvious in the *psd* seedling. *psd* seedlings typically have a group of dead cells (arrow) at the center of the meristem. (C,D) Longitudinal section through the shoot apical meristem of Ler (C) and *psd* (D) seedlings 8 days after planting. The Ler seedling has initiated 5-6 leaves, whereas the *psd* seedling has initiated 3-4 leaves. Leaves one and two are indicated by *. (E) Ler (left) and *psd* (right) seedlings 15 days after planting. Note that the first two leaves of the *psd* seedling are more elongated than the first two leaves of the Ler seedling. scale bar, 50 μm.

with abaxial trichomes at the correct time in shoot development, despite the fact that *psd* plants make few or no leaves lacking abaxial trichomes and *amp1-1* and *amp1(cop2)* plants make a large number of such leaves. This observation is inconsistent with the hypothesis that abaxial trichome production is regulated by the size of the shoot. Rather, it supports the hypothesis that this trait is regulated either by a temporal change in the character of the shoot meristem, or by factors that change independently of the growth of the shoot.

Abaxial trichome production is sensitive to photoperiod and some flowering time mutations

Vegetative phase change has been correlated with the attainment of reproductive competence in other species (Hackett, 1985; Zimmerman et al., 1985). If this is also true in *Arabidopsis*, and if abaxial trichome development is a marker for the vegetative phase of the shoot, then this trait is likely to be influenced by factors that regulate flowering. We found that abaxial trichome production is indeed affected by environmental conditions and some mutations that affect flowering time.

Arabidopsis plants grown in short days (SD) flower later, with more rosette leaves, than those grown in long days or CL (Martínez-Zapater et al., 1994). Ws, Ler and Col plants grown

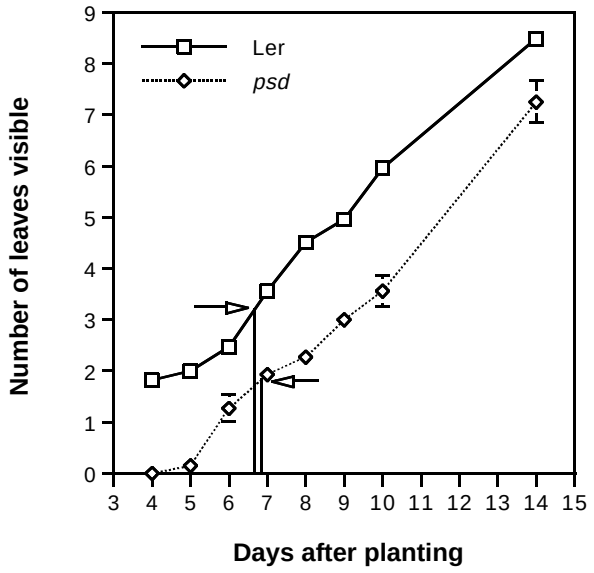

Fig. 4. Comparison of the rate of leaf initiation and the time at which abaxial trichomes are first produced in *psd* and wild-type Ler plants. Leaf initiation was measured in 45 Ler and 41 *psd* plants. The arrows indicate the average position of the first leaf with abaxial trichomes in each genotype (see Table 1). From this point, the average time at which abaxial trichome development was initiated was estimated (as indicated by the vertical lines). Error bars indicate 2× s.e.m.

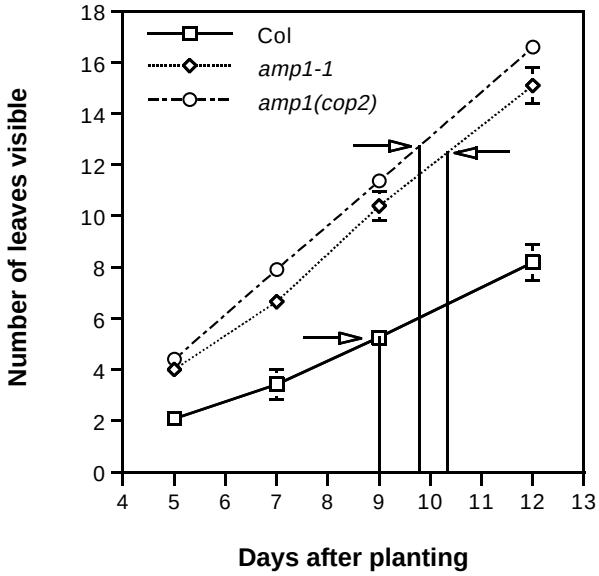

Fig. 5. Comparison of leaf initiation and the time at which abaxial trichomes are first produced in *amp1-1*, *amp1(cop2)*, and wild-type Col plants. Leaf initiation was measured in 11 Col, 23 *amp1-1*, and 36 *amp1(cop2)* plants. The arrows indicate the average position of the first leaf with abaxial trichomes in each genotype (see Table 1). From this point, the average time at which abaxial trichome development was initiated was estimated (as indicated by the vertical lines). Error bars indicate 2× s.e.m.

in SD developed abaxial trichomes at significantly later leaf positions than plants grown in CL (Table 1, section d). Furthermore, six of seven late flowering mutations we examined delayed abaxial trichome production. Data from two separate experiments are shown (Table 2). *co-2*, *fd-1*, *gi-3*, *fca-1*, *fve-1* and *fpa-1* produced significantly more juvenile leaves than wild-type plants in both experiments, while *ft-1* did not. The additional leaves must reflect a true temporal delay in abaxial trichome production because none of these mutations was found to affect the rate of leaf initiation (data not shown). The extent of the delay varied considerably. *fd-1* delayed abaxial trichome production by one leaf, while *fpa-1* plants doubled the number of leaves without abaxial trichomes. Further differences between the mutations become apparent when their relative effects on trichome production and flowering time are compared. *fpa-1*, for example, had a smaller delay in flowering (as indicated by total rosette leaf number), than *gi-3*, *fca-1* and *fve-1* although it produced more leaves without abaxial trichomes than any of these mutants. The observation that abaxial trichome development is delayed in late flowering mutants supports the use of abaxial trichome production as a marker for vegetative phase change, and indicates that vegetative phase change and reproductive development have some regulatory factors in common. However, the lack of a general correlation between the delay in abaxial trichome production in different mutants and the extent to which flowering is delayed indicates that there are additional components unique to the regulation of each of these processes.

The conclusion that abaxial trichome production and flower induction are separable processes is supported by the phenotype of recessive mutations in the *TERMINAL FLOWER1* (*TFL1*) gene. *tfl1* mutations accelerate the transition within the inflorescence from the production of nodes with bracts and inflorescence branches to the production of nodes with single flowers, and also accelerate flowering (Shannon and Meeks-Wagner, 1991, 1993; Schultz and Haughn, 1993). To determine if *TFL1* controls the timing of vegetative phase change, we examined the effect of the *tfl1-10* mutation on abaxial trichome production. No significant difference was observed in the number of leaves without abaxial trichomes in *tfl1-10* and wild-type (Col) plants (Table 1, section e), or in their rate of leaf initiation (data not shown). A related result was reported by Weigel and Nilsson (1995), who assessed abaxial trichome development in transgenic plants overexpressing *LEAFY*, a gene that is negatively regulated by *TFL1* (Weigel et al., 1992). They found that these plants, which resemble *tfl1* mutants in many respects, produced abaxial trichomes at the same time as untransformed controls.

Table 2. The effect of late flowering mutations on rosette development

	Experiment 1		Experiment 2	
Genotype	Juvenile leaves*	Total rosette leaves*	Juvenile leaves*	Total rosette leaves*
Ler	2.7±0.3	7.1±0.4	3.4±0.4	6.9±0.2
ft-1	3.0±0.4†	9.3±0.7	4.0±0.4	12.7±0.7
co-2	3.4±0.5	10.8±0.6	4.0±0.0	13.6±0.5
fd-1	4.1±0.2	9.4±0.3	4.2±0.3	11.0±0.7
gi-3	4.5±1.0	15.8±0.7	4.9±0.3	20.8±2.5
fca-1	5.0±0.7	13.9±0.6	5.6±0.4	19.8±1.5
fve-1	5.1±0.4	13.2±0.5	5.1±0.5	13.0±2.0
fpa-1	7.3±0.7	12.1±0.6	6.0±0.6	11.9±0.4

*Each value is the average ± 2 × s.e.m. All values are significantly different from the control (P<0.05, Student's t-test) unless otherwise indicated.
†Not significantly different from Ler (P>0.05, Student's t-test).

The regulation of trichome production by gibberellins

Gibberellins (GA) have been found to regulate phase change in several species (Hackett, 1985; Zimmermann et al., 1985; Evans and Poethig, 1995). To examine the role of GA in abaxial trichome production, groups of Col plants were given regular applications of GA_3 beginning at different times in their development (Fig. 6). All plants responded by initiating abaxial trichomes precociously, with those receiving the earliest initial treatments producing the earliest trichomes. There was, however, a limit to this response in that plants never made abaxial trichomes earlier than leaf three, even when treated with GA_3 from the time of germination. This result indicates that GA promotes abaxial trichome production but suggests that leaves 1 and 2, unlike later rosette leaves, are incompetent to respond to this signal. This hypothesis is supported by the phenotype of *spindly* mutants, which undergo a constitutive GA response (Jacobsen and Olszewski, 1993). The *spy-3* allele caused abaxial trichomes to occur significantly earlier than wild type (Col) controls, but never earlier than leaf 3 (data not shown). In light of this effect, the phenotype of *spy-4* was striking. This mutation was isolated in Ws, an ecotype that normally makes abaxial trichomes under leaf 3. *spy-4* enhanced abaxial trichome production in that significantly more abaxial trichomes developed on leaf three in the mutants than in wild type (28.7±9.5 vs. 4.9±2.3 (P<0.001), but the mutation did not accelerate abaxial trichome production; all *spy-4* and Ws control plants formed abaxial trichomes on leaf three (Table 1, section f). Thus, both exogenously supplied GA_3 and the activated GA response conferred by *spy* mutations enhance abaxial trichome production on leaf 3 and later leaves, but neither has an effect on leaves 1 and 2.

Consistent with the stimulatory effect of GA on abaxial trichome production, we found that mutations that block different steps in the GA biosynthetic pathway (*ga1-3*, *ga4-1* and *ga5-1*) (Koornneef and van der Veen, 1980) and a mutation that confers insensitivity to GA (*gai*) (Koornneef et al., 1985) significantly delay abaxial trichome production (Table 1, sections g and h). In *ga1-3* mutants, which have the most extreme GA-deficient phenotype, abaxial trichomes did not develop on any of the rosette leaves or on bracts. We also found that the *ga1-3* mutation repressed adaxial trichome production (Fig. 7A). No adaxial trichomes formed on the first few rosette leaves of most mutant plants and although later rosette leaves developed adaxial trichomes in increasing numbers, the trichomes were restricted to the apical regions of the leaf blade. The normal distribution of both abaxial and adaxial trichomes was restored in *ga1-3* mutants by exogenous applications of GA_3, although a higher dose of this hormone was required to induce trichomes on the abaxial surface than on the adaxial surface. In plants treated with 10^{-7} M or higher concentrations of GA_3, the number of adaxial trichomes and the portion of the adaxial surface covered with trichomes increased at all positions (Fig. 7B). By contrast, abaxial trichomes only formed on *ga1-3* plants treated with at least 10^{-5} M GA_3. These results suggest that GA promotes trichome production on both surfaces of the leaf, but that the abaxial surface is less sensitive to this hormone than is the adaxial surface.

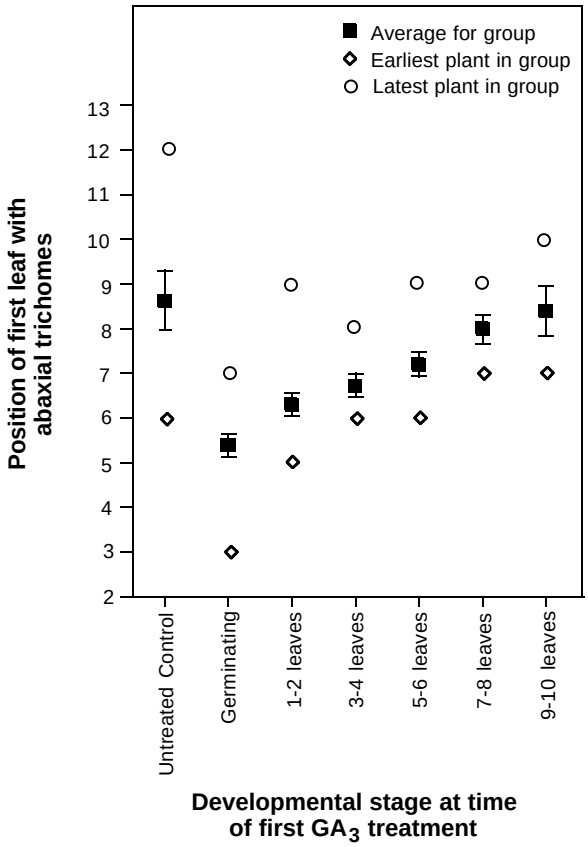

Fig. 6. Effects of GA_3 on abaxial trichome development in wild-type plants. The position of the first leaf with abaxial trichomes was assayed in groups of 12-50 plants treated with 10^{-5} M GA_3 beginning at different stages of development. The circles and diamonds indicate the range of this leaf position in each group. Error bars on the average values are 2× s.e.m.

Adaxial trichome loss

In addition to the changes in trichome distribution that occur during vegetative development, characteristic changes also occur

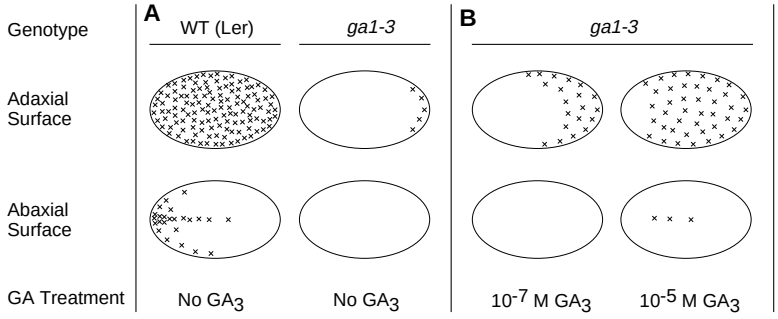

Fig. 7. Comparison of trichome development on the abaxial and adaxial surfaces of leaf 5 in wild-type Ler and *ga1-3* mutant plants and the response of *ga1-3* plants to GA_3 treatment. (A) Untreated plants. (B) Plants treated with GA_3. Each diagram shows the average number of trichomes observed on each surface (n = 5-10). The portion of each surface covered in trichomes is representative of genotype and treatment group. Note that average leaf size was not the same for each group and thus differences in trichome densities should not be inferred. The apical end of each leaf is to the right in this diagram.

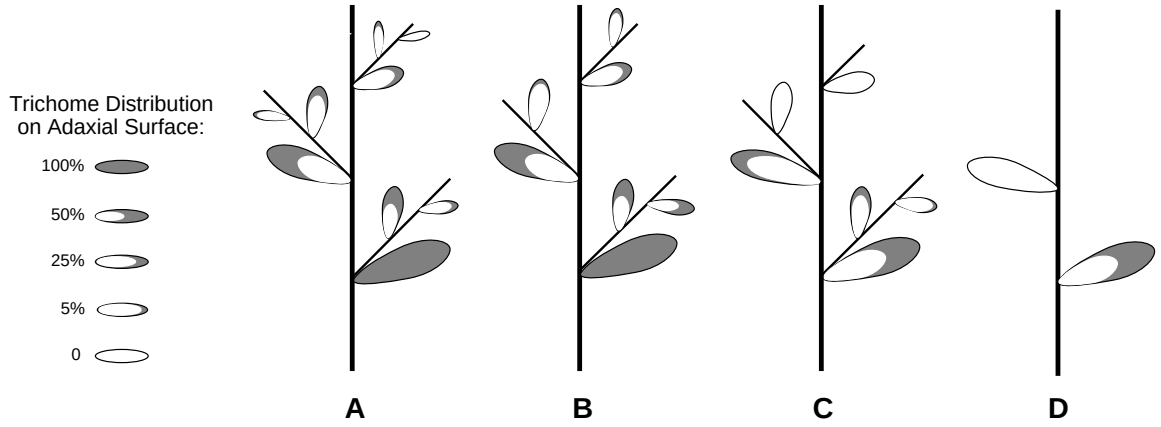

Fig. 8. Schematic diagram comparing adaxial trichome distributions on the bracts of wild-type Col, Ler and Ws plants and *tfl1-10* mutants. (A) Col, (B) Ler, (C) Ws, (D) *tfl1-10* (Col background). Vertical lines represent the primary inflorescence stems and diagonal lines represent coflorescence stems. Inflorescences and coflorescences from approximately ten plants of each strain were examined. The trichome distribution shown for each bract is representative of the bracts found at that position in each group of plants.

during the reproductive phase of shoot development. In *Arabidopsis*, modified leaves (bracts) are formed at the basal nodes of the primary inflorescence (primary bracts) and at the basal nodes of each inflorescence branch, or coflorescence (coflorescence bracts). The structure of a typical coflorescence is shown in Fig. 8A. All bracts produce abaxial trichomes, but bracts located at apical nodes of the inflorescence usually lack trichomes on all or part of their adaxial surface. Adaxial trichomes disappear first from the basal and medial region of the bract, and this region expands towards the tip of the bract on bracts located at successively higher nodes. This pattern of trichome loss also occurs within each coflorescence. However, coflorescences differ from each other in that the bracts on basal coflorescences have more adaxial trichomes than bracts at identical positions on apical coflorescences. Thus, the character of coflorescence bracts is correlated with the position of the coflorescence within the inflorescence. The Ws, Col and Ler ecotypes differ in the rate at which adaxial trichomes are lost from successive bracts (Fig. 8A-C), with Ws losing adaxial trichomes more rapidly than Col and Ler.

The loss of adaxial trichomes appears to be tied to the process of inflorescence development. We have never observed this phenomenon on the rosette leaves of wild-type plants under any growth conditions. Because other morphological changes that occur during inflorescence development are accelerated by mutations in *TFL1*, we examined *tfl1-10* mutants to determine if this mutation also accelerates adaxial trichome loss. While the first bract of wild-type Col plants was completely covered with adaxial trichomes, the first bract in about 70% of *tfl1-10* plants was at least partially devoid of adaxial trichomes (Fig. 8D). None of the second bracts in mutant plants had adaxial trichomes, compared with the 50% coverage normally seen on the second bract of control plants. The observation that *tfl1-10* accelerates both flower production and loss of adaxial trichomes from bracts supports the hypothesis that this latter event is triggered by floral induction.

DISCUSSION

The regulation of trichome differentiation in *Arabidopsis thaliana* has been the subject of intensive genetic analysis (Marks et al., 1981; Hülskamp et al., 1994; Larkin et al., 1996). Less is known about the basis for developmental variation in leaf trichome production (Telfer and Poethig, 1994; Chien and Sussex, 1996). Leaves produced early in rosette development do not have trichomes on their abaxial surface, whereas leaves produced later possess trichomes on both their adaxial and abaxial surfaces. Leaves that develop after the plant has been induced to flower (bracts) possess abaxial trichomes, but frequently lack trichomes on all or part of their adaxial surface. We believe that abaxial trichome production reflects global changes in the character of the shoot related to shoot maturation, and can thus serve as a marker of the adult phase in studies of the regulation of vegetative phase change in *Arabidopsis*. This conclusion is based on the following observations. In many plants, the types and the distribution of epidermal hairs vary according to the developmental phase of the shoot and are correlated with the appearance of many other less obvious phase-specific traits (Schaffalitzky de Muckadell, 1954; Wareing and Frydman, 1976; Brand and Lineberger, 1992; Lawson and Poethig, 1995). Furthermore, as we have shown here, abaxial trichome production in *Arabidopsis* is influenced by factors known to affect phase change in other species. These include genes involved in floral initiation, and GA. We suggest that adaxial trichome loss from bracts occurs as a consequence of floral induction and reflects the suppression of vegetative development in the inflorescence.

Changes in trichome distribution may be temporally regulated

Developmental variation in vegetative traits have been proposed to occur in response to a variety of developmental cues. These include the attainment of a critical overall size, the production of a set number of leaves, and the age of the shoot. The morphology of vegetative structures might also be determined by positional information within the meristem, as has been proposed for the determination of floral organ identity. By analogy to the positional information model of flower morphogenesis (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994), the vegetative shoot meristem could be divided into discrete domains by the overlapping expression of regulatory genes, with the character of a leaf being determined by the domain in which it arose.

To investigate these possibilities, we asked if mutations that affect the rate of shoot growth also affect the number of leaves that lack abaxial trichomes. We found that *psd*, a mutation that delays leaf production, results in the production of fewer leaves lacking abaxial trichomes than wild type. Conversely, mutations of *amp1*, which accelerate leaf production, cause an increase in the number of leaves without abaxial trichomes. In spite of their abnormal rate of leaf initiation, both *psd* and *amp1* mutants begin producing leaves with abaxial trichomes at approximately the same time as wild-type plants. If these mutations can be assumed to have no direct effect on trichome production, this result suggests that the production of abaxial trichomes does not depend on the prior production of a specific number of leaves or the attainment of a specific overall shoot size. It is also inconsistent with models in which the character of a leaf is influenced by its distance from the root since the production of abaxial trichomes occurs at a lower node in the case of *psd*, and at a higher node in the case of *amp1*. The phenotype of *psd* also fails to support the predictions of the positional information model. If the trichome pattern on a leaf were specified by where it arose within the shoot apical meristem, *psd* would not be expected to affect trichome production on the first leaves because they appear to arise at normal positions on the basal perimeter of the meristem. The phenotypes of *psd* and *amp1* are consistent with the temporal regulation of abaxial trichome production driven, perhaps, by temporal changes in factors produced by the shoot apical meristem or a specific organ (e.g. cotyledons or the root system).

A model for the regulation of trichome development

One way to account for the distribution of trichomes on rosette leaves is to postulate that there is a global inductive signal for trichome production, the level of which increases during the development of the rosette, and that the abaxial epidermis is less sensitive to this signal than the adaxial epidermis. Early in shoot development the level of this factor is sufficient to induce trichome production on the adaxial surface of the leaf, but not on the abaxial surface. Production of abaxial trichomes begins in developing leaves when the level of the inducer increases above the threshold sensitivity of the abaxial epidermis. This model implies that all rosette leaf primordia have the same sensitivity to the inductive signal but differ in their expression of abaxial trichomes according to the level of the inductive signal in the plant. An alternative is that leaves initiated at different times in development have different capacities to respond to the signal. These two models are not mutually exclusive, and other explanations are possible. Fig. 9 illustrates a case in which the pattern of abaxial trichome development in the rosette depends on both the changing level of an inductive signal and on differences in the competence of the first two and later rosette leaves to respond to that signal.

Our results, and those of Chien and Sussex (1996) suggest that the 'trichome inducer' may be GA and that the abaxial and abaxial surfaces do indeed differ in their sensitivity to this hormone. *ga1-3* mutants, which contain a null mutation in the enzyme that controls the first committed step in GA-biosynthesis (Sun et al., 1992; Zeevaart and Talón, 1992), produce no abaxial trichomes, and have few or no adaxial trichomes on rosette leaves. This phenotype is corrected by exogenous applications of GA_3, although a higher concentration of this hormone is required to restore trichomes to the abaxial surface of a given leaf than to the adaxial surface.

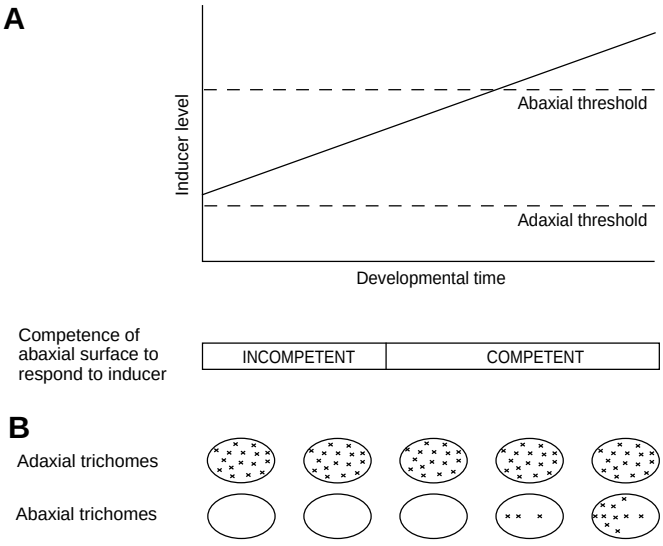

Fig. 9. A model of phase change regulation in *Arabidopsis*. (A) The level of a trichome inducer increases in the plant with time, and must exceed certain threshold levels before the abaxial and adaxial surfaces of developing leaves can respond and produce trichomes. The competence of the abaxial surfaces of leaves to respond to this inducer also changes. The first two leaves are not competent to respond to inducer levels above the threshold, but later leaves are. (B) The pattern of trichome development on rosette leaves predicted by the parameters shown in A.

Our observation that leaves one and two do not produce abaxial trichomes in response to exogenously applied GA_3 or to mutations in *SPINDLY* suggests that abaxial trichome production on these leaves is regulated in a qualitatively different way than it is on later leaves. This could arise if changes in the developmental state of the shoot apex cause the first two leaf primordia to be inherently different from later leaf primordia in their developmental potential (i.e. have different identities). Cells in the abaxial epidermis of the first two leaf primordia might, for example, lack a critical component of the GA signal transduction pathway. It is also possible that all leaf primordia have the same developmental potential, but that the global levels of other developmentally regulated stimuli or inhibitors within the plant influence their sensitivity to GA.

The effects of GA on trichome production in *Arabidopsis* are in agreement with the effect of GA on phase-specific traits in other herbaceous species. In maize, for example, GA-deficient mutations delay the appearance of adult vegetative traits, including epidermal hairs on the leaf blade, and prolong the expression of many juvenile traits; exogenous applications of GA_3 have the opposite effect on these traits (Evans and Poethig, 1995). GA is also regarded as an important regulator of phase change in woody species (Zimmerman et al., 1985; Pharis, 1990). Thus, while the model described here is couched in terms of trichome development, it may be applicable to other phase-specific traits as well.

The relationship between trichome production and reproductive development

Short day conditions delay flowering in *Arabidopsis* and also delay the production of leaves with abaxial trichomes. This

result is in agreement with that of Chien and Sussex (1996), and is consistent with previous observation that other developmental changes in leaf morphology are delayed in SD (Martínez-Zapater et al., 1995). Most of the late flowering mutations that we examined also delay the production of abaxial trichomes. These results imply that vegetative phase change and reproductive development are coordinated in *Arabidopsis*, as they appear to be in many other plants (Zimmerman et al., 1985; Hackett, 1985).

Based on physiological experiments and double mutant analyses, the late flowering genes FCA, FVE and FPA have been proposed to promote flowering by increasing the level of, or response to, GA (reviewed in Martínez-Zapater et al., 1994). Given that GA promotes abaxial trichome production, it is not surprising that mutations in these genes delay abaxial trichome production as well as flowering. The lack of correlation in these mutants between the extent of delays in flowering and abaxial trichome production could reflect the involvement of different GA species in these two processes. The effects of SD conditions on abaxial trichome production may also be mediated by GA, as photoperiodic conditions have been shown to affect GA biosynthesis and/or the sensitivity to this hormone in a number of species (Zeevaart, 1983; Gilmour et al., 1986; Pharis et al., 1987). The other late flowering mutations that we examined (*ft-1*, *co-2*, *fd-1*, and *gi-3*) are in genes proposed to function independently of GA (reviewed by Martínez-Zapater et al., 1994). The observation that three of these mutations delay abaxial trichome production is significant because it implies that GA is not the only factor common to the regulation of vegetative phase change and flowering.

The mechanism by which flowering and abaxial trichome production are linked remains unclear. Coordination of flowering and abaxial trichome production would occur if (a) the two processes were independent but were regulated by common factors, (b) the factors responsible for abaxial trichome production either induced reproductive maturation or were a prerequisite for it, or (c) floral induction or the development of reproductive competence induced abaxial trichome production. The identification of mutants that affect both of these processes provides an entrée into the genetic analysis of this problem. We anticipate that the analysis of these and other flowering time mutants, in combination with mutants isolated on the basis of their effect on trichome distribution, will allow the nature of the coordination to be determined.

Floral initiation affects trichome development on bracts

Bracts can be distinguished from rosette leaves not only by their position on the inflorescence stem, but also by their relatively small size, lack of petiole and by the reduced cover of trichomes on their adaxial surface. The phenotypes of wild type and *tfl1-10* mutants indicate that the disappearance of adaxial trichomes in the inflorescence is likely to occur as a result of floral induction. Indeed, adaxial trichome loss might serve as a useful marker of floral induction under conditions in which flowers do not develop, or when the inflorescence develops abnormally.

Hempel and Feldman (1994) showed that primary bracts develop from leaf primordia present on the meristem at the time of floral induction and proposed that variation in the character of bracts within the inflorescence could reflect the differences in the age of these primoridia at the time of floral induction. The pattern of adaxial trichome loss from bracts is consistent with this model. Leaf tissues mature in a apical-basal gradient (reviewed by Telfer and Poethig, 1994), with differentiated structures such as trichomes appearing first in apical regions of developing leaf primordia. Thus, differences in the maturation state of tissues within a leaf could lead to the production of bracts in which adaxial trichomes are suppressed to varying extents. The region devoid of trichomes would be expected to be more extensive on apical bracts than on basal bracts because the primordia of apical bracts would have been younger, and therefore less determined, at the time of floral induction.

The observation that coflorescence bracts can have more adaxial trichomes than the most apical bract on the main stem is unexpected because coflorescence bracts are initiated later than the primary bracts (Hempel and Feldman, 1994). If axillary buds become florally induced at the same time and to the same extent as the primary meristem, then coflorescence bracts should always have fewer adaxial trichomes than primary bracts. The observation that this is not the case supports the argument that axillary meristems present at the shoot apex at the time of floral induction become induced after the primary meristem, and perhaps independently of this event (Hempel and Feldman, 1994).

Prospects for the genetic analysis of phase change using trichome distribution as a phase marker

Our studies have shown that trichome distribution is regulated at a number of different levels. Because of this complexity, the simple division of rosette development into juvenile and adult phases based on abaxial trichome production may not accurately reflect the full range of developmental programs expressed during vegetative development. By this single criterion, wild-type Col plants grown in CL, for example, produce 4 or 5 juvenile leaves. However, we have shown that the first two leaves can be distinguished from other juvenile leaves in that they cannot be induced to produce abaxial trichomes by any of the experimental or environmental treatments described in this paper. This distinction can be interpreted to mean that the juvenile phase is composed of two subphases: an early juvenile phase during which abaxial trichomes cannot be induced, and a late juvenile phase during which leaves are competent to produce abaxial trichomes if exposed to the correct stimuli. This is consistent with the observations that in maize the first juvenile leaves are morphologically and anatomically different from later juvenile leaves (Bongard-Pierce et al., 1996) and that *gl15*, a maize mutation that accelerates the appearance of adult vegetative traits, does not affect these first juvenile leaves (Evans et al., 1994; Moose and Sisco, 1994). It remains to be determined if variation in the production of abaxial trichomes on competent *Arabidopsis* leaves (leaves 3 and above) is dictated solely by the level of an inducer such as GA, or if other, possibly inherent, differences distinguish late juvenile leaves from adult leaves.

We recognize that mutations affecting trichome development can alter trichome distribution in ways that are not phase-specific, complicating the use of trichome distribution as a phase marker in mutant genetic screens. However, we have been able to identify genes that affect other key phase-specific traits (e.g. leaf shape, floral initiation) in screens for mutations

that accelerate or delay abaxial trichome production (A. Telfer, K. M. Bollman and R. S. Poethig, unpublished). Genes that influence the timing of adaxial trichome loss have also been identified. The information presented in this paper provides a framework for interpreting the role of these and other genes in vegetative phase change and inflorescence development.

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