Transgenic study of parallelism in plant morphological evolution

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Developmental constraint is indicated when one finds that similar genetic mechanisms are responsible for independent origins of the same derived phenotype. We studied three independent origins of rosette flowering within the mustard family and attempted to evaluate the extent to which the same mechanisms were involved in each transition from the ancestral phenotype, inflorescence flowering. We used transformation to move a candidate gene, *LFY*, and its cis-regulatory sequences from rosette-flowering species into an inflorescence-flowering recipient, *Arabidopsis thaliana*, in place of its endogenous *LFY* gene. The transgenic phenotypes of experimental and control lines (containing an *A. thaliana LFY* transgene) and the expression driven by the cis-regulatory sequences show that changes at the *LFY* locus might have contributed to the evolution of rosette flowering in two of the three lineages. In the third case, changes upstream of *LFY* are implicated. Our data suggest that changes in a single developmental regulatory program were involved in multiple origins of the same derived trait but that the specific genetic changes were different in each case.

Parallelism refers to the independent evolution of the same derived trait via the same developmental changes, whereas convergence refers to superficially similar traits that have a distinct developmental basis (1). The most compelling reason to distinguish these two phenomena is because they serve to document different phenomena. Convergence provides evidence of the efficacy of natural selection, whereas the occurrence of parallelism shows that the path of evolution is constrained to certain channels determined by the structure of developmental programs (2, 3).

Strict parallelism, where the identical mutation occurs repeatedly, is documented in cases of biochemical adaptation to toxins (4–6). When dealing with morphology, however, the concept of parallelism is usually relaxed to include cases of different mutations to the same target locus and even changes to different genes within the same developmental pathway. Under this broader definition, parallelism can be elucidated by using comparative gene expression data (7–10). In such studies, however, there is always the concern that changes in gene expression might be far downstream of the ultimate genetic causes of morphological homoplasy. A few studies have used classical genetic data to study homoplasy (11–14), and some studies have provided strong evidence in favor of parallelism (13, 14). Here we use a transgenic approach that is not limited to cases in which study species are crossable (15). Although interspecies transformation has been used to elucidate male song evolution in *Drosophila* (16) and the origin of self-compatibility in *Arabidopsis thaliana* (17), it has not to our knowledge been applied to the problem of parallel evolution.

Most species of Brassicaceae, including *A. thaliana*, bear flowers in an inflorescence, an elongated portion of stem on which the leaves that would otherwise subtend the flowers are suppressed (Fig. 1 *a* and *b*). Phylogenies of Brassicaceae (18–20) suggest that inflorescence flowering is ancestral but that a number of independently derived lineages have evolved a striking modification to plant architecture, rosette flowering. Rosette-flowering plants produce solitary flowers in the axils of rosette leaves, and flowers are elevated on elongated pedicels rather than by elongation of internodes in the primary shoot axis (Fig. 1 *a* and *c–e*). We investigated three rosette-flowering species: *Ionopsidium* (*Jonopsidium*) *acaule*, *Idahoa scapigera*, and *Leavenworthia crassa*. *Ionopsidium* includes six Mediterranean species, of which two are rosette-flowering. *Idahoa* contains only one species of diminutive annual from the northwestern United States. *Leavenworthia* includes eight rosette-flowering, winter annual species from the southeastern United States.

The difference between rosette and inflorescence flowering can be attributed at least in part to differences in the fate of axillary meristems in the rosette, which take on a floral identity in rosette-flowering taxa but a shoot identity in inflorescence-flowering taxa. We therefore focused this study on a candidate locus, *LEAFY/FLORICAULA* (*LFY*), that plays a role in the regulation of shoot meristem identity and plant architecture in several model species and has been suggested to be a key player in the evolution of inflorescence architecture (21, 22). *lfy* mutants show proliferation of inflorescence meristems and the formation of shoot–flower intermediates in place of flowers (23). Ectopic expression of *LFY* in *A. thaliana* results in the formation of some rosette flowers (22). Furthermore, prior work on the rosette-flowering *Ionopsidium acaule* showed that *LFY* is expressed in the shoot apical meristem (SAM) (24). This contrasts with *A. thaliana* and other plants with racemose inflorescences, which show *LFY* expression in flowers and young leaves but never in the inflorescence meristem (23, 25).

LFY is orthologous to the *Antirrhinum* gene *FLO* (25) and encodes a DNA-binding transcription factor that promotes floral meristem identity by activating floral organ identity genes (23, 26). Vegetative SAMs of *A. thaliana* are prevented from taking on a floral identity by the activity of *TFL1*, which indirectly represses *LFY* (27). Because *LFY* also represses *TFL1*, the balance of expression of these two genes is thought to play a key role in determining the floral versus vegetative fate of SAMs (21, 22).

In this study we cloned *LFY* homologs with intact cis-regulatory regions from the three rosette-flowering species and introduced them into *lfy* mutant *A. thaliana* plants. Comparison of the resulting transgenic lines with control lines containing the *A. thaliana LFY* gene plus its cis-regulatory sequences was made to see whether the exogenous *LFY* genes can rescue *lfy* mutants and, if so, whether the transgenic plants show features normally associated with rosette flowering.

Materials and Methods

Plant Materials. *Ionopsidium acaule* lines were described previously (24). Seeds of *Idahoa scapigera* were collected by D.A.B. in
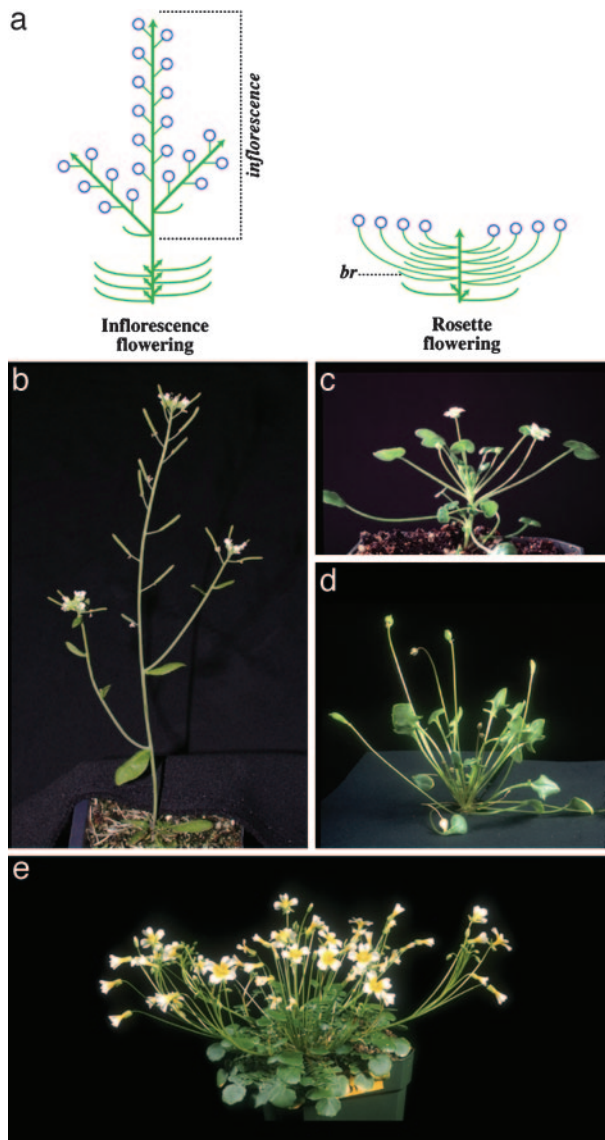

Fig. 1. Comparison of the plant architecture of inflorescence- and rosette-flowering species. (*a*) Diagram of inflorescence- and rosette-flowering architectures. Leaves (curved lines), shoot meristems (arrows), and flowers (circles) are shown. Bracts (br) are leaves that directly subtend flowers. (*b–e*) Photographs of inflorescence-flowering *A. thaliana* (ecotype Landsberg *erecta*) (*b*) and the three rosette-flowering members of Brassicaceae studied here, *Ionopsidium acaule* (*c*), *Idahoa scapigera* (*d*), and *L. crassa* (*e*).

Pullman, WA (voucher, Baum 365; Gray Herbarium, Cambridge, MA). Seeds of *L. crassa* were obtained from a cultivated source (voucher, Baum 379; Wisconsin State Herbarium, Madison). Wild-type and *lfy-6 A. thaliana* seeds were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus).

Cloning, Transformation, and Phenotype Scoring. We amplified partial *LFY* homologs from genomic DNA using a series of degenerate primers situated in highly conserved regions of the gene. This strategy has been used successfully to isolate *LFY* homologs from a diversity of other Brassicaceae (H.-S.Y., R. Oldham, and D.A.B., unpublished data), suggesting that this approach should allow one to identify all functional *LFY* paralogs. Amplified products were cloned and sequenced, and the data were used to design primers for genome walking (Clon-

tech), allowing us to obtain flanking genomic sequences both 5′ and 3′ of the coding region. Based on this extended region of sequence, primers were designed to amplify an ≈7-kb DNA fragment of each gene containing ≈3 kb upstream of the *LFY* ORF and ≈1 kb downstream. These fragments likely contain a majority of the important cis-regulatory elements because 2.3 kb of 5′ noncoding DNA has been reported to contain all elements necessary for proper expression of *LFY* in *A. thaliana* (28). Using high-fidelity PCR (PCR using *Pfu* DNA polymerase, Stratagene) and UV-free gel extraction (Invitrogen), we cloned *AthLFY*, *IacLFY* (GenBank accession no. AY219226), *IscLFY1* (Gen-Bank accession no. AY219228), and *LcrLFY* (GenBank accession no. AY219227) in the pCR-Blunt II-TOPO vector (Invitrogen). Six independent *LFY* clones from each rosette-flowering species (three in each orientation) and four clones from *A. thaliana* (two in each orientation) were then moved into the binary vector pCAMBIA3300, which includes the *BAR* selectable marker conferring Basta resistance.

A. thaliana Landsberg *erecta* heterozygous *lfy-6*/*LFY* plants were transformed with the 22 clones by using the floral dip method (29). An empty vector pCAMBIA3300 was used as an additional control. T1 seeds were selected by spraying 0.2 mg/liter Basta. Resistant plants were genotyped at the endogenous *LFY* locus by using cleaved amplified polymorphic sequences (CAPS) markers developed by D. Weigel (www.salk.edu/LABS/pbio-w/CAPS.html). The primers used did not amplify the exogenous genes (*IacLFY*, *IscLFY1*, or *LcrLFY*). To genotype the endogenous *LFY* locus for *AthLFY* transgenic lines, we developed a new primer set [forward, 5′-GGT TCC TCC CTA AAA ACT CTT CAA AAT CCC-3′, and reverse, 5′-GTC CCT CTA AAC CAC CAA GTC GCA TCC C-3′). The forward primer is situated outside the cloned region, thus ensuring that only the endogenous *LFY* is amplified, allowing CAPS analysis to be conducted by means of a second PCR. Wild-type and *lfy-6* plants were distinguished by using the restriction enzyme *Bst*API, which generates 345- and 25-bp fragments for the wild type and a single 370-bp product for *lfy-6*. We selected resistant T1 plants that were determined to be *lfy-6*/*lfy-6* at the endogenous locus and used them for analysis in the T2 generation. We characterized T2 populations in long-day conditions (16 h light/8 h dark). The segregation ratio of Basta-sensitive/Basta-resistant in T2 and T3 plants was used to estimate the number of transgene loci (in most cases, one or two).

GUS Fusion Constructs and Staining. The 5′ fragment of the genes was amplified by high-fidelity PCR from genomic DNA using the same forward primers used to clone the 7-kb *LFY* fragments and reverse primers that anneal to the middle of the first exon of each *LFY* homolog. The resulting 3.3-kb fragments were cloned into pCR-Blunt II-TOPO vector (Invitrogen) and subsequently moved to pBI101 vector (Clontech) in frame. Wild-type *A. thaliana* plants were transformed by the floral dip method, and transformants were selected on agar plates in Murashige and Skoog medium (ICN) containing 50 μg/ml kanamycin. *GUS* expression was determined in whole-mount shoot apices. Tissue was incubated in 0.5 mg/ml X-Gluc staining solution (50 mM potassium phosphate buffer, pH 7.2/0.1% Triton X-100/0.1% 2-mercaptoethanol/4 mM potassium ferricyanide/4 mM potassium ferrocyanide), first on ice under vacuum for 15 min, then at 37°C for 12 h. Then tissues were transferred and incubated in 70% (vol/vol) ethanol at 4°C until the chlorophyll was completely extracted. Stained whole-mount specimens were examined and photographed with an Olympus SZX12 dissecting microscope.

Analysis of Cis-Regulatory and Intron Sequences. Alignments of the cis-regulatory regions were conducted manually in MACCLADE VERSION 4.05 (30) with the help of the motif-searching algorithms
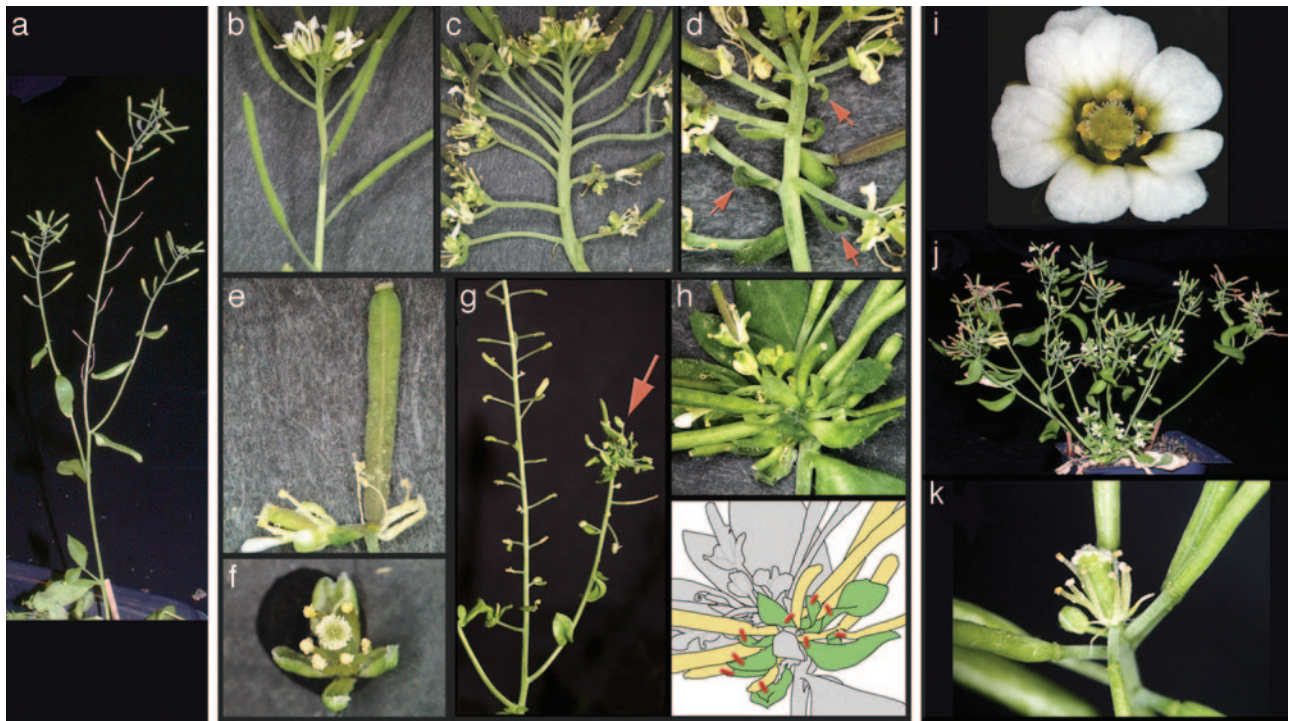
Fig. 2. Phenotypes of *A. thaliana lfy* mutants containing *LFY* transgenes. (*a*) *IacLFY* replaces endogenous *AthLFY* function, resulting in a wild-type architecture. (*b–d*) Compared with inflorescences from control *AthLFY* transformants (*b*), inflorescence elongation was inhibited in *IscLFY1* transformants (*c*), and bracts (arrows) are often formed (*d*). (*e* and *f*) *IscLFY1* lines showed modified floral architecture demonstrating an *apetal1*-like phenotype (*e*) and a reduction in petal number to an average of 1.38 ± 0.50, often showing complete apetaly (*f*). (*g*) Aerial rosettes are produced on some secondary inflorescence shoots of *IscLFY1* transgenic plants (arrow). (*h*) The structure of one dissected rosette (*Upper*) is diagrammed (*Lower*) to show that each fruit/flower (yellow) has a subtending bract (green) indicated by a linking red line. (*i*) *LcrLFY* rescued the *lfy* mutation but caused flowers with 6.65 ± 1.27 petals rather than the typical four petals (sepal and stamen number is unchanged). (*j*) *LcrLFY* modifies inflorescence architecture in *A. thaliana*. (*k*) All of the inflorescences are prematurely terminated by partial, terminal flowers, resulting in the release of determinate axillary shoots from the rosette.

implemented in MEME (http://meme.sdsc.edu/meme/website/meme.html). For *AthLFY* and *LcrLFY*, a pairwise alignment was generated that spanned the entire intergenic region 5′ of *LFY*. For *IscLFY1* and *IacLFY*, it was only possible to align the ≈350 bp 5′ of the *LFY* start codon. Possible regulatory elements were detected by using the PLACE sequence scan web server (ref. 31; www.dna.affrc.go.jp/htdocs/PLACE) or manually in MACCLADE VERSION 4.05.

Results

LFY from *Ionopsidium acaule* Replaces *A. thaliana* LFY Function. One *LFY* ortholog, here named *IacLFY*, was present in *Ionopsidium acaule* [the two sequences reported previously, under the gene name *vcLFY* (24), seem to be alleles (data not shown)]. For five of the six *IacLFY* constructs, T1 transgenic plants showed rescue of the *lfy* mutant phenotype, as indicated by the production of flowers with petals and stamens in *lfy-6* homozygous plants. No systematic differences were seen between these constructs. The sixth line, however, never rescued the *lfy-6* phenotype, most likely because of a mutation introduced into the transgene during cloning. We focused on eight *A. thaliana lfy-6* lines with high fertility, representing all five functional constructs. These lines showed some floral defects (data not shown), reminiscent of weak *ap2* mutations (32), but showed a wild-type *A. thaliana* plant architecture (Fig. 2*a*), similar to control lines in which *lfy-6* was rescued with the *AthLFY* transgene (data not shown). This result suggests that the *IacLFY* locus did not change significantly during the evolution of rosette flowering. Consistent with this result, the 5′ regulatory sequences of *IacLFY* drove reporter gene expression in a pattern (Fig. 3*a*) similar to that reported for *A. thaliana* (28) but quite different from the native expression of

IacLFY, which includes the SAM (24). These data imply that normal expression of *IacLFY* in *Ionopsidium acaule* requires trans-acting regulatory elements. Thus, under the hypothesis that altered expression of *IacLFY* contributes to the development of rosette flowering (24), this effect must be mediated by genetic changes that lie upstream of *LFY*.

LFY from *Idahoa scapigera* Produced Aspects of Rosette Flowering in *A. thaliana*. There are two *LFY* genes in *Idahoa scapigera* (*IscLFY1* and *-2*), but only *IscLFY1* was studied here. We examined 16 independent *A. thaliana* lines derived from all six *IscLFY1* constructs. T1–T3 plants homozygous for the *lfy-6* allele but containing *IscLFY1* transgenes produced stamens, indicating at least partial rescue (Fig. 2*f*). However, the *IscLFY1* lines (unlike the control *AthLFY* lines) showed reduced petal number or complete apetaly, and most lines produced flowers resembling *apetala1* (*ap1*) mutants (33) (Fig. 2*e*). One interpretation is that rescue of the *lfy-6* mutation was incomplete in the outer floral whorls, resulting in the maintenance of inflorescence identity in this region. This phenomenon likely results from a failure of the *IscLFY1* upstream sequences to drive expression in the perianth: the *IscLFY1* 5′ regulatory sequences drive reporter gene expression only in the stamen and carpel whorl (Fig. 3*b*). It will therefore be interesting to determine whether the promoter region of the second *Idahoa LFY* gene, *IscLFY2*, drives a complementary expression pattern, with strong activity in the perianth.

Fourteen *IscLFY1* lines produced some plants with altered inflorescence architecture: shoots had noticeably shorter internodes (compare Fig. 2 *b* and *c*), and in many cases leaves subtended these flowers (Fig. 1*a* and Fig. 2*d*). Furthermore, an
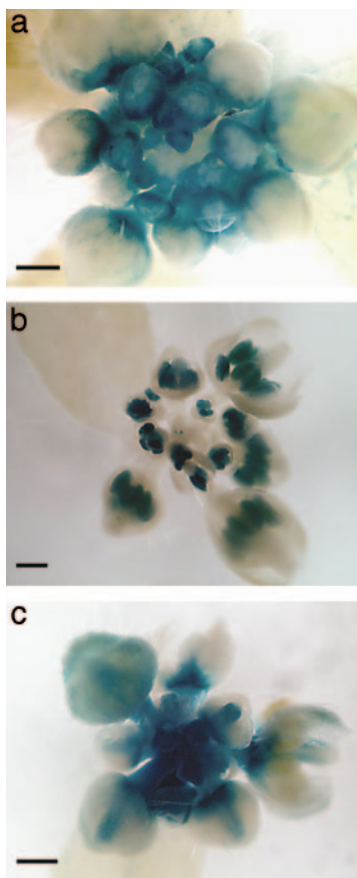
Fig. 3. Expression of *LFY*:*GUS* transgenes in wild-type *A. thaliana* plants. Inflorescence apices are shown. (*a*) *IacLFY*::*GUS* activity is detected in flower primordia and maintained mainly in sepals until approximately stage 7. GUS activity is limited to the bases of the sepals in later stages of the development. Note the absence of *IacLFY*::*GUS* expression in the SAM. (*b*) *IscLFY1*::*GUS* activity is visible in the carpel and stamen primordia until stage 9 and persists mostly in stamens at later stages of floral development. *IscLFY1*::*GUS* expression is absent from the SAM. (*c*) *LcrLFY*::*GUS* is strongly expressed in the SAM and throughout floral primordia. Expression gradually declines from stage 5 flowers but is maintained in carpels and petals. Stages of flower development are according to Smyth *et al.* (40). (Scale bars = 0.5 mm.)

average of 1.52 shoots per plant produced an extreme phenotype in which internode elongation was inhibited to such a degree that an aerial flowering rosette was formed (Fig. 2*g*). Superficially similar aerial rosettes have been reported in natural and mutant *A. thaliana* plants (34, 35). The latter structures comprise lateral inflorescences that make a rosette of vegetative leaves without flowers and, when a reproductive transition occurs, produce normal, elongated inflorescences with ebracteate flowers. In contrast, the aerial flowering rosettes of *IscLFY1* lines are in a reproductive state, being composed of bracteate (leaf-subtended) flowers (Fig. 2*h*). Thus, whereas the aerial rosettes reported previously are architecturally equivalent to inflorescence-flowering plants, the aerial flowering rosettes we observed resemble entire rosette-flowering plants. These observations suggest that a change at the *IscLFY1* locus might have contributed to the evolution of rosette flowering by suppressing internode elongation and derepressing bracts. The fact that *IscLFY1* converts only secondary shoots to rosette flowering rather than the whole plant implies that additional interacting genetic factors (possibly *IscLFY2*) also changed during the evolution of rosette flowering.

***LFY* from *L. crassa* also Altered *A. thaliana* Architecture.** *L. crassa* has a single *LFY* gene (*LcrLFY*). One of the constructs seemed to

have acquired a null mutation in the *LcrLFY* region during the PCR or the subsequent cloning process, because the Basta-resistant lines all showed a *lfy-6* phenotype. The remaining five constructs showed an ability to rescue the *lfy-6* mutation; however, because of mortality in these lines (probably unrelated to the transgenes), we were only able to establish six independent transgenic lines (representing four constructs). These lines showed good rescue of the floral defects of the *lfy-6* mutation, but three lines from two constructs produced flowers with additional petals (Fig. 2*i*). Two lines (from two constructs) showed a wild-type architecture, one line produced slow-growing and small plants with a wild-type architecture, and the other three lines (from two constructs) manifested a highly modified plant form. These plants produced partial terminal flowers (Fig. 2*k*) on the main axis after 6–9 lateral flowers had been produced. Secondary inflorescence branches also had terminal flowers, but the number of flowers produced before termination gradually increased during development (Fig. 2*j*). This phenotype somewhat resembles rosette flowering in that the axillary shoots in the rosette produce determinate rather than indeterminate axes (see *Discussion*).

The production of terminal flowers in *LcrLFY* lines resembles mutations in *TFL1*, a gene that acts to down-regulate *LFY* transcription in SAMs (21, 36, 37). Because the *LcrLFY* upstream sequences drive expression both in flower primordia and in the SAM (Fig. 3*c*), the observed phenotype could be due to a loss of *TFL1*-responsive cis-regulatory elements. Analysis of the upstream sequences revealed several potential regulatory elements that are missing in *LcrLFY* and should be considered as possible targets of *TFL1*-mediated repression in the SAM (Fig. 5, which is published as supporting information on the PNAS web site). For example, *LcrLFY* lacks the C box bZIP binding site that falls within the "distal fragment" of *AthLFY* that is required for normal expression (28).

Discussion

Introduction of *LFY* from the three rosette-flowering lineages into an *A. thaliana lfy* genetic background resulted in the rescue of *lfy* mutant phenotypes, at least with regard to stamen development and the production of intact flowers early in plant development (*lfy* mutants do not make flowers until late in development, and these lack both petals and stamens). This result implies a high degree of functional conservation in the *LFY* gene product, as suggested previously (e.g., ref. 22). With *IscLFY1* and *LcrLFY*, however, several transgenic lines manifested an architecture that is distinct from weak *lfy* mutant phenotypes. These results cannot be caused by changes in gene copy number because they were not observed in control lines containing the *AthLFY* transgene introduced by the same protocol, and they do not resemble the effects of adding extra copies of *LFY*, which primarily involve changes in flowering time (38). The altered shoot morphology can therefore be attributed to changes at the *LFY* locus that have evolved since the divergence of *A. thaliana* and *Idahoa/Leavenworthia*. Although these changes could have occurred on any phylogenetic branch between the rosette-flowering species and *A. thaliana*, we would suggest that, if the transgenes confer aspects of rosette flowering, it is most parsimonious to assume that the causal genetic changes occurred coincidentally with the evolution of rosette flowering. This inference should be tested ultimately by conducting equivalent experiments with the *LFY* genes of inflorescence-flowering mustard species.

To provide a framework for deciding whether transgenic phenotypes can be considered "aspects of rosette flowering," we present a simple developmental model. Inflorescence flowering in Brassicaceae can be represented by a two-stage program (Fig. 4*A*) involving (*i*) a vegetative phase during which the SAMs produce short internodes, expanded leaves,
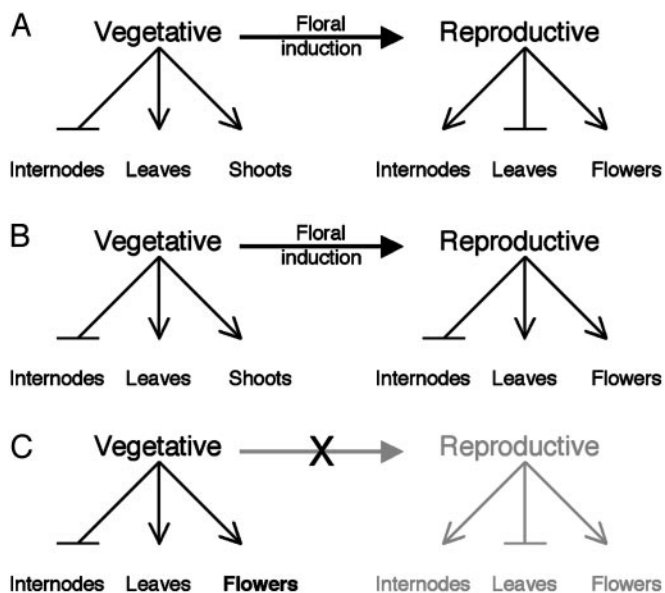
Fig. 4. Developmental model for the evolution from inflorescence flowering (*A*) to rosette flowering (*B* and *C*). Bold arrows mark the transition (floral induction) from the vegetative to the reproductive phase. Within a developmental phase, an arrow indicates promotion of the indicated developmental unit, whereas bars indicate inhibition. Rosette flowering can evolve either by altering the rules of development during the reproductive phase (*B*), as inferred for *Idahoa*, or by arresting development in the rosette stage but producing ectopic flowers in place of axillary shoots (*C*). See text for explanation.

and axillary meristems with vegetative identity (paraclades) and (*ii*) a reproductive phase during which SAMs produce long internodes, lack leaves (bracts), and produce axillary meristems with a floral identity. Although this model is oversimplified (for example, internode elongation spreads into the vegetative zone, resulting in the production of elevated secondary inflorescences and cauline leaves; ref. 39), it provides a basis for identifying two distinct paths by which rosette flowering could evolve. The first possibility is that the reproductive phase was modified such that internodes no longer expand and bracts are no longer suppressed (Fig. 4*B*). The alternative possibility is that the transition to the reproductive phase is postponed indefinitely but that flowering takes places within the rosette as a result of the homeotic conversion of axillary SAMs into flowers (Fig. 4*C*). Because both scenarios would result in the same phenotypic end point, they can only be distinguished by reference to intermediate steps in the evolutionary process. Such intermediate steps might be uncovered by using a transgenetic strategy involving the transformation of *A. thaliana* plants with only one of several genes that changed during the evolution of rosette flowering. Thus, under the first scenario, transformation experiments could result in plants showing internode suppression and bract production within the inflorescence, whereas the second scenario predicts partial conversion of axillary rosette shoots to floral identity.

Introduction of *IscLFY1* into *Arabidopsis* resulted in a tendency for internode compression and bract derepression within the inflorescence. This result suggests that the evolution of rosette flowering in *Idahoa* involved the developmental mechanism shown in Fig. 4*B*. The fact that an incomplete conversion to rosette flowering occurred shows that *IscLFY1* is one, but not the only, gene that causes rosette flowering. An obvious candidate for partnership in the evolutionary transition is *IscLFY2*. It is plausible, therefore, that *IscLFY1/IscLFY2* doubly transgenic

A. thaliana would show a more extreme conversion to rosette flowering.

In the case of *LcrLFY*, we observed conversion of axillary meristems to a more determinate fate caused by ectopic accumulation of *LFY* transcript. Although this phenotype is consistent with the second developmental model for the evolution of rosette flowering, other genetic factors are clearly missing, as shown by the fact that the determinate structures are short shoots rather than single flowers. Furthermore, to explain the phenotype of wild-type *Leavenworthia*, which lacks terminal flowers, there must be mechanisms in *Leavenworthia* that prevent the production of terminal flowers on the primary shoot meristem.

In the case of *Ionopsidium*, the failure to alter plant architecture suggests that the evolution of rosette flowering did not involve changes at the *LFY* locus *per se*. Nonetheless, the fact that the native expression pattern of *IacLFY* (24) is similar to the expression driven by the *LcrLFY* promoter implies that *Ionopsidium* evolved rosette flowering via a similar developmental mechanism, but, in this case, trans-, rather than cis-, regulation was responsible. Candidate trans-regulatory loci in *Ionopsidium* include *AP1* and *TFL1*.

Taken together, our data suggest but by no means prove that the *IscLFY1* and *LcrLFY* loci contributed directly to the evolution of rosette flowering. Additionally, our study makes plausible the hypothesis that the evolution of rosette flowering in *Ionopsidium acaule* resulted from changes in the trans-regulation of *IacLFY*. Although it remains a formal possibility that changes at the *LFY* locus arose independently of the origin of rosette flowering (and only coincidentally mimic aspects of rosette flowering when placed in *Arabidopsis*), the level of certainty achieved in this study is far greater than what could have been achieved by using only comparative gene expression studies, illustrating the potential value of the evolutionary transgenic strategy.

What do our data say about parallelism? If for parallelism one requires that the same genetic changes occurred in different lineages, our data would refute parallelism. In only two of the three lineages is the *LFY* locus itself implicated, and in these two cases our data suggest alternate development mechanisms (Fig. 4). Nonetheless, we wish to propose that the evolution of rosette flowering is most readily understood within the framework of parallelism and developmental constraint. The possibility that *LFY* has played a role in three independent origins of the same phenotype (two involving changes at the *LFY* locus, one involving upstream changes) suggests that this gene could be situated at such a position in the developmental program that it can readily respond to selection for different plant architectures. If evolutionary constraint were minimal, it would be inconceivable that the same gene should be involved, even indirectly, in three of three independent origins of the same trait. Therefore, the repeated involvement of *LFY* implied by our data is incommensurable with a claim of strict convergence but rather suggests developmental constraint and hence parallelism (3). Thus, despite the obvious capacity to achieve the same end point by means of different genetic changes, our results show that the evolution of morphology is not random but highly structured by the intrinsic organization of developmental programs.

1. Patterson, C. (1982) in *Morphological Characters and Homology*, eds. Joysey, K. A. & Friday, A. E. (Academic, London), pp. 21–74.
2. Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. & Wolpert, L. (1985) *Q. Rev. Biol.* **60,** 265–287.
3. Gould, S. J. (2002) *The Structure of Evolutionary Biology* (Belknap, Cambridge, MA).
4. Crandall, K., Kelsey, C., Imamichi, H., Lane, H. & Salzman, N. (1999) *Mol. Biol. Evol.* **16,** 372–382.
5. Bernasconi, P., Woodworth, A. R., Rosen, B. A., Subramanian, M. W. & Siehl, D. L. (1995) *J. Biol. Chem.* **270,** 17381–17385, and erratum (1996) **271,** 13925.
6. Thompson, M., Shotkoski, F. & ffrench-Constant, R. (1993) *FEBS Lett.* **325,** 187–190.
7. Burke, A. C., Nelson, C. E., Morgan, B. A. & Tabin, C. (1995) *Development (Cambridge, U.K.)* **121,** 333–346.
8. Averof, M. & Patel, N. (1997) *Nature* **388,** 682–686.
9. Bharathan, G., Goliber, T. E., Moore, C., Kessler, S., Pham, T. & Sinha, N. R. (2002) *Science* **296,** 1858–1860.
10. Gompel, N. & Carroll, S. B. (2003) *Nature* **424,** 931–935.
11. Wittkopp, P. J., Vaccaro, K. & Carroll, S. B. (2002) *Curr. Biol.* **12,** 1547–1556.
12. Hoekstra, H. E. & Nachman, M. W. (2003) *Mol. Ecol.* **12,** 1185–1194.
13. Sucena, E., Delon, I., Jones, I., Payre, F. & Stern, D. L. (2003) *Nature* **424,** 935–938.
14. Eizirik, E., Yuhki, N., Johnson, W. E., Menotti-Raymond, M., Hannah, S. S. & O'Brien, S. J. (2003) *Curr. Biol.* **13,** 448–453.
15. Baum, D. A. (2002) in *Developmental Genetics and Plant Evolution*, eds. Cronk, Q. C. B., Bateman, R. M. & Hawkins, J. A. (Taylor & Francis, London), pp. 493–507.
16. Wheeler, D. A., Kyriacou, C. P., Greenacre, M. L., Yu, Q., Rutila, J. E., Rosbash, M. & Hall, J. C. (1991) *Science* **251,** 1082–1085.
17. Nasrallah, M. E., Liu, P. & Nasrallah, J. B. (2002) *Science* **297,** 247–249.
18. Koch, M., Haubold, B. & Mitchell-Olds, T. (2001) *Am. J. Bot.* **88,** 534–544.
19. Koch, M., Haubold, B. & Mitchell-Olds, T. (2000) *Mol. Biol. Evol.* **17,** 1483–1498.
20. Galloway, G., Malmberg, R. & Price, R. (1998) *Mol. Biol. Evol.* **15,** 1312–1320.
21. Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R. & Coen, E. (1997) *Science* **275,** 80–83.
22. Weigel, D. & Nilsson, O. (1995) *Nature* **377,** 495–500.
23. Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. & Meyerowitz, E. M. (1992) *Cell* **69,** 843–859.
24. Shu, G., Amaral, W., Hileman, L. C. & Baum, D. A. (2000) *Am. J. Bot.* **87,** 634–641.
25. Coen, E. S., Romero, J. M., Doyle, S., Elliott, R., Murphy, G. & Carpenter, R. (1990) *Cell* **63,** 1311–1322.
26. Parcy, F., Nilsson, O., Busch, M. A., Lee, I. & Weigel, D. (1998) *Nature* **395,** 561–566.
27. Ratcliffe, O. J., Amaya, I., Vincent, C. A., Rothstein, S., Carpenter, R., Coen, E. S. & Bradley, D. J. (1998) *Development (Cambridge, U.K.)* **12,** 1609–1615.
28. Blazquez, M. A. & Weigel, D. (2000) *Nature* **404,** 889–892.
29. Clough, S. J. & Bent, A. F. (1998) *Plant J.* **16,** 735–743.
30. Maddison, D. R. & Maddison, W. P. (2002) MACCLADE (Sinauer, Sunderland, MA), Version 4.05.
31. Higo, K., Ugawa, Y., Iwamoto, M. & Korenaga, T. (1999) *Nucleic Acids Res.* **27,** 297–300.
32. Jofuku, K. D., den Boer, B. G., Van Montagu, M. & Okamuro, J. K. (1994) *Plant Cell* **6,** 1211–1225.
33. Irish, V. F. & Sussex, I. M. (1990) *Plant Cell* **2,** 741–753.
34. Grbic, V. & Bleecker, A. B. (1996) *Development* **122,** 2395–2403.
35. Poduska, B., Humphrey, T., Redweik, A. & Grbic, V. (2003) *Genetics* **163,** 1457–1465.
36. Shannon, S. & Meeks-Wagner, D. R. (1991) *Plant Cell* **3,** 877–892.
37. Bradley, D., Vincent, C., Carpenter, R. & Coen, E. (1996) *Development (Cambridge, U.K.)* **122,** 1535–1544.
38. Blázquez, M., Soowal, L., Lee, I. & Weigel, D. (1997) *Development (Cambridge, U.K.)* **119,** 721–743.
39. Hempel, F. D. & Feldman, L. J. (1995) *Plant J.* **8,** 725–731.
40. Smyth, D. R., Bowman, J. L. & Meyerowitz, E. M. (1990) *Plant Cell* **2,** 755–767.