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Research Advances in the Molecular Regulatory Mechanisms of Dimorphic Flower Development in Viola philippica

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Abstract. Dimorphic cleistogamy, in which a single plant produces both open chasmogamous flowers and closed cleistogamous flowers, provides a useful system for studying how environmental cues reshape floral development. Viola philippica is a suitable model for this question because it alternates seasonally between the two flower types and because photoperiod manipulation can induce or switch flower type under controlled conditions. In this species, floral dimorphism is expressed mainly through quantitative differences in corolla and stamen growth, organ number, and maturation state, rather than through classic homeotic changes in organ identity. The main developmental question therefore lies after organ initiation: how growth, maturation, and partial arrest are regulated in specific floral whorls. Available evidence links photoperiod signaling with hormone regulation, particularly gibberellin metabolism and signaling, and with reduced expression of floral B-function genes during cleistogamous development. Transcriptomic and small RNA studies in related Viola species also point to regulatory modules involving photoperiod integrators, hormone signaling components, and organ-specific transcriptional regulators. Functional evidence in V. philippica, however, remains limited. Here, we summarize current knowledge of morphological divergence, developmental timing, environmental and endogenous signal integration, and gene regulatory models for dimorphic flower development in V. philippica, and identify experimental directions needed to move from correlation to causal mechanism.

Keywords: Viola philippica, dimorphic cleistogamy, photoperiod, gibberellin, floral B-function

1. Introduction

Viola philippica(*V. philippica*) offers a tractable system for studying floral dimorphism because its flower type responds reproducibly to photoperiod. The same genetic background can produce chasmogamous or cleistogamous flowers when daylength is manipulated, which makes it possible to separate developmental regulation from much of the background environmental variation [1]. The two flower types differ mainly in degree rather than identity. Petal and stamen number, organ expansion, and maturation state change substantially, but canonical homeotic conversion has not been observed. The main developmental problem lies after organ specification: later growth and maturation programs are curtailed in selected whorls.

Dimorphic flowering describes the production of two structurally and functionally different flowers on one plant. In cleistogamous species, open flowers generally allow outcrossing, whereas closed flowers usually self-fertilize. The paired strategy has arisen repeatedly in angiosperms and often occurs under variable environments, unreliable pollination, or limited resources. Viola species commonly show seasonal cleistogamy, and intermediate flowers can occur between the fully open and strongly reduced forms [2].Those intermediates are important because they make the system developmental, not merely categorical.

The unresolved issue is how photoperiod and other environmental inputs are translated into flower-type-specific growth programs. Evidence from *V. philippica* and related Viola species connects daylength responses with gibberellin signaling, reduced B-function gene expression, and small-RNA-associated regulatory modules. These data define plausible regulatory routes, but causal tests in *V. philippica* remain limited [3]. The sections below review the available morphological, developmental, hormonal, and transcriptional evidence, with emphasis on where correlation still needs to be converted into mechanism.Preparing your paper

2. Phenotypic divergence and developmental basis of dimorphic flowers in *Viola philippica*

2.1. Morphological divergence between chasmogamous and cleistogamous flowers

A well-structured paper follows a clear hierarchy of sections, with different levels of headings to organize content logically. Generally, a conference paper may include the following sections: *Introduction, Literature Review, Methodology, Results, Discussion, Conclusion, and References*. Fourth- and lower-level headings should not be used in your paper. Note that the styles for headings will automatically add numbers for each section. Use sentence case for the section headings. Only the first word should be capitalized, and the rest should be lowercase except for proper nouns, abbreviations, and acronyms.

The morphological differences between chasmogamous and cleistogamous flowers in *V. philippica* are readily quantifiable. Chasmogamous flowers have a typical five-petal corolla, including a spur at the base of the lower petal. Five stamens surround the pistil and form a conical structure, with the two lower stamens bearing conspicuous nectaries. Each stamen contains four pollen sacs. Cleistogamous flowers are much smaller. Their corolla is absent or reduced to rudimentary structures, the floral architecture is simplified, and only two functional stamens remain (Figure 1). Nectaries are absent, and each stamen has two pollen sacs. Filaments are also more visible in cleistogamous and intermediate flowers, whereas they are less conspicuous in chasmogamous flowers [4].

Intermediate flowers can develop under particular photoperiod regimes. They usually show petal and stamen numbers between those of chasmogamous and cleistogamous flowers, often with one to three underdeveloped petals and two to five developing stamens. Some stamens also show partial suppression of microsporogenesis, so male reproductive development may be especially sensitive during the transition between flower types. This graded series argues against a simple binary switch at meristem identity or organ initiation. A more plausible model is thresholded control of organ growth and maturation, in which environmental inputs interact with endogenous growth-regulatory networks.

Figure 1. Morphological comparison between chasmogamous and cleistogamous flowers in *Viola philippica*

2.2. Floral developmental trajectory and key divergence stages

Developmental observations indicate that flower-type divergence in *V. philippica* does not begin with whether floral organ primordia are initiated. Instead, the two flower types diverge mainly during later development, after organogenesis is largely complete. Scanning electron microscopy and morphological staging divide floral development into floral meristem formation, organogenesis, and subsequent maturation. During organogenesis, chasmogamous and cleistogamous flowers do not show homeotic changes. The major differences arise later, when petal and stamen growth is inhibited and organ number is reduced. This places the main mechanistic focus on stage-specific regulation of cell division, cell elongation, tissue differentiation, and reproductive organ maturation.

This staging logic also has practical value for experimental design. If divergence is late and growth-centered, sampling should target the window after primordia initiation but before full organ expansion and maturation. Candidate regulators should therefore include not only canonical floral homeotic genes, but also growth regulators, hormone pathway components, cell-cycle genes, and tissue-specific factors involved in filament elongation, anther maturation, and corolla expansion.

2.3. Reproductive strategy and ecological adaptive significance

The mixed mating system in *V. philippica* combines two reproductive functions. Chasmogamous flowers can attract pollinators and increase the chance of outcrossing, which may increase genetic variation in heterogeneous environments. Cleistogamous flowers provide reproductive assurance when pollinators are scarce or resources are limited, and they may reduce the cost of reproduction by lowering investment in showy petals and nectar [5]. The adaptive significance of this system depends on plasticity in flower-type production. Understanding how environmental variation is sensed and translated into different flower-type ratios is therefore relevant to both developmental biology and evolutionary ecology.

3. Environmental responses and integration of endogenous signals underlying dimorphic flower formation

3.1. Light, temperature, and seasonal regulation

Photoperiod is the clearest environmental cue identified for flower-type determination in *V. philippica*. Short days favor chasmogamous flowers, whereas long days promote cleistogamous development. Intermediate daylengths can produce mixed phenotypes, and the proportion of cleistogamous flowers rises as daylength increases. This experimental response matches field phenology: chasmogamous flowers are most common in early spring, while cleistogamous flowers become dominant during summer and early autumn.

Daylength is not the only environmental variable acting on seasonal cleistogamy in Viola. Total irradiance, canopy shade, temperature, and water status can all affect whether plants produce open or closed flowers. Photoperiod functions as the primary timing signal, but it operates within a larger environmental input set rather than as a single independent switch.

Temperature probably modifies flower-type output through effects on growth rate, developmental timing, and resource allocation. Reported shifts in temperature regimes are accompanied by changes in the timing and relative abundance of chasmogamous and cleistogamous flowers. For *V. philippica*, photoperiod remains the better-supported cue, but photoperiod-by-temperature interactions still require controlled experiments.

3.2. Nutritional status and carbon metabolism signals

Nutritional status and carbon metabolism can influence flower development both as resource supplies and as signaling states. In related Viola species, flower-type-linked differentially expressed genes are enriched for carbohydrate and fatty acid metabolism [3]. Floral dimorphism may involve physiological reallocation as well as floral regulatory genes. The reduced corolla and loss of nectaries in cleistogamous flowers would be expected to alter source-sink balance, and sugar metabolism or sugar signaling could place limits on organ expansion. In *V. philippica*, however, this remains an inference from expression data. Direct tests should measure metabolic flux, sugar signaling components, hormone levels, and targeted perturbations during the photoperiod-sensitive transition.

3.3. Phytohormone regulatory networks

Hormone pathways provide one route by which daylength can affect floral development in *V. philippica*. Among the pathways examined so far, gibberellin (GA) has the strongest experimental support. Long-day treatment induces cleistogamous flowers, and cleistogamous buds contain higher active GA levels than chasmogamous buds produced under short days. Active GA biosynthesis appears to act downstream of, or at least alongside, photoperiod-dependent flower-type induction [1].

GA action differs by organ and developmental context. GA biosynthesis-related genes are upregulated during pistil development under both long- and short-day treatments, consistent with a role in pistil formation across flower types. In stamens, however, GA accumulation is higher under short days, when anthers and adjacent petals develop more fully. Long-day buds show lower GA levels in anthers and petals, and these organs remain poorly developed. Pharmacological data make the pathway harder to read: blocking GA biosynthesis does not restore chasmogamous development under long days, whereas exogenous GA under relatively short days can partially shift flowers toward cleistogamy. That shift coincides with reduced B-function gene expression and suppression of petal and stamen growth. A linear photoperiod-GA-organ growth pathway is unlikely. More plausible tests would examine local GA metabolism, DELLA-mediated sensitivity, and downstream growth programs separately in pistils, stamens, and petals.

In this developmental context, B-function genes appear to do more than assign organ identity. Their expression level may also set the growth and maturation capacity of petals and stamens after organ initiation. VpTM6-1, VpTM6-2, and VpPI show highest expression in chasmogamous flowers, lowest expression in cleistogamous flowers, and intermediate expression in transitional forms, consistent with a graded photoperiod-dependent response.

AP3 ortholog comparisons across Viola species support the same interpretation. AP3 expression falls during long-day-induced cleistogamous development, whereas species without floral dimorphism maintain more stable AP3 expression. Sequence comparisons further identify differences in light-responsive cis-elements in AP3 regulatory regions. Photoperiod-dependent modulation of B-function gene expression may involve cis-regulatory divergence rather than only trans-acting pathway changes.

4. Molecular regulatory mechanisms of dimorphic flower development

4.1. Expression and roles of canonical floral homeotic genes

Canonical floral homeotic genes provide the starting point for interpreting dimorphic flower formation. In the ABC model, combinations of A-, B-, and C-function genes specify floral organ identity, with B-function genes such as AP3 and PI controlling petal and stamen identity [6]. In *V. philippica*, however, comparisons between chasmogamous and cleistogamous flowers show no homeotic transformations. Dimorphism is therefore unlikely to result from organ identity re-specification. The evidence instead points to post-initiation regulation of organ growth and maturation.

Reduced expression of B-function-related genes at later developmental stages appears to restrict petal and stamen development. In this setting, B-function genes may act not only as identity genes but also as quantitative regulators of organ growth and maturation. Expression analyses of VpTM6-1, VpTM6-2, and VpPI show a gradient: expression is highest in chasmogamous flowers and lowest in cleistogamous flowers, with intermediate levels associated with photoperiod-dependent transitional forms.

Comparative work on AP3 orthologs across *Viola* species supports this model. AP3 expression is reduced during long-day-induced cleistogamous development, whereas stable expression is maintained in species without dimorphic flowers. Regulatory-sequence analyses also identify divergence in light-responsive cis-elements within AP3 loci. These findings suggest that photoperiod-dependent regulation of B-function gene expression may have a cis-regulatory basis.

4.2. Signal integration and gene regulatory networks mediated by non-MADS transcription factors

MADS-box genes cannot by themselves account for the coordinated reduction of petals, stamens, nectaries, and reproductive structures in *V. philippica*. Non-MADS transcription factors and signaling integrators are also needed in the model. Transcriptome network analyses in related Viola species identify photoperiod pathway components, including gigantea, as hubs during chasmogamous development. This places circadian and daylength pathways close to floral organ growth regulation. GAIP-B-like DELLA genes have been proposed to connect GA signaling with downstream B-function modules, and transient reporter assays indicate that DELLA proteins can repress B-class promoter activity (Figure 2). In *V. philippica*, DELLA expression is photoperiod-responsive and may be involved in filament development in cleistogamous flowers [7].

Organ-specific regulators may further shape the network. Unusual Floral Organs and YABBY5 are associated with petal and nectary development, which is consistent with corolla suppression and nectary loss in cleistogamous flowers. Small RNA regulation adds another layer. miRNAs such as miR393 and miRN2005 target auxin signaling components, including TIR1 and ARF2, and AP2-like factors have also been implicated in flower-type determination [8] .

Other transcription factor families, including bHLH, NAC, and SPL, may act as downstream executors or integrators of developmental and hormonal signals. bHLH modules are plausible regulators of anther development and microsporogenesis, whereas NAC and SPL pathways may contribute to organ growth and developmental timing. Their roles in *V. philippica* remain hypotheses until tested with functional and cell-type-resolved approaches [9-11].

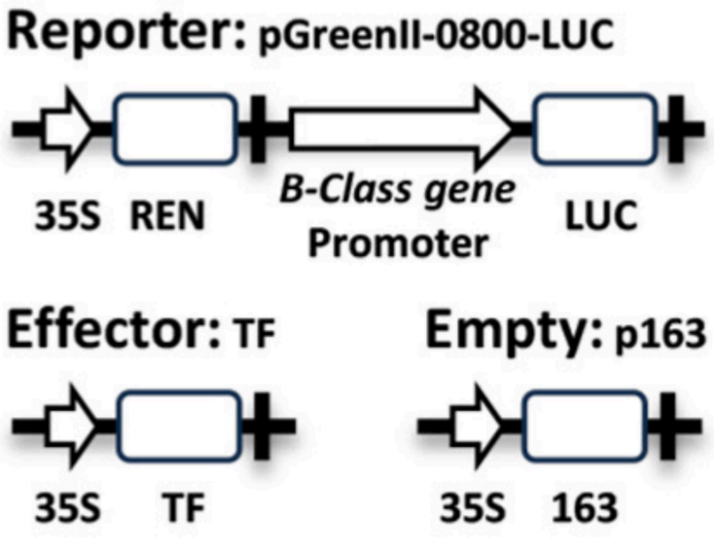

Figure 2. Dual-luciferase reporter system used to test repression of B-class gene promoters by DELLA factors

4.3. Epigenetic regulatory mechanisms (DNA methylation, histone modifications, and small RNAs)

Epigenetic regulation is a plausible layer in dimorphic flower plasticity because it can support environmental memory, threshold responses, and reversible developmental states. In *V. philippica*, the strongest current evidence lies in the small RNA layer; DNA methylation and histone modification have been studied less directly. Flower-type-associated miRNAs and their targets in Viola include hormone signaling components and transcription factors, fitting the role of small RNAs as regulators of network state. In other plant systems, DNA methylation and histone modifications contribute to floral transition by acting through photoperiod, vernalization, gibberellin, age, and ambient-temperature pathways that converge on floral integrators such as Flowering Locus T and Suppressor of Overexpression of Constans 1 (Figure 3) [12]. Work on epialleles at cly1 in grasses shows that altered DNA methylation can affect floral organ development and produce cleistogamy-related phenotypes. Grass cleistogamy usually involves failure of spikelet opening with largely intact organs, which differs from the organ reduction observed in *V. philippica*. Even so, it provides evidence that epigenetic states can influence cleistogamous morphology. For *V. philippica*, methylome profiling, chromatin accessibility mapping, and causal perturbation across photoperiod-induced transitions are needed to test this possibility.

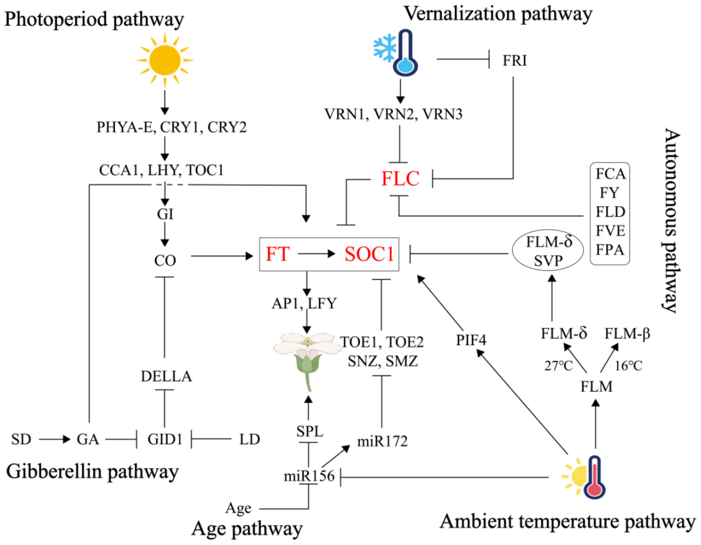

Figure 3. Integration of major flowering regulatory pathways converging on FT and SOC1

4.4. Regulatory network investigation from a multi-omics perspective

Multi-omics approaches are useful because dimorphic flower development is unlikely to be explained by a single gene. In *V. philippica*, current advances include photoperiod-controlled morphological and expression analyses, quantification of GA levels, expression analysis of GA biosynthesis genes, and comparative studies of B-function gene expression and cis-element variation. These data link environmental input, developmental gene expression, and floral phenotype.

Transcriptome network studies in related Viola species provide a broader view of the system. Weighted gene co-expression analyses show that modules associated with chasmogamous flower formation are enriched for photoperiod integrators and floral development genes, whereas modules associated with cleistogamous flower formation are enriched for stress responses, translation, and metabolism. Flower type may therefore be linked to systemic physiological state, including resource allocation and defense-related trade-offs. Future work in *V. philippica* should combine spatiotemporal multi-omics datasets from induction to organ maturation with hormone quantification, cell-type-resolved transcriptomics, and chromatin-level data. Such datasets would help move the field from correlation-based networks toward experimentally testable intervention targets.

5. Conclusions and outlook

The key gap in *V. philippica* is the stepwise causal route from environmental input to floral organ reduction. Photoperiod, GA signaling, and B-function gene expression are all implicated, but the cell types and thresholds that convert long-day exposure into cleistogamous morphology are not yet known. GA levels also do not map simply onto organ development: inhibiting GA biosynthesis fails to reverse long-day-induced cleistogamy. This points to parallel pathways, compensatory regulation, or upstream controls that have not been identified. Current models remain heavily gene-centered, while the phenotype itself involves organ growth, tissue maturation, and reproductive architecture.

Two questions should guide the next phase of work. First, how do photoperiod perception and circadian timing reshape transcriptional regulation and hormone homeostasis before B-function gene expression changes? Second, which cellular processes actually produce organ reduction? Reduced cell division, restricted elongation, programmed cell death, and altered differentiation are all plausible, and they need not be mutually exclusive. Candidate regulators include bHLH, NAC, and SPL transcription factors, together with miRNA modules that connect hormone signaling with developmental timing [11].

Progress will require functional tests layered onto multi-omics data. Transcriptomic and co-expression analyses can nominate regulators, but they cannot establish causality alone. Transformation systems reported in related Viola species, including Agrobacterium-mediated transformation and Rhizobium rhizogenes-based regeneration, provide a basis for Clustered Regularly Interspaced Short Palindromic Repeats editing and transgenic validation [13, 14]. For *V. philippica*, stable transformation, cis-regulatory assays, spatially timed perturbation, and epigenetic editing would allow direct tests of photoperiod-responsive regulatory elements and their effects on B-function gene expression [15].

Dimorphic flower regulation also matters beyond developmental mechanism. Environmentally driven shifts in chasmogamous and cleistogamous flower ratios can alter population genetic structure and adaptive potential under climate variation. In breeding, cleistogamy may help maintain genetic stability or control pollination. A clearer photoperiod-hormone network in *V. philippica* could inform both evolutionary interpretation and reproductive trait improvement in economically important Viola species.

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