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Stylar steroids: Brassinosteroids regulate pistil development and self-incompatibility in *Primula*

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Manipulation of active brassinosteroid content in the developing flower of *Primula* dictates style length and female incompatibility type. A new study reveals the dual effects of brassinosteroids on establishing both the morphology of the pistil and mate recognition in self-incompatible heterostylous *Primula forbseii*.

Self-incompatible (SI) reproductive mechanisms in plants allow for improved chances of outcrossing events, ensuring continued genetic variability within populations, and avoiding the risks of inbreeding depression[1]. Several mechanisms are known to play a role in ensuring successful outcrossing, including combinations of: temporal separation of stamen and pistil maturity (dichogamy)[1]; molecular recognition and rejection of self pollen at the stigmatic surface (sporophytic self-incompatibility, SSI)[2,3] or within the stylar tissue (gametophytic self-incompatibility, GSI)[4,5]; and physical separation of the male and female organs that gives rise to polymorphic flowers (herkogamy)[1,5–10].

The development of heterostyly is a well-documented and highly studied area of herkogamous plant SI and has been observed across a broad range of angiosperm families, including in members of the rosid and asterid eudicot clades and in the monocots[1,5–10]. In distylous mating systems, stamens and pistils are arranged reciprocally such that flowers will either display short styles with high anthers (S-morph or 'thrum') or long styles with low anthers (L-morph or 'pin')[5–10] (Figure 1A). In this way, pollen deposition on animal pollinators by one morph will allow for efficient transfer to flowers of the reciprocal morph for outcrossing[1]. In addition to herkogamy, distylous plant species have another mechanism for the inhibition of non-self-pollen at the stigmatic surface or within the style as a backup against incorrect pollen deposition[5,8]. These mechanisms operate by a 'lock-and-key' system involving protein–protein interactions that either dictates the delivery of resources to the dry pollen at the stigmatic surface (SSI)[2,3,5] or determines pollen tube survival as it progresses through the stylar tissue (GSI)[4,5].

The establishment of floral morphs is dictated by the *S*-locus supergene cluster, which includes at least three sub-loci: the *A*-locus responsible for determining anther position; the *G*-locus responsible for determining style length; and the *P*-locus, which dictates pollen size and number[5–10]. In several *Primula* species, the presence of the *S*-locus in the hemizygous (*S*/*s*) condition results in the development of the S-morph, whereas a lack of the *S*-locus (that is, homozygous recessive, *s*/*s*) results in L-morph flowers[5–8]. Molecular characterization of the genes encoded by the *S*-locus has identified critical candidates for sub-loci genes, including the *A*-locus gene *GLOBOSA2* (*GLO2*, not to be confused with the compatibility factor Glyoxalase 1, GLO1, in *Brassica* SSI)[3,7], encoding a MADS-box transcription factor found to regulate anther position, and the style-specific *G*-locus genes *PfCYP734A50*, encoding a cytochrome P450 enzyme[6], and *TsBAHD*, encoding an acyl transferase[9], both of which are involved in the inactivation of brassinosteroid plant hormones (BRs) by hydroxylation or acetylation, respectively[11] (Figure 1B). Following

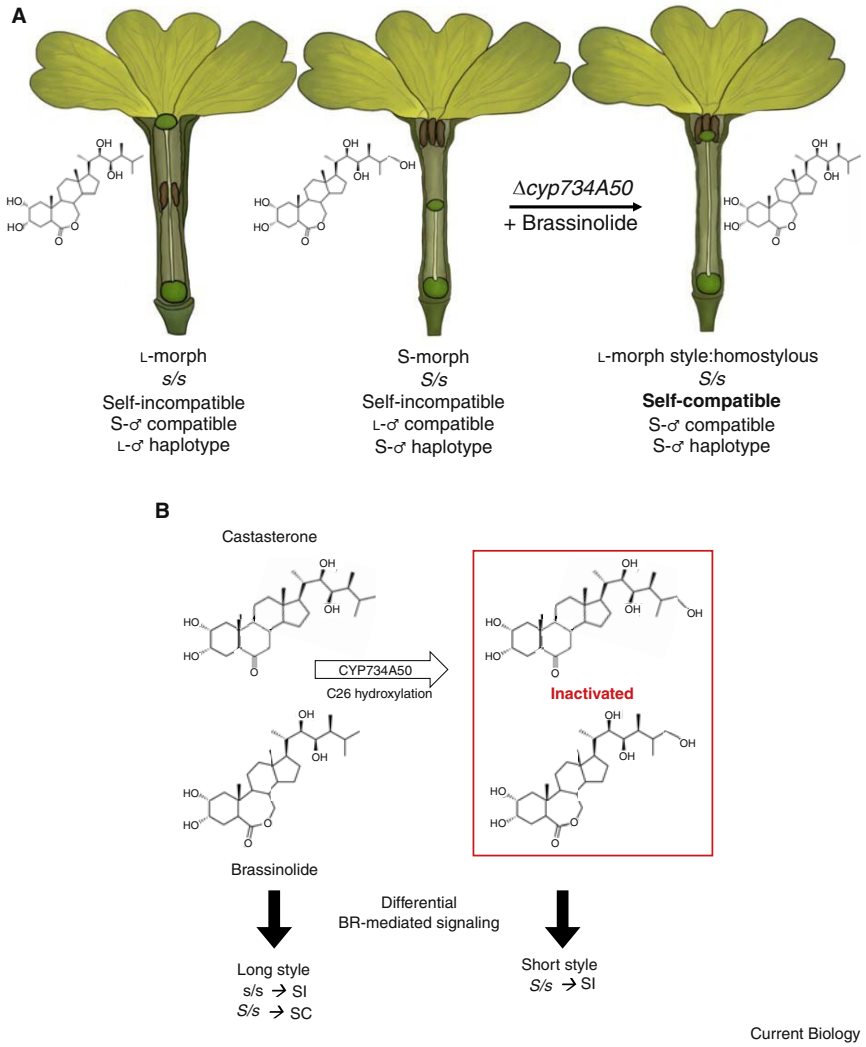

Figure 1. Manipulation of active brassinosteroids within the styles of heterostylous *Primula* dictates stylar morphology and compatibility type.
(A) Morphology and mating type of the L- and S-morph flowers and the result of either *cyp734A50* mutation or application of brassinolide on S-morph flowers[6,8]. (Artwork hand-drawn by Lisa Samuel, requisitioned by Marcus Samuel.) (B) Inactivation of castasterone and brassinolide by CYP734A50 dictates stylar morphology and impacts compatibility type[6,8,11]. Hydroxylation of castasterone or brassinolide at the C26 position by CYP734A50 results in their inactivation[11]. CYP734A50, encoded by the *S*-locus, hydroxylates active brassinosteroids within the style and results in short-styled S-morph flowers (*S/s*). Lack of the *S*-locus (*s/s*) results in brassinosteroid overaccumulation and the development of long-styled L-morph flowers. Both L- and S-morph flowers are self-incompatible (SI), but deletion of *CYP734A50* or application of brassinolide to *S/s* flowers results in homostylous, long-styled, self-compatible (SC) flowers[8]. Compatibility type and style development are likely the result of brassinosteroid-mediated signaling within the style[8,10].

these discoveries, recent studies in both *Primula* and *Turnera* spp. have identified BRs as being critical for stylar development, which are subject to control by the *G*-locus[5–10].

BRs play important roles in floral development and reproduction, through their ability to both regulate pollen development and growth[12,13] as well as dictate ovule development[14] in *Arabidopsis thaliana*. A new study in this issue of *Current Biology* by Huu *et al.*[8] has established that inactivation of BRs in the pistil by CYP734A50 activity can modify both style length and mate recognition (Figure 1A). Following their previous work[6], the researchers turned their focus to the effects of BRs on herkogamy and the molecular mechanisms underlying SI in *Primula*.

Huu *et al.*[8] first assessed two *Primula forbseii* mutant lines in which the *CYP734A50* gene was deleted but the remaining genes of the *S*-locus were fully intact. These *cyp734a50* mutants had homostylous flowers with consistent L-morph styles and high anthers that were self-compatible. *cyp734a50* mutant pistils were also compatible with S-morph pollen and rejected L-morph pollen. The *cyp734a50* pollen SI type was unchanged as it retained its S-morph identity and was only accepted on L-morph stigmas (Figure 1A).

The researchers then sought further confirmation of the function of the *CYP734A50* gene by assessing the effects of viral-induced gene silencing (VIGS) on S-morph flowers. Suppression of *CYP743A50* by VIGS resulted in the same stylar phenotype as the *cyp734a50* mutants in that the compatibility type largely matched L-morph flowers. Full seed set was observed when pollinated with pollen from S-morph flowers and self-pollen, and only partial seed set was observed from L-morph pollen[8]. This was suggested to result from the incomplete suppression of *CYP743A50*. The pollen of the VIGS-*CYP* S-morph flowers retained its identity, only setting seed on L-morph stigmas. This confirmed that the deletions observed in the identified *cyp734a50* mutants were responsible for the observed phenotypes, and not further loss of the *S*-locus or surrounding genomic content[8].

Application of brassinolide (BL, a physiologically active BR) was used to determine whether the overaccumulation of BRs as expected in the *cyp734a50* mutants would have any effect on the expression of remaining *S*-locus genes. No specific changes to the expression levels of the remaining *S*-locus transcripts were observed[8]. From the authors' previous work[6] and from experiments in *Turnera*[10], inactivation of BR through CYP734A50 and BAHD activity appears restricted to the stylar tissue and, owing to the low cell–cell mobility of BRs[11], likely has little effect on levels of BRs in the surrounding floral organs. Exogenous BR was previously reported to have little effect on anther position and therefore was unlikely to affect the expression of *GLO2*[6].

To establish that a lack of BRs is responsible for the S-morph mating type, BL was applied to developing S-morph flowers. This led not only to an increase in style length to L-morph height, but also to a switch in the female self-incompatibility type from the S-morph to the L-morph (again accepting S-morph and self pollen) without altering pollen compatibility type (Figure 1). In contrast, treatment of L-morph flowers with propiconazole (PPZ, a BR biosynthesis inhibitor[15]) conferred a short-style phenotype, although the mating type following this treatment was unaffected. It was suggested that the length of this PPZ treatment regime only allowed for changes to stylar length, but this could allow the flowers to reaccumulate BRs prior to pollination and confer the expected mating type. Extended PPZ treatment damaged flowers and impeded further mating experiments. Isolation of a BR-defective mutant in *Primula* could be helpful in elucidating whether simply the absence of BR instead of local degradation of BR could result in homostylous, self-compatible S-morph flowers. Therefore, absence or inactivity of *CYP734A50* or an overaccumulation of active BRs within the flower promotes lengthening of the style and switching of the female self-incompatibility type (S-morph to L-morph), without altering the male mating type or stamen filament length (Figure 1).

To determine whether the presence of BRs has a differential effect on the germination and growth of S- and L-morph pollen, *in vitro* pollen germination assays were performed in media supplemented with varying concentrations of BL[13]. Although an increase in S-morph pollen tube growth was observed at concentrations between 10 and 500 nM BL, pollen germination showed little change across these treatments. The increased growth of S-morph pollen tubes on BR may contribute to reproductive success as the pollen tubes will interact with a greater amount of BR within the L-morph style. However, the lack of active BR within the S-morph style did not appear to be sufficient to selectively promote L-morph pollen tube growth and impede that of self and S-morph pollen. This suggests that differences in active BR accumulation alone are insufficient to differentially impact pollen germination and growth of self and cross-pollen and that the molecular mechanism of SI is likely the result of downstream signaling dependent on active BR accumulation[8]. A similar result was observed in heterostylous *Turnera* where differential accumulation of active BL between the morphs did not account for the differing germination and growth observed between self and cross-pollen[10].

The molecular mechanism of SI in another *Primula* species is a mixture of SSI and GSI, with selection of pollen occurring either at the stigmatic surface alone or within the style or both[5]. Further investigation into the effects of differential accumulation of active BRs within the style could shed light on key factors behind the SI response; this should be considered for future research. Heterostyly has been studied for over 150 years as a model for SI; however, the molecular bases of these morphological differences and SI mechanisms have only recently begun to be dissected[1,5–10]. The findings by Huu *et al.*[6,8] have furthered our understanding of BR-dependent stylar development and mate selection and have set the stage for the identification of the molecular mechanism underpinning self-pollen recognition in *Primula*.

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