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NEWS AND COMMENTARY

Delineation of the *S* locus in *Turnera subulata*

Homing in on heterostyly

PM Gilmartin and J Li

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The majority of plants are hermaphrodite and produce both male and female gametes. In addition to the various contrivances by which plants facilitate outcrossing—using wind, insects and other animals—an elaborate range of mechanisms has evolved to prevent self-fertilization. One such mechanism, known as floral heteromorphy, results in the development of different forms of self-incompatible flowers on different individual plants (Darwin, 1877). Development of the distinct floral morphs with different anther height and style lengths is orchestrated by the *S* locus (see Richards, 1997). In species such as *Turnera subulata* (white alder), *Fagopyrum esculentum* (buckwheat) and *Primula vulgaris* (primrose), which produce two forms of flower with long and short styles, this phenomenon is also known as distyly and is coordinated by two alleles of the *S* locus (see for example Matsui *et al*., 2004; Li *et al*., 2007; Labonne *et al*., 2009). In contrast, most self-incompatibility (SI) systems, in which self-pollination is prevented by rejection of pollen following molecular self-recognition, occur in otherwise indistinguishable homomorphic flowers (see for example Hiscock and McInnis, 2003). To date, much greater progress has been made toward a detailed molecular understanding of homomorphic SI in a range of species than SI associated with floral heteromorphy. However, the recent study by Labonne *et al*. (this issue) provides a key step forward toward the identification of genes located at the *S* locus that control floral heteromorphy in distylous *T. subulata*. In this species, long-styled plants are homozygous recessive (*ss*) and short-styled plants are heterozygous (*Ss*) with respect to the two *S* locus alleles.

In their paper, Labonne *et al.* (this issue) present an X-ray deletion mutagenesis screen of *T. subulata*. Although mutagenesis has been successfully used in studies of homomorphic SI systems, this is the first published systematic screen in a heterostyled plant aimed at defining the *S* locus. This study focused on the identification of mutants that affect heteromorphic flower development, whereas previous studies in *F. esculentum* have focused on the identification of self-fertile mutants (see for example Matsui *et al*., 2004 and the references therein). In *Primula*, self-fertile and homostyle plants resulting from recombination within the *S* locus have defined different genetic functions at the *Primula S* locus responsible for style length (*G*), anther position (*A*), pollen size (*P*) and both pollen (I^P) and style (I^S) incompatibility phenotypes demonstrating the presence of a co-adapted linkage group of genes rather than the presence of a single master regulator (Dowrick, 1956; Richards, 1997). The existence of a similar co-adapted linkage group has been proposed in *F. esculentum* (see Matsui *et al*., 2004 and the references therein).

The Shore laboratory has pioneered the development of *T. subulata* as a model for the study of heteromorphy, and the scale and scope of this current study is impressive. The data presented are an accumulation of nearly a decade of study starting with the generation of genetically defined parental lines, an unusual homozygous (*SS*) short-styled plant, which provided the pollen for X-irradiation, and the long-styled (*ss*) pollen recipient, through to the analysis of 3982 progeny plants. All progeny from this cross should have been short styled with genotype *Ss*, but 10 long-styled mutants were obtained, suggesting deletion of the dominant *S* allele, together with a short-homostyle and a long-homostyle plant.

In parallel to the mutagenesis studies, the Shore laboratory have painstakingly identified a number of *S* locus-linked genes and DNA markers (Labonne *et al*., 2009), together with morph-specific proteins (Athanasiou and Shore, 1997), which were used to delimit the extent of the *S* locus deletions within these 12 mutants. As would be predicted by analogy to the known architecture of the *Primula S* locus (Dowrick, 1956; Richards, 1997), the long-styled plants seem to be derived from pollen in which the entire dominant *S* allele has been deleted. Subsequent analysis of the short homostyle did not reveal a simple explanation, but analysis of the long homostyle indicates that the dominant alleles responsible for development of a short style (*G*) and large pollen grains (*P*), but not that which determines high anthers (*A*), have been lost. These data provide evidence of a coadapted linkage group in *Turnera*, often referred to as a 'supergene', as previously proposed in *Fagypyrum* and *Primula* (see for example Richards, 1997 and Matsui *et al*., 2004).

Although the mutant screen could have been extended to identify mutants showing the expected short-styled phenotype that were rendered self-fertile following mutagenesis, the large deletions defined in those plants analyzed suggest that it is unlikely that such self-fertile short-styled plants would have been identified, as this would have required deletion of genes responsible for pollen or stigma SI functions (I^P or I^S) within the *S* locus without loss of the genes (*G*, *P* or *A*) responsible for heteromorphic architecture (Dowrick, 1956; Richards, 1997). What is remarkable, however, is that in the three heteromorphic species currently under investigation, *P. vulgaris*, *F. esculentum* and *T. subulata*, which fall within three distinct orders, namely, Ericales, Caryophyllales and Malpighiales, in different clades of the core Eudicots, independent evolution has resulted in similar predicted *S* locus architectures.

The identification of morph-specific genes and proteins in *Primula* (McCubbin *et al*., 2006) and *Turnera* (Athanasiou and Shore, 1997) that are not associated with the *S* locus provide an opportunity to characterize the regulatory functions of the *S* locus controlling heteromorphy in different species. Indeed, a further key observation from Labonne *et al*. (2010) is the finding that expression of the two morph-specific *Turnera* proteins known to be encoded by genes not associated with the *S* locus is absent in the long-styled and long-homostyle deletion mutants. This observation defines a regulatory role of the *S* locus in the modulation of unlinked genes that encode morph-specific characteristics.

A number of unknowns remain to be elucidated in *Turnera* and other heteromorphic models, such as the relationship between genetic map distance and physical map distance in each species, and the extent of recombination suppression within the *S* locus. Some of the *S* locus deletions presented in Labonne

et al. (2010) span up to 7 Mb, others are smaller but result in the loss of only one DNA marker and so the size cannot be estimated. However, a previous study suggested that the locus could be as compact as 32 kb (Labonne *et al*., 2009). Knowledge of the physical size of the *S* locus in *Primula* and *Fagopyrum* also remains elusive, but with rapid progress being made in the analysis of the *S* locus-linked genes, generation of genetic maps and screening of BAC libraries (Li *et al*., 2007, 2010; Labonne *et al*., 2008, 2009; McCubbin, 2008; Yasui *et al*., 2008), it is only a matter of time before sequences corresponding to each *S* locus are available for comparison of gene organization, function and genetic architecture. The current paper by Labonne *et al*. (this issue) takes us a step closer to homing in on the genes that control floral heteromorphy; one of the most remarkable mechanisms for preventing self-pollination and promoting outbreeding in plants.

Conflict of interest

The authors declare no conflict of interest.

Professor PM Gilmartin and Dr J Li are at the School of Biological and Biomedical Sciences, University of Durham, Durham, DH1 3LE, UK.

e-mail: philip.gilmartin@durham.ac.uk

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