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Article Addendum

Sex Determination in Plants

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Addendum to:

Premature Arrest of the Male Flower Meristem Precedes Sexual Dimorphism in the Dioecious Plant Silene latifolia

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ABSTRACT

Most dioecious plant species are believed to derive from hermaphrodite ancestors. The regulatory pathways that have been modified during evolution of the hermaphrodite ancestors and led to the emergence of dioecious species (with separate sexes) still remain unknown. *Silene latifolia* is a dioecious plant species harbouring XY sex chromosomes. To identify the molecular mechanisms involved in female organ suppression in male flowers of *S. latifolia*, we looked for genes potentially involved in the establishment of floral organ and whorl boundaries. We identified *Arabidopsis thaliana* homologs of *SHOOTMERISTEMLESS* (*STM*) and *CUP SHAPED COTYLEDON 1* (*CUC1)* and *CUC2* genes in *S. latifolia*. Our phylogenetic analyses suggest that we identified true orthologs for both types of genes. Detailed expression analyses showed a conserved expression pattern for these genes between *S. latifolia* and *A. thaliana*, suggesting a conserved function of the corresponding proteins. Both orthologs showed clear differences in their expression pattern between males and females or hermaphrodites suggesting their possible involvement in the sex determination pathway in *S. latifolia*.

SEX DETERMINATION IN PLANTS

Mechanisms underlying sex determination in plants are largely unknown. *Silene latifolia* is a dioecious species (with separate male and female individuals) which harbours XY sex chromosomes and constitutes an important model for sex determination in plants.[1-4] Chromosome deletion experiments have shown that the Y chromosome of *Silene latifolia* carries two loci involved in sex determination: the first involved in the suppression of female organ development and the second involved in the activation of male organ development. Several attempts to identify the corresponding genes and the regulatory pathways controlled by these have been unsuccessful.[2,5,6]

In the dioecious species *Silene latifolia*, four whorls of floral organs are observed in both male and female floral meristems, as it is the case for any hermaphrodite species: sepals, petals, stamens (male reproductive organs) and carpels (female reproductive organs). At an early stage, the flower meristem is similar in male and female plants (undifferentiated). As soon as all floral organ primordia are initiated, the female territory in the centre of the flower meristem is significantly smaller in male compared to female flower buds. Later, a filament develops in male flower, in place of female organs. In female flower buds, stamens are initiated but rapidly degenerate, whereas five fused carpels (female organs) develop in the centre.[7] In this study, we investigated the possible mechanisms that may lead to female organ arrest in male flowers of *S. latifolia.*

CANDIDATE GENES

Since we observed a whorl-specific arrest in cell proliferation in the early male flower meristem, we suspected a precocious arrest of the flower meristem in male individuals. We therefore decided to look for the orthologs in *Silene latifolia,* of two central genes in meristem function in *A. thaliana*: *WUSCHEL* (*WUS*) and *SHOOTMERISTEMLESS* (*STM*). We also looked for *CUP SHAPED COTYLEDON1 (CUC1)* and *CUC2* which have been shown to participate in meristem homeostasis in concert with *STM*.[8] Despite several attempts, we could not identify any WUS ortholog in *S. latifolia* but our phylogenetic reconstructions suggest that we have indeed identified orthologs of *A. thaliana STM* and *CUC1* and *CUC 2* in *S. latifolia*. We called them *SlSTM* and *SlCUC*.

We performed comparative in situ hybridization on young flower buds from male, female and hermaphrodite mutant plants (obtained by deleting portion of the Y chromosome[9]), using *SlSTM* and *SlCUC* as probes. First of all, the expression patterns observed reveal that Sl*STM* and *SlCUC* are likely to control apical meristem functions in *S. latifolia* in a way similar to *STM* and *CUC1* and 2 in *A. thaliana*. In addition, our results reveal a clear difference in the pattern of expression between males and females or hermaphrodites, before any morphological difference become apparent. In the male flower meristem, we observed a precocious disappearance of *SlSTM* in the centre and a concomitant unexpected expression of *SlCUC*. These observations reflect an early arrest of meristem function in male flowers and could be the cause of the lack of cell divisions observed. Our results make *SlSTM* and *SlCUC* strong candidates for being involved in sex determination in *Silene latifolia* and therefore open new perspective for molecular mechanism of sex determination in plants in general.

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