Identification of a sex determining gene in a dioecious plant *Silene latifolia*[†]

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Silene latifolia is a dioecious flowering plant with XY-type sex chromosomes belonging to the Caryophyllaceae family. Since its discovery in 1923 as one of the first species with sex chromosomes discovered in higher plants, it has been used for studies of sex chromosome evolution and sex-determining mechanisms in plants.[1,2] Two sex-determining genes have been postulated to be present on the Y chromosome: the male-promoting gene (SPF) and the female-suppressor gene (GSF). However, the Y chromosome is 1arge (approx. 660 Mb) and most of it comprises a non-recombining region and occupied by repetitive sequences, which has prevented genome sequence assembly and rendered the identification of sex-determining genes difficult.

We irradiated *S. latifolia* with heavy-ion beams to obtain several hermaphroditic (GSF-deficient) and asexual (SPF-deficient) mutants, and mapped the shared deleted regions of mutants as two sex-determining regions, respectively.[3] Genome sequencing and RNA-seq were performed on two of the hermaphroditic mutants with small deletion sizes. Sequences were then compared between male, female, and mutants. Consequently, we found three genes that were present and expressed only in males. One gene was commonly deleted in both of the 11 hermaphroditic mutants and females, which was identified as a candidate gene for *GSF*. Further, a Blast search revealed that this gene was highly homologous to the Arabidopsis *CLV3* gene, which regulates the size of the shoot apical meristems (SAMs) and flower bud primordia.[4]

clv3 mutants exhibited increased carpel size, while transgenic plants with overexpression of *CLV3* showed suppressed carpel development, suggesting that this gene is a promising candidate for the GSF. Furthermore, in addition to the Y copy (*GSFY*), we found its X-lined gametolog (*GSFX*). RT-PCR results showed that both *GSFY* and *GSFX* were expressed in the shoot apical meristems and young flower buds. The translation product of *CLV3* is known to act as a peptide with 12 amino acid residues in the final product. Therefore, we compared the amino acid residue sequences of GSFY and GSFX peptides and found that the sixth glycine residue in GSFX was mutated to an alanine residue. As this mutation is the same as that of the Arabidopsis *clv3-1/clv3-5* mutant (CLV3m), *GSFX* was considered to be a loss-of-function type. When these peptides were artificially synthesized and treated to the SAMs of *S. latifolia*. GSFY was found to be active in suppressing SAM size, similar to CLV3, where GSFX and CLV3m were inactive. Next, when *GSFY* was introduced into *Arabidopsis thaliana*, a hermaphroditic plant, the carpel development was inhibited. However, introduction of *GSFX* did not inhibit development of the carpel. Furthermore, treatment of flower buds of *S. latifolia* with the GSFY peptide also inhibited the development of the pistils. These results indicate that *GSFY*, a homolog of the *CLV3* gene, is a likely candidate of the sex-determining gene that suppresses gynoecium development in *S. latifolia*, and that *GSFX* on the X chromosome is dysfunctional.

We have previously shown that, in contrast to *GSFY, SlWUS1*, which is considered to function in enlarging the carpels, is present on the X chromosome but not on the Y chromosome in *S. latifolia*.[5] This suggests that the function of the X chromosome functions is to promote female development. In fact, there is a report in the old literature that the creation of XXXXY pentaploid chromosomes cause the carpels to enlarge and produce hermaphroditic flowers.[6] The manner in which the Y chromosome acquired the dominant *GSFY* during the evolution of sex chromosomes has been the greatest mystery; however, this system, wherein the X chromosome enlarges the carpels and the Y chromosome reduces its size, can successfully explain the emergence of the dominant GSFY (Fig. 1).

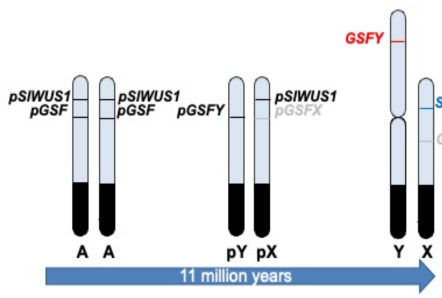

Fig. 1. Model for the evolution of *S. latifolia* sex chromosomes with *GSFX* dysfunction and loss of *SlWUS1* in the Y chromosome.

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