

Evolution of Sex Chromosomes and Gynoecium Suppression in Plants

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Summary White campion (*Silene latifolia*, Caryophyllaceae) is a classical model species for studies of sex determination and sex chromosome evolution in dioecious plants. Deletion mapping in this species revealed the presence of two Y-linked sex determining genes—the stamen promoting factor (*SPF*) gene and a gynoecium suppressing factor (*GSF*), which inspired the development of the classic ‘two genes’ model for dioecy evolution. We recently identified a Y-linked *GSFY* gene that encodes a *CLAVATA3* homolog and causes gynoecium suppression in *S. latifolia* via *WUSCHEL*-*CLAVATA* feedback loop. Interestingly, the *WUSCHEL* homolog in *S. latifolia* (*SIWUSI*) is also sex-linked and both *GSFY* and *SIWUSI* are located in the oldest part of the sex chromosomes, suggesting that selection to prevent recombination between these genes may have contributed to the origination of sex chromosomes in this species. The *WUS*-*CLV3* pathway is also involved in the sexual differentiation of gynoecium development in kiwifruits and melon, indicating that this pathway plays central role in gynoecium suppression in dioecious and monoecious plants.

Keywords *Silene latifolia*, Sex chromosome, Gynoecium suppression, Plant sex determination, *CLAVATA3*, *WUSCHEL*.

Most angiosperm plants have both stamens and pistils in a flower, making them hermaphrodites. However, about 6% of all angiosperms are dioecious, meaning they have male flowers with stamens and female flowers with pistils in different individuals (Renner and Ricklefs 1995). Some of these dioecious plants have sex chromosomes (Ainsworth 2000, Matsunaga and Kawano 2002, Vyskot and Hobza 2004). The emergence of the sex chromosomes has occurred independently in different plant species (Charlesworth 2002). Dioecious plants are thought to evolve from hermaphroditic plants via two main pathways; one is via gynodioecy (Fig. 1A; Westergaard 1958, Charlesworth and Charlesworth 1978, Dufaÿ *et al.* 2014) and the other is via monoecy (Renner 2014, Renner and Müller 2021).

The evolution of dioecy via gynodioecy involves at least two separate mutations, causing male and female sterility. The first is a recessive mutation that suppresses stamens or pistils in hermaphroditic populations, leading to evolution of gynodioecy or androdioecy, respectively.

The second is a dominant mutation that suppresses pistils or stamens, resulting in evolution of dioecy from gynodioecy or androdioecy, respectively (Weiblen *et al.* 2000, Charlesworth and David 2004, Dorken and Barrett 2004, Renner 2016). This ‘two-genes’ or ‘two-mutations’ model was supported by the studies in asparagus (Harkess *et al.* 2020) and kiwifruit (Akagi *et al.* 2019), where sex is determined by two sex determination genes; one is a stamen promoting factor and the other is a pistil suppressing factor.

If the two sterility mutations involved in dioecy evolution are segregating independently, a fraction of the population will inevitably inherit both mutations, making them completely sterile. Thus, recombination suppression between the two sex-determining genes is an essential feature of the two-genes model, which explains why non-recombining sex-determining region (SDR) evolves on sex chromosomes (Charlesworth and Charlesworth 1978). In case of both kiwifruit and asparagus, recombination suppression between the X- and Y-chromosomes in the SDR is achieved via hemizyosity of the Y-specific SDR (Akagi *et al.* 2019, Harkess *et al.* 2020). However, hemizyosity of the SDR is not universal in dioecious plants. For example, our recent work in *Silene latifolia* revealed that the Y-linked sex-determining gene *GSFY* has a dysfunctional X-linked

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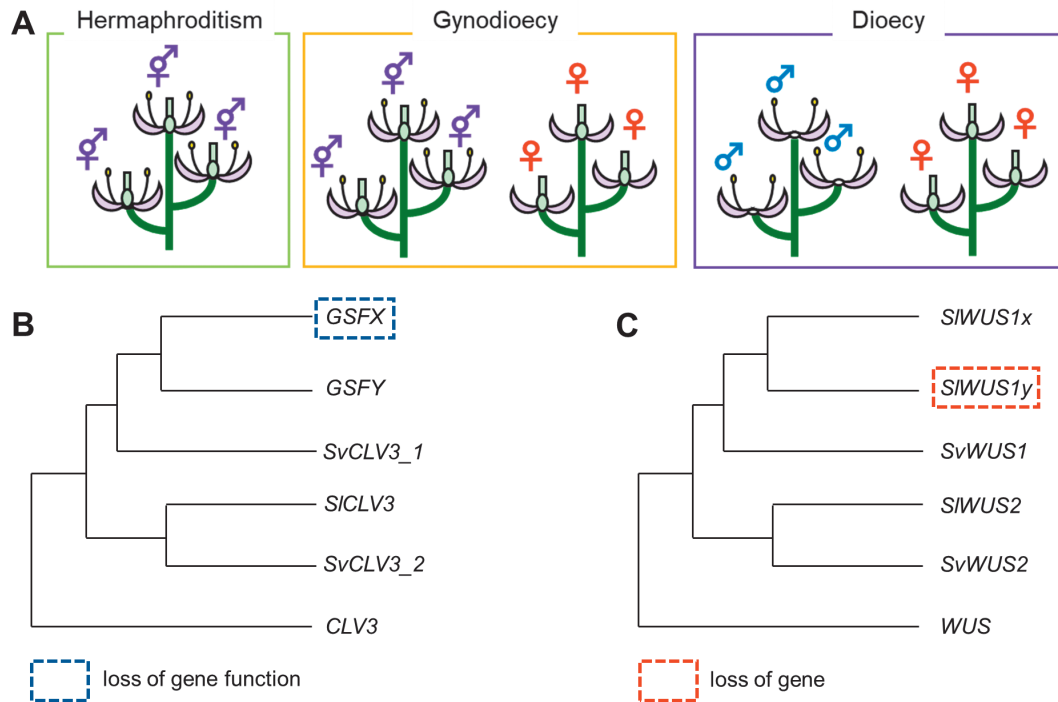


Fig. 1. Schematic diagram of gene loss in the *S. latifolia* sex chromosome evolution. (A) Diagram of three mating systems; hermaphroditism, gynodioecy, and dioecy. (B, C) Phylogenetic relationships of *CLV3* (B) and *WUS* (C) orthologs between hermaphroditic *A. thaliana*, gynodioecious *S. vulgaris*, and dioecious *S. latifolia*. Duplication of both *CLV3* and *WUS* genes occurred before the separation of the two *Silene* species. In *S. latifolia*, gene loss of *SIWUS* from the Y chromosome and dysfunctionalization of *GSFX* on the X chromosome occurred. Branch lengths are not proportional to genetic distance.

gametolog *GSFX* (Kazama *et al.* 2022) and earlier studies in this species reported intact and apparently functional X- and Y-linked gametologs for many sex-linked genes (Filatov *et al.* 2000, Atanassov *et al.* 2001, Moore *et al.* 2003, Filatov 2005). In many cases the sequence divergence between the X- and Y-linked copies is very low (<5% at silent sites, *e.g.*, Filatov *et al.* 2001) and it remains unknown what prevents recombination between the homologous X- and Y-linked genes in *S. latifolia* and other dioecious species with non-hemizygous SDRs (Charlesworth 2023).

S. latifolia is a dioecious plant of the Caryophyllaceae family, which evolved separate sexes and heteromorphic sex chromosomes *via* gynodioecy about 11 million years ago (Westergaard 1958, Desfeux *et al.* 1996, Kazama *et al.* 2003, Krasovec *et al.* 2018, Kobayashi *et al.* 2021), which makes it a convenient model species for studies of sex determination and sex chromosome evolution in plants (Bernasconi *et al.* 2010). Early genetic work in this species revealed the presence of two Y-linked sex-determining genes (Westergaard 1946), which contributed to the development of the two-genes model of dioecy evolution (Westergaard 1958, Charlesworth and Charlesworth 1978). More recent analysis of hermaphroditic and asexual *S. latifolia* mutants confirmed the presence and revealed the approximate locations of the gynoeecium suppression and stamen promotion genes on the Y-chromosome (Farbos *et al.* 1999, Lardon *et al.* 1999, Kazama *et al.* 2016, Aonuma *et al.* 2021). Extremely

large size of *S. latifolia* SDR (>500 Mb) makes identification of sex-determining genes difficult, but recently, we identified and functionally characterised the *S. latifolia* Y-linked gene *GSFY* responsible for the gynoeecium suppression (Kazama *et al.* 2022).

S. latifolia *GSFY* is a homolog of Arabidopsis *CLAVATA3* (*CLV3*) gene that encodes a small peptide consisting of 12 amino acids (Kazama *et al.* 2022), which is involved in shoot apical meristem development (Jennifer *et al.* 1999, Kondo *et al.* 2006). It is also known that *CLV3* acts in inflorescence meristems. In the Arabidopsis *clv3* mutants, a larger gynoeecium develops, whereas overexpression of *CLV3* shows smaller gynoeecium (Ulrike *et al.* 2000). Interestingly, the *S. latifolia* *GSFX*—the X-linked gametolog of *GSFY*, was expressed in shoot apical meristem (SAM) and small flower buds (Kazama *et al.* 2022). However, *GSFX* is dysfunctional as it encodes a peptide with the same change of amino acid as found in dysfunctional Arabidopsis *clv3-1* and *clv3-5* mutants (Song *et al.* 2012). The transformation of the *GSFX* gene and synthetic peptide treatments demonstrated that this X-linked copy is dysfunctional, while the Y-linked *GSFY* is functional and suppresses gynoeecium development (Kazama *et al.* 2022). Therefore, the evolution of gynoeecium suppression in *S. latifolia* likely involved defunctionalisation of *GSFX* copy (Fig. 1B).

Another gene that likely plays a role in *S. latifolia* gynoeecium suppression is *SIWUS1* that encodes Arabidopsis *WUSCHEL* (*WUS*) homolog (Kazama *et al.*

2012, 2022). In contrast to *CLV3*, *WUS* promotes gynoecium growth (Somssich *et al.* 2016) and *Arabidopsis wus* mutant has a smaller floral meristem, often resulting in the absence of a pistil (Laux *et al.* 1996). In *S. latifolia*, *GSFY* and *SIWUS1* likely jointly control gynoecium suppression via the *WUS-CLV3* feedback loop (Kazama *et al.* 2022), where the *CLV3* mRNA production is activated by *WUS* protein, while the expression of *WUS* is repressed by *CLV3*. Both of these genes are located in the oldest part of *S. latifolia* sex chromosomes (Kazama *et al.* 2022, Filatov 2023), consistent with their role in evolution of separate sexes and sex chromosomes in *S. latifolia* about 11 million years ago (Krasovec *et al.* 2018).

Analogous to *S. latifolia*, the *SyGI* gene in kiwifruit is related to *WUS-CLV3* pathway (Fig. 1); *SyGI* is a homolog of *Arabidopsis Response Regulator (ARR)* gene (Akagi *et al.* 2018), which is a cytokinin responsive gene that regulates the *WUS-CLV3* pathway (Argueso *et al.* 2010, Shi and Vernoux 2022). Similarly, in monoecious plants, cucumber and *Cucumis melo*, the *WUS-CLV3* pathway regulates carpel number (Che *et al.* 2020, Wang *et al.* 2022). In hermaphroditic plants, *Solanum lycopersicum L.*, the ortholog of *WUS*, *SIWUS* promotes carpel size (Li *et al.* 2017). Therefore, the *WUS-CLV3* may be one of the central pathways in controlling the size of carpels in monoecious and dioecious plants. This hypothesis builds parallels between sex-determination pathways of animals and plants. In animals, the upper sex-determination genes vary, while their intermediate steps converge on the genes *doublesex* or *DMRT1* (Kato *et al.* 2011). Further studies of the *WUS-CLV3* pathways will provide a deeper insight not only into the meristem maintenance but also into the floral sexual differentiation.

In *S. latifolia* the *WUS-CLV3* pathway directly controls gynoecium suppression, which requires interaction of the Y-linked *GSFY* and X-linked *SIWUS1* (Kazama *et al.* 2022). The former has a dysfunctional X-linked gametolog *GSFX*, while the latter lacks a Y-linked gametolog (Kazama *et al.* 2012). Based on linkage analysis of the *SIWUS1* orthologs in a gynodioecious related species, *S. vulgaris*, *SvWUS1* originally existed on both the proto-X and -Y chromosomes (Fig. 1A; Kazama *et al.* 2012). Thus, evolution of gynoecium suppression in *S. latifolia* involved two gene losses—dysfunctionalization of *GSFX* on the X- and disappearance of *SIWUS1* from the Y-chromosome (Fig. 1B, C). This led to sex-specific gene dosage balance that ensures gynoecium suppression only in males (Kazama *et al.* 2022). Recombination between these genes on proto-X and proto-Y chromosomes would have changed gene dosage balance, which would have disrupted gynoecium suppression achieved via joint action of Y-linked *GSFY* and X-linked *SIWUS1*. Thus, it may have been advantageous to suppress recombination between these genes, which could have

contributed to evolution of the non-recombining SDR in this species. Although plausible, this scenario for sex chromosome evolution and recombination suppression remains purely speculative and needs to be tested in the future. The actual mechanism(s) causing recombination suppression between the X- and Y-chromosomes in *S. latifolia* males also remain unclear. Molecular mechanisms of recombination suppression and their evolution on nascent sex chromosomes are poorly understood (Bergero and Charlesworth 2009, Charlesworth 2023) and their analysis in *S. latifolia* would significantly advance our understanding of sex chromosome evolution generally.

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