

A new physical mapping of the *Silene latifolia* Y chromosome[†]

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The majority of angiosperms are hermaphrodites and only 6% are dioecious (with separate males and females); some of these plants have sex chromosomes. Plant sex chromosomes are excellent for studying the early stages of sex chromosome evolution because they have evolved independently in different species, more recently than in animals.

Dioecious plants have sex-determining systems that are thought to involve two different genes, a stamen promoting gene and a pistil-suppressing gene. The dioecious plant, *Silene latifolia*, has such two sex determining genes on the Y chromosome (gynoecium-suppressing function; GSF, and stamen-promoting function; SPF). These sex-determining genes have not yet been identified, and their evolution has not yet been studied. Maps of the genes on the X and Y chromosomes are needed to identify Y-linked sex-determining genes, compare gene orders in the X and Y, and determine whether the Y has lost regions present on the X chromosome. However, most of the Y chromosome is non-recombining, making genetic mapping impossible. Mapping Y-chromosome deletion mutants is therefore the only way to map the Y chromosome. Previous Y chromosome maps^{1),2)} used small numbers of markers. We developed a new *S. latifolia* Y map with more markers to achieve high reliability and developed a new deletion mapping software tool, DelMapper.

We collected 41 mutants with Y-chromosome deletions from progeny plants derived from seeds or pollen irradiated by carbon-ion beams or γ -rays as described previously.³⁾ The mutants include 15 hermaphrodites, one female-like mutant, 10 asexuals, 14 mutants with non-maturing anthers, and one male plant. These mutants were genotyped for the presence/absence of deletions at 71 Y-linked markers by PCR amplification (STS-markers). Hermaphroditic mutants must have deletions of the GSF, while asexual mutants have lost the SPF. Our use of numerous genic markers improves our ability to locate the GSF and SPF regions in comparison with the use of non-genic markers.

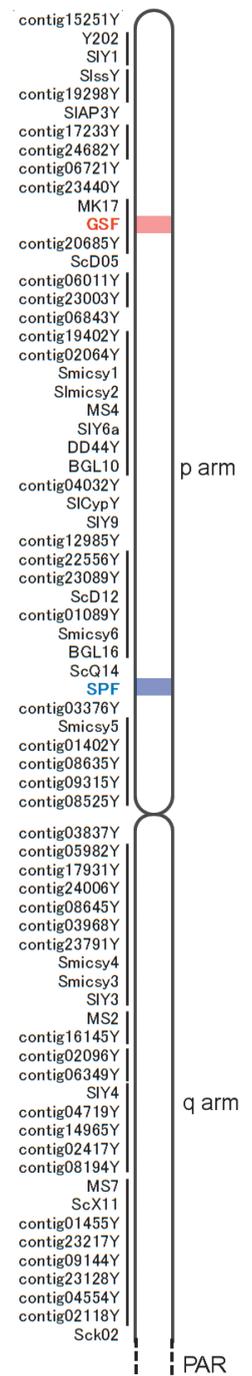
To find the marker order, DelMapper obtains the best fit to the data by solving a “Travelling Salesman problem”, minimizing the number of chromosome breaks required. Minimization of the chromosome breaks is a standard approach for deletion mapping, and the novelty of our method is that it can deal with many markers. We verified this approach by testing DelMapper using simulated datasets

with known gene orders. The accuracy of DelMapper depends strongly on the number of individuals with mutant phenotypes, and on the total number of deletions in the dataset, but the number of markers used is less important. Our simulations allow us to determine the accuracy of maps made using DelMapper.

The analyzed data included 442 instances of deleted markers among the 41 mutants. Based on our simulations, the accuracy of a map based on such a dataset is expected to be 90%. Our new Y-chromosome map infers that markers MK17 and ScQ14 are the closest to the GSF and SPF genes, respectively,^{1),2)} and it locates both of them on the putative p arm (the chromosome arm that does not include the pseudo autosomal region (PAR): see Fig. 1). We locate GSF between MK17 and genic contig20685Y, in an interior region of the p arm. SPF is located between ScQ14 and the genic contig 03376Y. This new map with these added genic markers provides improved ability to locate the GSF and SPF regions, in comparison with the previous maps.

Our new map will greatly facilitate the isolation of sex-determining factors and will be instrumental in other research focusing on sex chromosomes.

Fig.1 The new *S. latifolia* Y map. Markers indicated by bars are closely linked and their orders were not determined.



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