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Note

Comparison of the X and Y Chromosome Organization in *Silene latifolia*

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ABSTRACT

Here we compare gene orders on the *Silene latifolia* sex chromosomes. On the basis of the deletion mapping results (11 markers and 23 independent Y chromosome deletion lines used), we conclude that a part of the Y chromosome (covering a region corresponding to at least 23.9 cM on the X chromosome) has been inverted. The gradient in silent-site divergence suggests that this inversion took place after the recombination arrest in this region. Because recombination arrest events followed by Y chromosome rearrangements also have been found in the human Y chromosome, this process seems to be a general evolutionary pathway.

CHROMOSOMAL rearrangements are thought to have played a central role in the evolution of human sex chromosomes (Lahn and Page 1999). Inversion events have contributed to the genetic isolation and subsequent deterioration of the human Y chromosome (Lahn *et al.* 2001). Because the human Y chromosome is highly degenerated, dioecious plants with evolutionary young sex chromosomes (*Silene latifolia*, Vyskot and Hobza 2004; *Carica papaya*, Liu *et al.* 2004) are much more convenient for studying the early stages of the Y chromosome evolution. In *S. latifolia*, the presence of putative evolutionary strata (Nicolas *et al.* 2005), and also the data supporting the Y chromosome rearrangement in comparison with the X chromosome (Moore *et al.* 2003), have been reported recently. However, in the previous research, a low number of both X- and Y-linked genes were available, and a lack of an X chromosome map did not enable us to analyze the process of structural differentiation of the sex chromosomes in detail.

Lebel-Hardenack *et al.* (2002) have characterized Y chromosome deletions in a collection of X-ray-induced sexual phenotype mutants. They used AFLP markers that cosegregated with the wild-type Y chromosome to determine the extent of deletions in the Y chromosome of each mutant and built up a deletion map of the Y chromosome. Moore *et al.* (2003) improved this map by adding three Y-linked genes possessing copies on the X chromosome: *SlY1* (Delichere *et al.* 1999), *SlY4* (Atanassov *et al.* 2001), and *DD44Y* (Moore *et al.* 2003). The map consisted of several groups of DNA markers and an isolated key marker, *L26*. Group A markers were shown to be linked to the gynoecium suppressor, and the male plant lacking key group A markers was hermaphroditic. On the other hand, the marker *L26* was shown to be closely linked to the stamen-promoting function, as it was absent in nearly all Y chromosome deletion mutants with early and intermediate stamen arrest, but was present in all other mutants examined. Loss of the group C markers was shown to be linked to the late stamen developmental arrest and thus connected with the loss of male fertility function(s).

In this study, we used previously described Y-linked genes (*SlAP3Y*, Matsunaga *et al.* 2003; *SlY3*, Nicolas *et al.* 2005; *SlssY*, Filatov 2005a,b) and Y-linked PCR markers (*Bam37* and *Bgl10*, Donnison *et al.* 1996; *ScD05, ScQ14, ScX11*, and *ScK02*, Zhang *et al.* 1998; *ORF285*, Nakao *et al.* 2002; *MS4*, Obara *et al.* 2002) to refine the Y chromosome map using the Y chromosome deletion mutants described by Lebel-Hardenack *et al.* (2002). We performed PCR on genomic DNA samples isolated from the set of Y-deletion mutants using primers designed to amplify the Y-linked allele of each gene or Y-linked marker. The results of deletion mapping are summarized in supplementary Table S1 at http://www.genetics.org/supplemental/. The results were first evaluated by Fisher's exact tests (supplementary Table S2 at http://www.genetics.org/supplemental/) and logarithm of the odds (LOD) of linkage scores.

The gene *SlY4* (Atanassov *et al.* 2001) was found to

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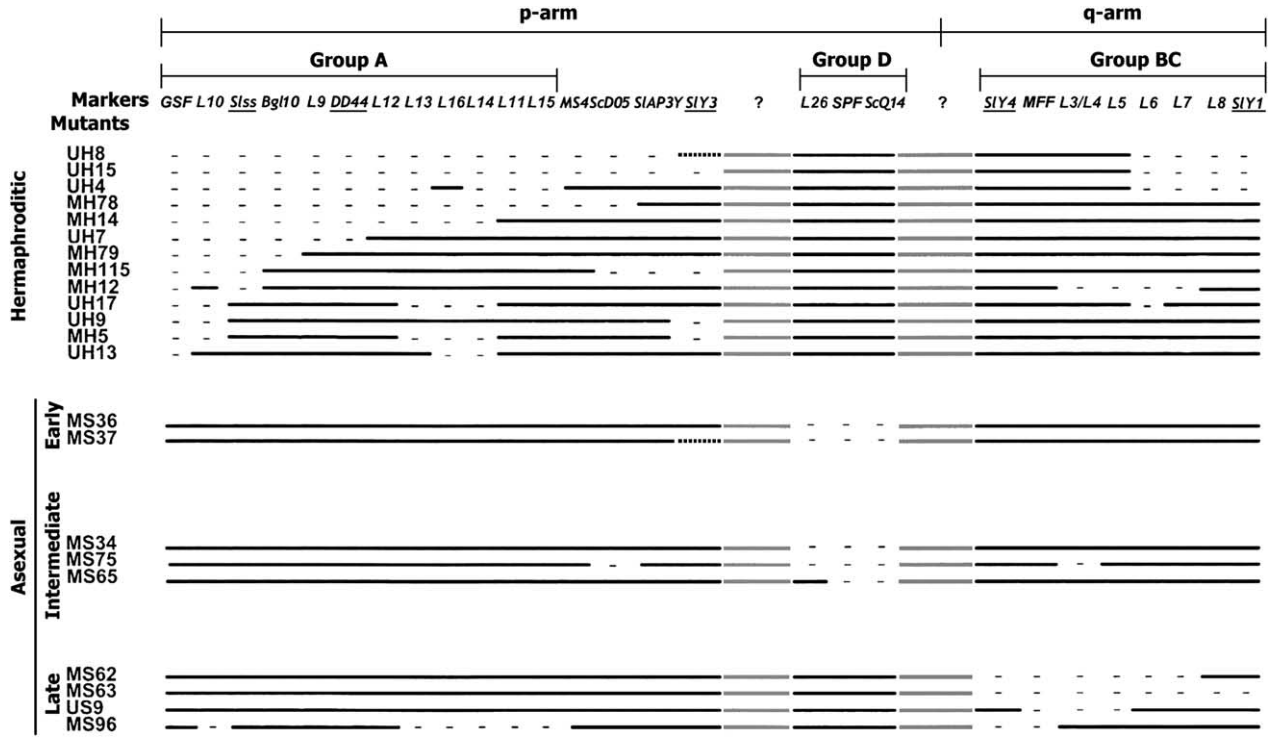

FIGURE 1.—Marker order on the *S. latifolia* Y chromosome based on the combination of the maximum-likelihood algorithm and the minimal break criterion (modified from LEBEL-HARDENACK *et al.* 2002 and MOORE *et al.* 2003). The extent of the bar corresponding to each mutant name represents the markers present in each mutant, while dashes indicate that the marker was missing. Dotted lines indicate where the presence of the marker was not studied. Lines over the marker names represent linkage groups with LOD >3. Because it was not possible to order all markers under the criterion $P < 0.05$ of Fisher's exact test, unordered markers (including the markers *ScK02, ORF285, ScX11*, and *Bam37*) are represented by "?." The pseudoautosomal region is expected to be at the right end. Genes having copies on the X chromosome are underlined. GSF, gynoecium-suppressing function(s); SPF, stamen-promoting function(s); MFF, male-fertility function(s).

be significantly associated with gene(s) responsible for late stamen development (MOORE *et al.* 2003). The genes *DD44Y* (MOORE *et al.* 2003) and *SlssY* (FILATOV 2005a,b) are significantly associated with gene(s) responsible for gynoecium suppression (MOORE *et al.* 2003; this study). We have found an association of *SlY3* with group A (supplementary Table S2 at http://www.genetics.org/supplemental/). The marker ordering using a combination of the maximum-likelihood algorithm with the equal retention probability model and the minimal break criterion also confirms this interpretation (Figure 1).

The marker *ScQ14* is closely linked to the stamen-promoting function, as it was absent in all Y chromosome deletion mutants with early and intermediate stamen arrest and was present in all other mutants examined. This linkage was proven statistically significant by Fisher's exact test (supplementary Table S1 at http://www.genetics.org/supplemental/). This marker is also closely linked to the marker *L26* (LOD >3). The markers *L26*, *ScQ14*, and the stamen-promoting function form a group in which markers are associated with LOD >3 but <4. To maintain the names of the linkage groups published before (LEBEL-HARDENACK *et al.* 2002), we named the linkage group formed of *L26*, *ScQ14*, and the stamen-promoting function group D.

The combination of the maximum-likelihood algorithm with the equal retention probability model and the minimal break criterion enabled us to correct the map by MOORE *et al.* (2003) and to put together the male-fertility function, the markers from groups B and C, and the genes *SlY1* and *SlY4* with LOD >3 but <4. In the schematic of the Y chromosome deletion map (Figure 1), the newly formed group is named group BC.

Because the deletions cannot continuously cover the whole Y chromosome (due to the need for an intact centromere to transmit the chromosome to daughter cells), it is not possible to construct a whole-chromosome deletion map. Moreover, the marker *Bam37* was present in all 22 mutants examined (DONNISON *et al.* 1996; this article), and LEBEL-HARDENACK *et al.* (2002) also found one marker present in all the mutants examined. To identify which markers and marker groups are present on a respective Y chromosome arm, we performed PCR on genomic DNA isolated from a male hairy-root culture with a complete deletion of the q arm of the Y chromosome (the cell line MD151). PCR analysis using *DD44*-specific primers confirmed the presence of the p arm, and nonamplification of the *SlY4* and *SlY1* confirmed the absence of the opposite q arm (MOORE *et al.* 2003). In our experiments on this cell

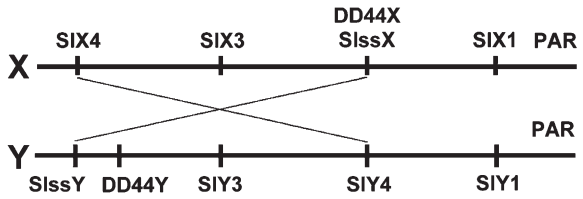

FIGURE 2.—Schematic of *S. latifolia* sex chromosomes (not in scale). Thin lines connect genes in the supposed inversion boundary.

line, specific primers amplified the genes *SlssY, SlY3*, and *SlAP3Y*, which are parts of group A, where *DD44Y* is also present. The results are summarized as supplementary Table S1 at http://www.genetics.org/supplemental/).

So far, the positions (in respect to the centromere) of only two (gynoecium-suppressing function and male-fertility function) of three sex determination functions are known (WESTERGAARD 1958; MOORE *et al.* 2003). Mapping of the marker *ScQ14* (which is closely linked to the stamen-promoting function) on the hairy-root mutant lacking the q arm enabled the localization of this third sex determination function on the p arm. We conclude that both the stamen-promoting function and the gynoecium-suppressing function are located on the p arm, whereas the male-fertility function is present on the q arm.

The gynoecium-suppressing function is located at the subtelomeric region of the p arm as deduced from the distribution of deletions in the hermaphroditic mutants (LEBEL-HARDENACK *et al.* 2002). The male-fertility function is probably located near the pseudoautosomal region (PAR), because the male-fertility function is a part of group BC, which also includes the gene *SlY1*. The gene *SlY1* stopped recombination with its X-linked copy, *SlX1* (DELICHERE *et al.* 1999), very recently (NICOLAS *et al.* 2005), and *SlX1* is closely linked to the PAR (NICOLAS *et al.* 2005). Moreover, the FISH pattern of three repetitive sequences used as markers at the subtelomeric part of the Y chromosome q arm is similar to the corresponding part of the X chromosome (LENGEROVA *et al.* 2004). A prominent rearrangement of the most recently evolved nonrecombining part of the Y chromosome is thus unlikely.

The deletion mapping approach enabled us to compare the positions of several Y-linked genes with their X-linked copies. The genetic mapping data revealed the order of the X-linked copies: PAR, *SlX1*, (*D44X* + *SlssX*), *SlX3*, and *SlX4* (FILATOV 2005a,b; NICOLAS *et al.* 2005). However, the order of their Y-linked copies is rather different. The most likely order on the q arm is PAR, *SlY1*, and *SlY4*, and the gene order on the p arm most probably is centromere, *SlY3*, *DD44*, and *SlssY* (Figure 2). This difference is apparently caused by a chromosomal rearrangement on the Y chromosome. The gene order observed on the Y chromosome could arise as a single chromosomal inversion covering the large region from *SlY4* to *SlssY*. The corresponding region on the X chromosome (from *SlssX* and *DD44X* to *SlX4*) covers ~23.9 cM (NICOLAS *et al.* 2005). Because the genes *DD44X/Y*, *SlX/Y3*, and *SlX/Y4* fall into several evolutionary strata, as revealed by silent nucleotide substitution divergences (NICOLAS *et al.* 2005), the considered inversion event occurred after the recombination arrest in the whole region between the genes *DD44X/Y* and *SlX/Y4*. Another possible explanation is that several independent Y chromosome rearrangements were involved in the Y chromosome rearrangement, but it is less likely.

As processes of successive recombination arrest (envisaged by the strata model in LAHN and PAGE 1999) associated with large chromosome rearrangements resulted in formation of the current human Y chromosome (SKALETSKY *et al.* 2003), this might be a general evolutionary pathway common to both animals and plants, which evolved sex chromosomes independently. The *S. latifolia* sex chromosome system appears to support the view that the inversion was a consequence of recombination arrest and not the cause.

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