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Evolutionary History of *Silene latifolia* Sex Chromosomes Revealed by Genetic Mapping of Four Genes

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ABSTRACT

The sex chromosomes of dioecious white campion, *Silene latifolia* (Caryophyllaceae), are of relatively recent origin (10–20 million years), providing a unique opportunity to trace the origin and evolution of sex chromosomes in this genus by comparing closely related *Silene* species with and without sex chromosomes. Here I demonstrate that four genes that are X-linked in *S. latifolia* are also linked in nondioecious *S. vulgaris*, which is consistent with OHNO's (1967) hypothesis that sex chromosomes evolve from a single pair of autosomes. I also report a genetic map for four *S. latifolia* X-linked genes, *SIX1*, *DD44X*, *SIX4*, and a new X-linked gene *SlsX*, which encodes spermidine synthase. The order of the genes on the *S. latifolia* X chromosome and divergence between the homologous X- and Y-linked copies of these genes supports the “evolutionary strata” model, with at least three consecutive expansions of the nonrecombining region on the Y chromosome (NRY) in this plant species.

IT has been suggested that sex chromosomes evolve from a pair of homologous autosomes (proto sex chromosomes), which stop recombining with each other and gradually diverge into mostly nonhomologous X and Y chromosomes (OHNO 1967; BULL 1983). Alternatively, sex chromosomes could have evolved in several stages, through consecutive translocations of autosomal fragments to the (proto)-sex chromosomes and gradual expansion of the nonrecombining region. Y-linked genes advantageous in males and detrimental in females may possess a selective advantage, promoting translocation of autosomal regions containing sexually antagonistic genes to the Y chromosome, as well as further expansion of the nonrecombining region on the Y chromosome (CHARLESWORTH and CHARLESWORTH 1980; BULL 1983; RICE 1987). Indeed, the distal regions of the human sex chromosomes are autosomal in marsupials (SPENCER *et al.* 1991), suggesting that modern mammalian sex chromosomes have formed due to translocations of fragments of autosomes to the sex chromosomes (WATSON *et al.* 1991). The *Drosophila* Y chromosome also appears to contain a patchwork of genes translocated from autosomes (CARVALHO 2002), casting doubts on the generality of OHNO's (1967) hypothesis that sex chromosomes evolve from a single pair of autosomes.

Genes with homologous X- and Y-linked copies on

the human sex chromosomes fall into four groups, depending on silent X/Y divergence (K_s), suggesting that different genes spent different amounts of time in the nonrecombining region of the Y chromosome, the NRY (LAHN and PAGE 1999). On the basis of this observation, it was suggested that the cessation of recombination on the mammalian Y was not a single event, but occurred in several stages spanning a timescale of 240–350 MY and creating four “evolutionary strata” with differing levels of divergence between the X- and Y-linked homologs (LAHN and PAGE 1999). Mouse (SANDSTEDT and TUCKER 2004) and bird (HANDLEY *et al.* 2004) sex chromosomes were also reported to have evolutionary strata. It is not clear, however, whether the stepwise formation of the sex chromosomes is a general process or is confined to mammals and birds.

Studying the origins of sex chromosomes in model organisms, such as *Drosophila*, mouse, human, and chicken is extremely difficult, as they arose hundreds of millions of years ago. The Y (or W in chicken) chromosomes in these species have diverged from the X (or Z) and lost almost all functional genes due to such processes as Muller's ratchet and genetic hitchhiking, leading to the accumulation of deleterious mutations and gene loss in the nonrecombining regions (CHARLESWORTH and CHARLESWORTH 2000). Many organisms, however, have much younger sex chromosomes [*e.g.*, plants *Silene latifolia* (FILATOV *et al.* 2000) and *Carica papaya* (LIU *et al.* 2004) and the stickleback fish (PEICHEL *et al.* 2004)].

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TABLE 1

Primers used for PCR amplification and sequencing of four genes in *S. latifolia* and *S. vulgaris*

Species	Gene	Primers		
		PCR forward	PCR reverse	Sequencing
<i>S. latifolia</i>	<i>SIX1</i>	SIX1 + 11	SIX1-7	N/A
	<i>DD44X</i>	DD44_3F	DD44XYR2.1	N/A
	<i>SlssX</i>	c2B12 + 1	c2B12-2	c2b12 + 1
	<i>SIX4</i>	SIX4 + 11	SIX4-3	N/A
<i>S. vulgaris</i>	<i>SvXY1</i>	SIXY1 + 3	SIXY1-10	N/A
	<i>SvDD44</i>	DD44XY + 1	DD44XYR2.1	DD44XYR2.1
	<i>Svss</i>	c2B12 + 6	c2B12-4	c2B12 + 9
	<i>SvXY4</i>	SIXY4 + 12	SIXY4-8	XY4 + 12

In particular, the plant genus *Silene* is very convenient for studying the early stages of sex chromosome evolution because sex chromosomes in this genus have been found only in a small cluster of dioecious *Silene* species (section *Elisanthe*: *S. latifolia*, *S. dioica*, *S. diclinis*, *S. heuffelii*, and *S. marizii*), where males are the heterogametic sex (XY) and females are homogametic (XX) (WESTERGAARD 1959). The rest of the *Silene* genus is nondioecious (except *S. otites*, which apparently evolved dioecy independently from *Elisanthe*), allowing a comparison of closely related species with and without sex chromosomes and a tracing of the origin of the sex chromosomes.

Silent divergence between dioecious *S. latifolia* and nondioecious *S. vulgaris* is ~20%, suggesting that the *S. latifolia* sex chromosomes are probably no older than 10–20 million years (FILATOV and CHARLESWORTH 2002). Degeneration has probably started on the *S. latifolia* Y chromosome, as one of the first isolated X-linked genes, *MROS3X*, has a degenerate Y-linked copy (GUTTMAN and CHARLESWORTH 1998). However, four further genes isolated from the X [*SIX1/Y1* (DELICHÈRE *et al.* 1999; FILATOV *et al.* 2000), *SIX4/Y4* (ATANASSOV *et al.* 2001), *DD44X/Y* (MOORE *et al.* 2003), and *SlssX/Y* (FILATOV 2005)] have intact X- and Y-linked homologs, suggesting that genetic deterioration of the *S. latifolia* Y-linked genes is at a very early stage. The silent divergence between the homologous *S. latifolia* X- and Y-linked genes varies from as little as 1.7% for the *SIX1/SIY1* to ~16% for the *SIX4/SIY4* genes, suggesting that the cessation of recombination between the proto-X and proto-Y chromosomes has been gradual, rather than the result of a single event (ATANASSOV *et al.* 2001; FILATOV and CHARLESWORTH 2002), and resembles the evolutionary strata discovered on the human sex chromosomes (LAHN and PAGE 1999). It is not known, however, whether the order of the genes on the X supports the evolutionary strata model. The difference in X/Y divergence of the *S. latifolia* sex-linked genes may also be due to consecutive

TABLE 2

Primer sequences

Name	Sequence
SIX1 + 11	AAGCTCACAAATGCTGATCTTCACTG
SIX1-7	ACTTGCAACGACTTCACCTTTGAG
SIXY1 + 3	AGGCTCGTTTCTCCCTTTGTG
SIXY1-10	TCCAGCAGAGCTTGAACAGTC
DD44_3F	CTTTGCTACCAAGGCTCCTG
DD44XY + 1	ATGTCAATGGCGAACCCGCAT
DD44XYR2.1	CTCCATCTGTCTTTGCCCTGG
c2B12 + 1	GTCCGTTGCAAAGGCTCTTC
c2B12-2	ACTCACGGACAGGTCTTTTGC
c2B12 + 6	GARATNAGYCCNATGTGGCCNG
c2B12-4	CAAAAGTAGATTGACGGAAACAGC
c2B12 + 9	GTAATCATTTTTGCCATCATCTCTT
SIX4 + 11	GTGGCCTGGCGTCTATGTG
SIX4-3	AATTACCGAAGACAGTAAAGCGTC
SIXY4 + 12	TCCAGAGGAACAAATACAAACAG
SIXY4-8	CTGAATGGGTAATACTCCGA

translocations of fragments of autosomes to the sex chromosomes. Such translocations are probably quite common because they were reported for many insect (BACHTROG and CHARLESWORTH 2002; JACOBS 2004), mammalian (WATSON *et al.* 1991; PACK *et al.* 1993), and plant (AINSWORTH 1999) species. Thus, without knowing the order of the genes on the *Silene* X chromosome, and without comparisons with the closely related nondioecious *Silene* species, it is difficult to infer the sequence of events that has led to the formation of the *Silene* sex chromosomes.

To shed light on the evolutionary history of the *S. latifolia* sex chromosomes, I constructed and compared genetic maps for X-linked genes in *S. latifolia* and their homologs in nondioecious *S. vulgaris*. For this study I used three published X-linked genes, the *SIX1* (DELICHÈRE *et al.* 1999; FILATOV *et al.* 2000), *SIX4* (ATANASSOV *et al.* 2001), and *DD44X* (MOORE *et al.* 2003) as well as a new X-linked gene, the *SlssX* encoding spermidine synthase (FILATOV 2005). The *MROS3X* (GUTTMAN and CHARLESWORTH 1998) has not been used because this gene is a member of a multicopy gene family with multiple autosomal copies (KEJNOVSKY *et al.* 2001), which would complicate genetic mapping and interpretation of the results.

To establish the relative positions of the *SIX1*, *SIX4*, *DD44X*, and *SlssX* genes on the *S. latifolia* X chromosome, I tested cosegregation of these genes in the F₁ progeny of the genetic cross described previously (“family 5” in FILATOV 2005). The fragments of the four X-linked genes were PCR amplified using primers shown in Tables 1 and 2. Conveniently, the female parent used in the cross was heterozygous for the PCR fragment size differences in three of four genes, *SIX1*, *DD44X*, and *SIX4*. Thus, the segregation of the alleles in these genes was inferred from the electrophoretic mobility of PCR products on 1.5% agarose gels. For the

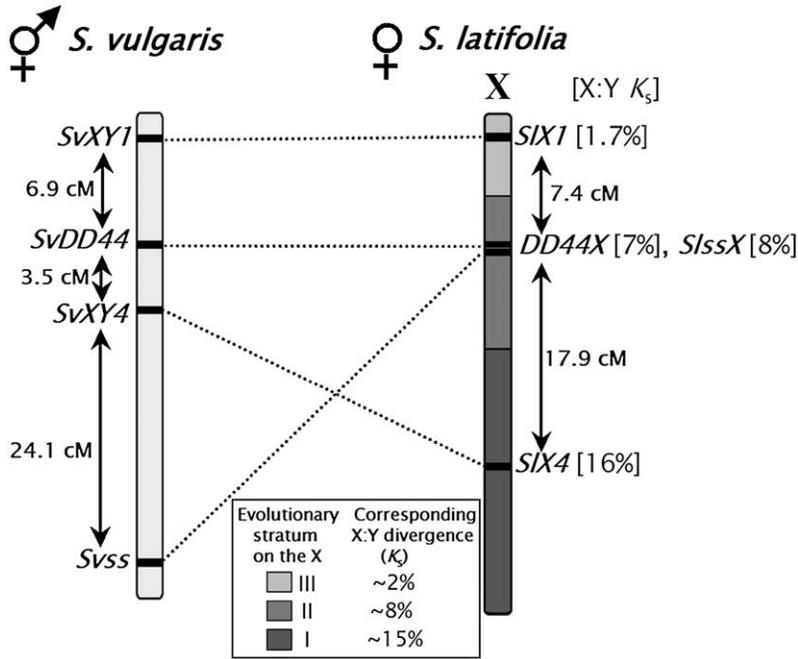


FIGURE 1.—Genetic map for four X-linked genes in dioecious *S. latifolia* and their homologous genes in nondioecious *S. vulgaris*. The percentages in brackets show intron divergence (K_s) of X-linked genes from homologous genes on the *S. latifolia* Y chromosome.

SlsX, however, no size difference of the PCR products was detected. Direct sequencing of the *SlsX* PCR product from the maternal and paternal individuals of the family 5 (FILATOV 2005) revealed two nucleotide positions heterozygous in the female and hemizygous in the male. As none of these polymorphisms were located at restriction sites, the segregation of the *SlsX* gene was tested by direct sequencing of the PCR products of all the F_1 progeny.

The four X-linked genes were genotyped in the parents and in 136 F_1 progeny (40 males and 96 females). No recombinants were found between the *SlsX* and *DD44X* genes, demonstrating that the two genes are tightly linked (<1 cM). The *SIX1* and *SIX4* genes are the most distant from each other (24.3 cM), while the distances from the *SlsX*/*DD44X* cluster to the *SIX1* and the *SIX4* genes are shorter: 7.4 and 17.9 cM, respectively (see Figure 1 and Table 3). Hence, the most likely order of the four genes on the X chromosome is *SIX1*, *SlsX*/*DD44X*, and *SIX4*. This order corresponds to what would be expected from the evolutionary strata model: the youngest (*SIX1*/*Y1*, $K_s = 1.7\%$) and the oldest (*SIX4*/*Y4*, $K_s = 16\%$) sex-linked genes are at the two ends of the map, while the two genes with intermediate X/Y silent divergence (*SlsX*/*Y*, $K_s = 8\%$ and *DD44X*/*Y*, $K_s = 7\%$) are located in the middle (Figure 1).

The order of the three *S. latifolia* genes, *SIX1*, *DD44X*, and *SIX4*, mapped in an independent genetic cross (NICOLAS *et al.* 2005) is consistent with the results reported in the current study. However, the genetic distances between these genes reported here are somewhat shorter, compared to those observed by NICOLAS *et al.* (2005). The reasons for this discrepancy are unclear. It may well reflect the variation in recombination

rate among the *S. latifolia* individuals. If the maternal individual in the *S. latifolia* cross reported here is heterozygous for an inversion located between the *SIX1* and *SIX4* genes, this could explain the shorter genetic distances reported in the current study. Interestingly, according to the map reported by NICOLAS *et al.* (2005), the *SIX1* gene is the closest to the pseudoautosomal region, which is also consistent with the evolutionary strata model.

To establish whether the *S. latifolia* sex chromosomes originated from a single pair of autosomes or were assembled from fragments of several autosomes, I conducted a segregation analysis of four *S. vulgaris* genes—*SvXY1*, *Svss*, *SvDD44*, and *SvXY4*, which are homologous to the *S. latifolia* sex-linked genes *SIX1*/*Y1* (DELICHERE *et al.* 1999; FILATOV *et al.* 2000), *SlsX*/*Y* (FILATOV 2005), *DD44X*/*Y* (MOORE *et al.* 2003), and *SIX4*/*Y4* (ATANASSOV *et al.* 2001), respectively. If *S. latifolia* sex chromosomes have evolved from a single pair of autosomes, these genes would be expected to be linked in nondioecious *S. vulgaris*. *S. vulgaris* is gynodioecious (females and hermaphrodites) and lacks sex chromosomes. The genetic control of gynodioecy in this species is nuclear cytoplasmic (CHARLESWORTH and LAPORTE 1998) and apparently originated independently from that of dioecy in *S. latifolia*.

For the segregation analysis in *S. vulgaris* I used 58 F_1 progeny of the cross between two *S. vulgaris* individuals grown from seeds kindly provided by D. Charlesworth (University of Edinburgh). Two parental *S. vulgaris* individuals and 58 F_1 progeny were genotyped for the *SvXY1*, *Svss*, *SvDD44*, and *SvXY4* genes. In the *S. vulgaris* cross, only the maternal parent was heterozygous for the size of the PCR product of the *SvXY1* gene, which allowed

TABLE 3

The number and proportion (in parentheses) of recombinants between the four genes in *S. latifolia* (above diagonal) and *S. vulgaris* (below diagonal) crosses

<i>S. latifolia</i> genes	<i>SIX1</i>	<i>DD44X</i>	<i>SlsX</i>	<i>SIX4</i>	—
<i>SIX4</i>	33 (0.24)***	24 (0.18)***	24 (0.18)***	—	<i>SvXY4</i>
<i>SlsX</i>	10 (0.07)***	0***	—	14 (0.24)***	<i>Svss</i>
<i>DD44X</i>	10 (0.07)***	—	16 (0.28)**	2 (0.04)***	<i>SvDD44</i>
<i>SIX1</i>	—	4 (0.07)***	19 (0.33)*	6 (0.10)***	<i>SvXY1</i>
—	<i>SvXY1</i>	<i>SvDD44</i>	<i>Svss</i>	<i>SvXY4</i>	<i>S. vulgaris</i> genes

Asterisks correspond to significance of linkage (deviation from independent segregation) tested by G-test (SOKAL and ROHLF 1995): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

me to study segregation of this variant on agarose gels. Segregations of all the other genes were studied by direct sequencing of the PCR products of the parents and F₁ offspring. The primers used for PCR amplification and sequencing are listed in Tables 1 and 2. The segregation analysis in the *S. vulgaris* cross demonstrated that all four genes are linked in *S. vulgaris*, supporting the hypothesis that *S. latifolia* sex chromosomes evolved from a single pair of autosomes (Figure 1 and Table 3). Interestingly, the distance between *SvDD44* and *SvXY4* reduced to only 3.5 cM, while the distance between the *SvDD44* to *Svss* increased to 27.6 cM, compared to the distances on the X chromosome of *S. latifolia*. This demonstrates that the order of the genes in *S. vulgaris* differs from that in *S. latifolia*, probably due to an inversion, including *Svss* and *SvXY4* genes.

Mapping of the four genes on the *S. latifolia* X chromosome and in the nondioecious *S. vulgaris* has demonstrated that, despite very different divergence between the homologous X- and Y-linked copies of these genes, all four genes are linked in both species, suggesting that *S. latifolia* sex chromosomes have evolved from a single pair of autosomes via formation and expansion of the NRY. Although only four genes were used in this study, they fall into at least three evolutionary strata and might represent a significant proportion of the *S. latifolia* sex chromosomes (~25 cM according to the map reported here and >50 cM according to NICOLAS *et al.* 2005). Nevertheless, it is still possible that parts of the *S. latifolia* sex chromosomes were translocated from the autosomes. Indeed, the autosomal *S. latifolia* *Slap3* gene was reported to have a functional Y-linked homolog in this species, which probably originated due to a translocation from the autosome to the Y chromosome (MATSUNAGA *et al.* 2003). Thus, translocation of genetic material to the sex chromosomes observed in many species (WATSON *et al.* 1991; PACK *et al.* 1993; AINSWORTH 1999; BACHTROG and CHARLESWORTH 2002; JACOBS 2004) may also be common in *Silene*. With only four genes it may be difficult to detect such translocations. Isolation and mapping of further sex-linked genes will lead to a much

more detailed picture of the evolution of *S. latifolia* sex chromosomes.

Originally, cessation of recombination between the proto X and Y chromosomes in the *S. latifolia* lineage probably occurred in the region including the *SIX4/Y4* gene. The formation of the original NRY (stratum I, containing *SIX4/Y4*) in *S. latifolia* was followed by at least two further expansions of the NRY region, creating stratum II, including the *SlsX/Y* and the *DD44X/Y* genes and, finally, stratum III, which includes the *SIX1/Y1* gene. Because a similar sequence of NRY expansions apparently resulted in formation of the human sex chromosomes (LAHN *et al.* 2001), this might be a general evolutionary pathway, which may be followed in most groups of animal and plant species, which evolved sex chromosomes independently.

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