Report

Current Biology

Gene Loss from a Plant Sex Chromosome System

Highlights

- We show that genes have been lost from the Y chromosome of the plant *Silene latifolia*
- Loss of Y gene expression is mainly due to gene loss rather than silencing
- Chromosome-wide dosage compensation has not yet evolved in this XY chromosome system

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In Brief

Degeneration of non-recombining sex chromosomes is predicted, but gene loss from plant Y chromosomes may be limited by selection in the haploid gametophytes. Bergero et al. document and estimate the extent of gene losses from a recently evolved plant Y chromosome and show that compensating mechanisms do not counteract loss of expression.

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Gene Loss from a Plant Sex Chromosome System

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SUMMARY

Sex chromosomes have evolved independently in numerous animal and plant lineages. After recombination becomes suppressed between two homologous sex chromosomes, genes on the non-recombining Y chromosomes (and W chromosomes in ZW systems) undergo genetic degeneration, losing functions retained by their X- or Z-linked homologs, changing their expression, and becoming lost [1, 2]. Adaptive changes may also occur, both on the non-recombining Y chromosome, to shut down expression of maladapted genes [3], and on the X chromosome (or the Z in ZW systems), which may evolve dosage compensation to increase low expression or compensate for poor protein function in the heterogametic sex [2, 4, 5]. Although empirical approaches to studying genetic degeneration have been developed for model species [3, 6], the onset and dynamics of these changes are still poorly understood, particularly in de novo evolving sex chromosomes. Sex chromosomes of some plants evolved much more recently than those of mammals, birds, and *Drosophila* [7–9], making them suitable for studying the early stages of genetic degeneration in de novo evolving sex chromosomes. In plants, haploid selection should oppose gene loss from Y chromosomes, but recent work on sex chromosomes of two plant species has estimated that Y-linked transcripts are lacking for 10%–30% of X-linked genes [10–12]. Here, we provide evidence that, in *Silene latifolia*, this largely involved losses of Y-linked genes, and not suppressed expression of Y-linked alleles, or gene additions to the X chromosome. Our results also suggest that chromosome-wide dosage compensation does not occur in this plant.

RESULTS AND DISCUSSION

To study genetic degeneration and its time course, we used the plant *Silene latifolia*. Silent site divergence between this plant's X and Y chromosomes is less than 25%, suggesting that recombination between the X and Y first stopped about 5–10 mega-annum (Ma) ago [13, 14]. Analyses of transcriptome sequences found that Y-linked alleles of many loci show excesses of non-synonymous substitutions, compared with their X-linked counterparts [10, 11]. Since adaptive evolution (the alternative explanation for non-synonymous substitutions) is unlikely to affect most Y-linked genes, this suggests that recombination suppression has already led to Y-linked genes losing function, despite the likelihood of purifying selection on some genes in the haploid phase. This evidence for genetic degeneration is consistent with evidence for a greatly reduced effective population size of the *S. latifolia* Y, based on much lower sequence diversity than for X-linked homologs or genes in genome regions in the species [15, 16], which is indirect evidence for the operation of processes causing ongoing genetic degeneration [17]. Such degeneration can potentially lead to Y-bearing pollen losing competitive ability, relative to X-bearing pollen, leading to female-biased progenies, as observed in many dioecious plants, including *S. latifolia* [18].

Using transcriptome sequences, we previously ascertained several hundred *S. latifolia* X-linked genes solely through one or more SNP variants segregating in the female parent of a family [10]. 435 of them are fully rather than partially sex-linked (see Supplemental Experimental Procedures) and have transcribed Y copies. In addition, we ascertained a set of 106 X-linked genes, with no detectable Y-linked transcripts. Depending on how many informative SNP variants were used to classify a gene as X-linked, we estimated that between 10% and 20% of X-linked genes have no (or very low) expression of their Y-linked alleles. Either (1) these genes' Y-linked copies were lost, or (2) their expression is very low, or else (3) Y-linked genomic copies of X-linked genes are lacking because these genes have been added to X chromosome regions that do not recombine with the Y. To test for gene loss, and distinguish this from the other possibilities, we used both a PCR-based approach and high-throughput genotyping assays (Illumina GoldenGate) applied to a set of 68 of these putatively X-linked genes that lacked Y transcripts (Table 1). Marker segregation in males from an F2 family indicated that 61 of these genes were indeed fully X linked (Table 1), and we used their segregation patterns to identify genes likely to be male hemizygous (candidates for gene losses from the Y chromosome; see Figure S1). Out of the 61 X-linked genes tested by at least one approach, 52 appeared to lack Y genomic copies, i.e., to be hemizygous in males (Figure 1; Table 1). Of 18 genes tested by both methods, all but one yielded identical conclusions with respect to hemizygosity, as did 15/16 tests that used different regions of the same gene (Table S1). Therefore, the agreement of tests using different primers is very high, giving confidence that the results are not due to PCR failures due to DNA sequence variants. The two methods yielded very similar overall proportions of hemizygous X-linked

Table 1. Numbers of Genes Tested for X Linkage and for Hemizygosity by the PCR and GoldenGate Tests

Result	Genes Tested	Method Used for Tests		
		Genes Tested by PCR Only	Genes Tested by GoldenGate Only	Genes Tested by Both PCR and GoldenGate
Genes tested	68	34	15	19
Autosomal or pseudoautosomal	7	2	4	1
Tested for hemizygosity				
Y copy present	9	5	0	4[a]
Hemizygous	52	27	11	14
Total tested for hemizygosity	61	32	11	18

The initial set included 106 putatively X-linked genes without detectable expression of corresponding Y-linked copies. 53 of these genes were chosen randomly for testing by PCR, and all 106 were submitted for primer design for GoldenGate genotyping, which yielded results for 34 genes.
[a]Both methods identified Y copies in three of these four genes. The exception was gene E849 (no Y copy was detected by PCR, but a Y-linked copy was detected by GoldenGate; see Table S1); this gene was categorized as probably having a Y-linked copy.

genes (42/50 = 84% by PCR and 25/29 = 86% by GoldenGate genotyping).

Although PCR and GoldenGate assays may, of course, sometimes fail to detect Y-linked alleles of X-linked genes, our PCR primers were designed to minimize such failures (see details in Supplemental Experimental Procedures), and the good repeatability just mentioned shows that such problems are rare. Our previous PCR tests, using the same primer design principles, failed to detect the Y copy of 1/13 (7.7%) of genes already known to have Y-linked copies, including several with high sequence divergence [10]. We further tested our ability to detect Y-linked copies, when present, by including 52 such genes with Y-linked copies in our GoldenGate assays; GoldenGate failed to detect seven of the genes (13.5%). Overall, therefore, we should have only slightly overestimated gene losses.

Hemizygosity in males could also arise by gene movement to a fully X-linked region, rather than gene loss. This has been detected in papaya [20]. We tested for this by genetically mapping orthologs of hemizygous genes in the related non-dioecious species, *Silene vulgaris*, in which the linkage group homologous with the *S. latifolia* fully sex-linked region is known [19]. All 16 genes that we could test in *S. vulgaris* (all of our *S. latifolia* genes tested for hemizygosity whose sequences in the parents of our *S. vulgaris* mapping families had variants that could be used for genotyping) indeed mapped to this *S. vulgaris* linkage group (Figure 1B), implying that they have been carried on the same chromosome since before these two species split, rather than having moved onto the X chromosome. These results indicate that gene additions onto the *S. latifolia* X chromosome are uncommon. Even using the most generous estimate of the number of genes added (using the upper 95% confidence interval from the binomial distribution of the proportion 0/16), at most 11 of the 52 hemizygous X-linked genes could represent additions to the X. The *S. latifolia* Y chromosome has therefore indeed lost genes.

Overall, we found Y-linked genomic sequences for only nine of the genes without detectable Y-linked transcripts that we tested (Table 1), some of which may have undergone mutations greatly reducing the Y alleles' expression. Therefore, lack of detectable Y expression largely represents losses of functional genes from the Y, and at most 15% of our tested genes are present but silenced. This places an upper limit on the number of Y-linked genes that are not expressed due to adaptive silencing.

The estimates described so far are for the proportion of genes lost among X-linked genes that have no detectable Y-linked transcripts. We also estimated the absolute proportion of gene losses, relative to the total number of X-linked genes (with or without Y-linked copies) inferred solely from X-linked variants (Supplemental Experimental Procedures). Using a corrected estimate of 76 hemizygous genes (which takes into account the untested candidate hemizygous genes; see Supplemental Experimental Procedures) and the inferred total number of 435 X-linked genes with Y copies, we obtained an estimate of gene loss of 14.5%.

We next examined the time course of Y chromosome degeneration. Sex chromosome pairs often include regions in which recombination became suppressed at different times. Such "evolutionary strata" were first discovered in mammalian XY pairs [21], and the *S. latifolia* XY sex chromosome pair has at least two strata [22], with the more recent stratum adjacent to the recombining pseudo-autosomal region (PAR). To test when gene losses from the *S. latifolia* Y chromosome occurred and whether the younger stratum has already experienced such changes, we used silent site divergence between Y- and X-linked sequences to indicate the different times of recombination suppression [21, 22]. We detected gene losses from both the old and young strata (Table 2; Figure 1A), including genes mapping near the PAR boundary, some of which were involved in the rearrangement shown in Figure 1B.

For genes in the younger stratum that have retained Y-linked copies, the mean X-Y sequence divergence is only 3.16% (based on 5,241 sites in 7 XY genes; standard error 0.89%), so non-detection of Y-linked copies is highly unlikely to be due to sequence differences causing failure of our PCR or GoldenGate tests, which might sometimes occur for the old stratum genes (although, as explained above, recombination stopped much more recently, even in this region, than in mammal or bird sex chromosomes). Therefore, our inability to detect genomic copies of 41 genes in the young stratum (or 34 if we exclude genes in the rearranged region; see Figure 1B and Table 2) strongly suggests either gene loss or large sequence divergence after pseudogenization (since a loss-of-function mutation causes loss of the selective constraints maintaining sequence similarity with the X-linked allele).

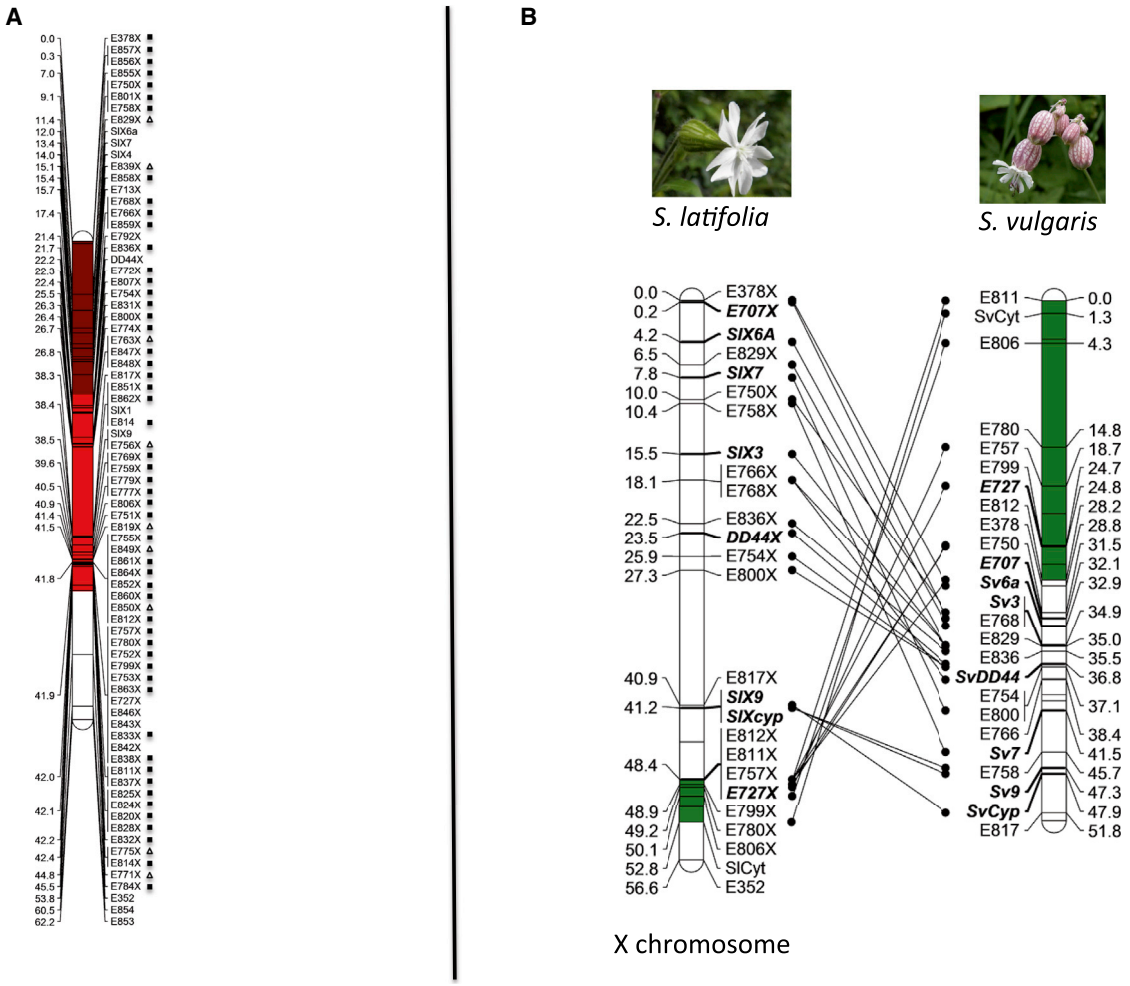

Figure 1. Genetic Mapping of X-Linked Genes with Lost or Silenced Y Copies

(A) 61 X-linked genes tested for hemizygosity were newly mapped to the *S. latifolia* X chromosome, using informative variants (Figure S1) genotyped by GoldenGate and/or PCR assays (Table S1). Genetic map distances are from segregation data in a family of 60 F2 plants. The old and young evolutionary strata (Table S2) are indicated in dark and light red, respectively. Solid black squares denote genes without Y copies in our genomic tests, and open triangles denote X-linked genes where we detected genomic Y copies, despite no Y-linked expression being detectable.

(B) Comparative mapping of 16 hemizygous genes in *S. latifolia* and their orthologs in *S. vulgaris*. All 16 genes map to *S. vulgaris* LG Sv12, which is homologous to the non-recombining part of the *S. latifolia* X chromosome [19]. Bold font indicates XY genes mapped in previous studies. A rearrangement (in green) has occurred, including eight genes, seven of them hemizygous in *S. latifolia*.

We also estimated the absolute proportions of gene losses in the two strata, using the corrected estimate of 76 hemizygous genes (Supplemental Experimental Procedures). To obtain the numbers of X-linked genes that are in the old and young strata, we estimated the relative proportions of 435 X-linked genes with Y copies in the two strata (Supplemental Experimental Procedures). 86% of a random subset of these 435 genes had X chromosome map locations within the young stratum (Table S4). We thus extrapolated the estimates of gene content for the two strata (374 and 61 genes for the young and old stratum, respectively) and combined it with our hemizygosity results to estimate the proportions of stratum 1 and 2 genes that have become hemizygous (Table 2). Although we detected more hemizygous genes in the young stratum than in the old stratum (41 versus 11, respectively), our genetic mapping of X-linked genes with Y copies shows that the younger stratum includes more genes (it either has a higher gene density or is physically larger) than the older one. Taking this into account, the estimate of gene losses from the old stratum is larger (though not significantly so) than that for the young one (Table 2).

The two strata also allow us to test whether gene loss is a secondary consequence occurring after silencing. This scenario predicts a higher proportion of silenced genes in the young stratum than in the old stratum, as the young stratum should include more genes that are silenced, but not yet lost. As explained above, we found only nine silenced genes among the genes with undetectable Y-linked transcripts that we tested, and Table 2 shows that these are represented in similar proportions in both strata. Although the number of silenced genes is small, and future work should apply this analysis to larger numbers, once more genes are ascertained, there is no sign from the current results that the young stratum is enriched for silenced genes.

Table 2. Time Course of Loss from the Y or Silencing, Based on Fully X-Linked Genes

Stratum	Potentially Hemizygous X-Linked Genes Tested	XY Divergence (K_s)	Genes Lost versus Non-expressed		Estimated Gene Loss	
			Y-Linked Copies Present, but Not Transcribed	Y-Linked Genomic Copy Not Detected	Genes Without Y-Linked Copies[a]	Estimated Proportions of Genes Lost
Old	13	20%–25%	2	11	16	0.21
Young	41	3%–8%	7	34	60	0.14[b]
Young (2a only)[c]	7	5.2%	0	7		

The numbers of genes without detectable expression of Y-linked copies and of gene losses, in the old and young recombination-suppressed chromosome strata (estimated as XY divergence, K_s). Strata 1 (old) and 2 (young) were defined as regions with non-overlapping confidence intervals of their K_s values (Table S2). Stratum 2a genes map close to stratum 2 genes on the *S. latifolia* X chromosome but are rearranged with respect to the homologous region of the *S. vulgaris* linkage group (Figure 1B, green region). See also Figure S2 and Tables S3 and S4 for inference of gene loss estimates.

[a]The estimated numbers of X genes without Y copies include the inferred number of gene losses (based on results from our test of hemizygosity) from the set of 31 genes not tested.

[b]This estimate was obtained including hemizygous genes from stratum 2a.

[c]XY divergence for stratum 2a is based on one XY gene, so the time when recombination with the *S. latifolia* X chromosome stopped is not certain.

The marker density in our genetic map is not yet sufficient to detect whether gene loss occurs on a gene-by-gene basis or in larger deletion events. However, a high proportion of the genes in a rearranged region (stratum 2a in Table 2 and green region in Figure 1B) appear to have been lost, suggesting the possibility of a large deletion. The modality of gene loss can potentially be investigated in future studies that use screening of bacterial artificial chromosome (BAC) clones to determine whether loss of an X-linked gene is often accompanied by loss of nearby genes.

Gene loss in XY chromosome systems is expected to lead to the evolution of dosage compensation largely because loss of Y chromosome copies of genes reduces these genes' expression in males, and this is often not completely recessive in its effect on fitness; dosage compensation may also sometimes be favored due to deleterious effects of unbalanced expression level of genes whose products participate in protein complexes [2, 23, 24]. Previous studies have estimated expression levels of X- and Y-linked alleles in *S. latifolia* female and male plants to test whether dosage compensation occurs in this species. One study [11] concluded that complete dosage compensation has not evolved in *S. latifolia*, based on 127 genes without detectable Y transcripts in RNA sequencing (RNA-seq) data, although partial dosage compensation could not be excluded. However, another study, using genes that still have detectable Y transcripts, concluded that dosage compensation has evolved [25]. We therefore examined the possibility of dosage compensation for our genes with well-supported X linkage and with Y copies either absent or not detectably expressed.

Tests using X-linked genes whose Y-linked copies have been lost have two advantages for testing for dosage compensation, compared with genes that still have Y copies. First, dosage compensation may be most likely to evolve in response to complete gene silencing or gene loss [2, 26] because such genes will lower fitness similarly to cases of aneuploidy [27]. Second, Y gene losses must affect both the haploid and diploid stages in plants, whereas low expression of Y-linked alleles might be tissue specific or differ between these phases. However, many plant genes are unlikely to undergo genetic degeneration. In *Arabidopsis thaliana*, and in other distantly related species, 60% of genes are expressed in male gametophytes [28–30] and are presumably important for functions in the haploid male gametophytes, including pollen competitive ability. Losses from plant Y chromosomes may therefore be largely restricted to around 40% of genes (those that are not expressed in male gametophytes), and losses of these genes are expected to trigger dosage compensation in males' diploid tissues, just as in animals.

To assess the evidence for dosage compensation, we estimated ratios of male to female expression from the transcriptome data for our genes. We compared ratios for our 99 X-linked genes without detectable expression of Y-linked copies with estimates for autosomal genes (Figures 2A and S3A). Figure 2A shows that the distribution of the male/female (M/F) ratios centers around the value of 0.5, as expected under no dosage compensation. These X-linked genes' median is 0.52, not significantly different from 0.5, the ratio expected without dosage compensation ($P = 0.36$, by bootstrapping), whereas the median M/F ratio for autosomal genes is, as expected, close to 1.

Although chromosome-wide dosage compensation does not seem to exist in this species, compensation might occur on a gene-by-gene basis. The distribution of M/F ratios for hemizygous genes (Figure 2A) has a long tail, reaching above 1, suggesting that some genes could be individually dosage compensated. To investigate this possibility, we compared male and female expression for hemizygous genes with different expression levels (Figure 2B). Dosage compensation is predicted to evolve preferentially for highly expressed genes because loss of expression in males of Y-linked genes whose X-linked copies are highly expressed in females (and, presumably, in the ancestor before suppressed recombination evolved in their genome region) is most likely to reduce males' fitness and therefore select for dosage compensation [31]. However, a trend for dosage compensation occurring largely for genes with low expression levels was reported in two bird species [32, 33]. The M/F ratios for 31 of the 33 most highly expressed genes in our dataset are consistent with the ratio expected without dosage compensation (the median does not differ significantly from 0.5; $P = 0.67$ by bootstrapping). A few genes in the

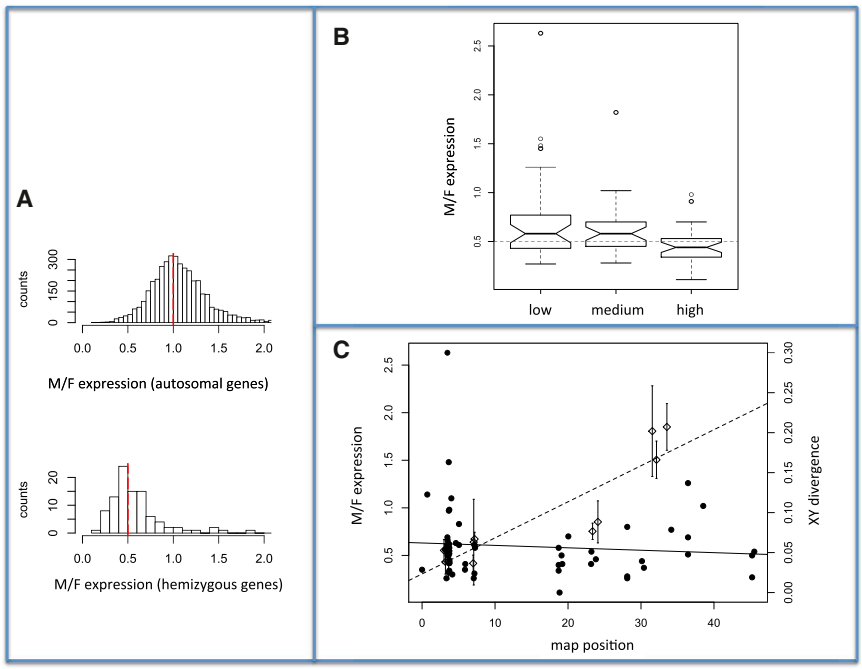

Figure 2. Tests for Dosage Compensation

(A) Expression estimates in males and females for 99 X-linked genes with lost or silenced Y copies and, for comparison, 4,050 autosomal genes. Male/female (M/F) expression ratios are shown for normalized read counts from our RNA-seq flower bud transcriptome data. Similar results were obtained from leaf transcriptome data (Figures S3A and S3B).

(B) M/F expression ratios of the 99 candidate hemizygous X-linked genes grouped by their expression levels. The cutoffs for the low, medium, and high expression categories were the 33rd and 67th percentiles of the expression values.

(C) Relationship between M/F ratios (black dots and left-hand y axis) and their map positions for 61 hemizygous genes, including nine with loss of Y expression. The right-hand y axis shows XY divergence estimates (K_s based on silent sites) for 12 genetically mapped X-linked genes that have Y-linked alleles (open diamonds; the error bars are 95% confidence intervals), which correlate with their map position (Pearson's r = 0.90, $P < 0.00008$), whereas M/F expression ratios do not (Pearson's r = −0.71, $P = 0.48$). See also Figure S3C for dosage compensation tests in XY genes with reduced Y expression.

less highly expressed sets have M/F ratios above 0.5 (4 and 5 in the low and intermediate expression categories, respectively), although the median for both sets is 0.58, not significantly different from 0.5 (p values 0.058 and 0.06, respectively). These few genes might indeed be dosage compensated, but their ratios could merely represent experimental noise (the variances of the ratios for the gene sets with low and medium expression are 0.237 and 0.078, respectively, versus 0.032 for the high expression set). Alternatively genes with M/F ratios greatly above 1 (five genes) may have male-biased expression. Our results are consistent with those from *Rumex hastatulus*, in which suppressed recombination evolved recently but in which X-linked genes without Y transcripts were detected, but dosage compensation was not detected either in a region that stopped recombining after a very recent Y-autosome fusion or one where recombination stopped somewhat earlier [12].

For comparison with the previous conclusion that dosage compensation has evolved in *S. latifolia* [25], we also analyzed another set of genes with expressed Y copies but whose expression in males is below 0.75 of the value for the X-linked allele. This set also includes 99 genes, and these also disagree with the previous conclusion for such genes [25], as we find no evidence for dosage compensation, even when their X-linked copies have high expression levels (Figure S3C).

Although we found no evidence for X chromosome-wide dosage compensation, compensating mechanisms might have evolved in just the older stratum. In human and rodent X chromosomes, regions where recombination suppression occurred longest ago (giving time for evolutionary responses to Y gene losses) indeed exhibit the highest proportions of dosage-compensated genes, whereas genes escaping X inactivation are mostly in the youngest stratum [34]. However, Figure 2C shows that we found no relationship between M/F expression ratios of the hemizygous and functionally hemizygous genes and their strata (inferred from their map positions on the *S. latifolia* X chromosome).

We conclude that dosage compensation has not evolved, although gene loss is non-negligible from this plant Y chromosome. We cannot currently test whether some of the losses of Y-linked genes occurred through deletions of whole Y chromosome regions versus individual gene losses (or extensive sequence changes after pseudogenization). The Y chromosome is 40% bigger than the X [35], suggesting that repetitive elements have accumulated on the Y [36], and ectopic recombination events between such sequences might have contributed to deletion of Y chromosomal regions [37, 38].

Our evidence indicates that neither silencing of maladapted Y-linked genes nor dosage compensation has yet evolved to any great extent in *S. latifolia*. Lack of major silencing of Y-linked genes is consistent with the lack of constitutive heterochromatin on the *S. latifolia* Y chromosome [35, 39], as heterochromatinization is generally associated with gene silencing [40]. The absence of dosage compensation in this 5- to 10-million-year-old plant sex chromosome system may appear to contrast with the rapid acquisition of dosage compensation in the *Drosophila miranda* neo-XY system, which evolved around 1–2 Ma ago [41]. However, the number of generations is probably much higher in *D. miranda*, which has several generations each year, whereas in *S. latifolia*, a generation probably corresponds to several years. Moreover, *D. miranda* has recruited pre-existing compensatory mechanisms that evolved in the ancestral XY system [26], whereas the *S. latifolia* sex chromosomes would have had to evolve a mechanism de novo.

EXPERIMENTAL PROCEDURES

Details of the mapping families, inference of male hemizygosity, and gene expression analyses are described in the Supplemental Experimental Procedures.

ACCESSION NUMBERS

The accession number for the RNA sequences reported in this paper is European Nucleotide Archive: PRJEB7338.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.03.015.

AUTHOR CONTRIBUTIONS

R.B. and D.C. designed the study. R.B. and S.Q. performed the experiments. R.B. analyzed the data with input from D.C. R.B. and D.C. wrote the manuscript. All authors approved the manuscript.

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REFERENCES

1. Bull, J. (1983). Evolution of Sex Determining Mechanisms. (Menlo Park: Benjamin/Cummings).
2. Charlesworth, B. (1996). The evolution of chromosomal sex determination and dosage compensation. Curr. Biol. *6*, 149–162.
3. Zhou, Q., and Bachtrog, D. (2012). Chromosome-wide gene silencing initiates Y degeneration in *Drosophila*. Curr. Biol. *22*, 522–525.
4. Itoh, Y., Replogle, K., Kim, Y.H., Wade, J., Clayton, D.F., and Arnold, A.P. (2010). Sex bias and dosage compensation in the zebra finch versus chicken genomes: general and specialized patterns among birds. Genome Res. *20*, 512–518.
5. Lee, H.S., and Chen, Z.J. (2001). Protein-coding genes are epigenetically regulated in *Arabidopsis* polyploids. Proc. Natl. Acad. Sci. USA *98*, 6753–6758.
6. Hughes, J.F., Skaletsky, H., Brown, L.G., Pyntikova, T., Graves, T., Fulton, R.S., Dugan, S., Ding, Y., Buhay, C.J., Kremitzki, C., *et al*. (2012). Strict evolutionary conservation followed rapid gene loss on human and rhesus Y chromosomes. Nature *483*, 82–86.
7. Carvalho, A.B. (2002). Origin and evolution of the *Drosophila* Y chromosome. Curr. Opin. Genet. Dev. *12*, 664–668.
8. Cortez, D., Marin, R., Toledo-Flores, D., Froidevaux, L., Liechti, A., Waters, P.D., Grützner, F., and Kaessmann, H. (2014). Origins and functional evolution of Y chromosomes across mammals. Nature *508*, 488–493.
9. Zhou, Q., Zhang, J., Bachtrog, D., An, N., Huang, Q., Jarvis, E.D., Gilbert, M.T.P., and Zhang, G. (2014). Complex evolutionary trajectories of sex chromosomes across bird taxa. Science *346*, 1246338.
10. Bergero, R., and Charlesworth, D. (2011). Preservation of the Y transcriptome in a 10-million-year-old plant sex chromosome system. Curr. Biol. *21*, 1470–1474.
11. Chibalina, M.V., and Filatov, D.A. (2011). Plant Y chromosome degeneration is retarded by haploid purifying selection. Curr. Biol. *21*, 1475–1479.
12. Hough, J., Hollister, J.D., Wang, W., Barrett, S.C., and Wright, S.I. (2014). Genetic degeneration of old and young Y chromosomes in the flowering plant Rumex hastatulus. Proc. Natl. Acad. Sci. USA *111*, 7713–7718.
13. Nicolas, M., Marais, G., Hykelova, V., Janousek, B., Laporte, V., Vyskot, B., Mouchiroud, D., Negrutiu, I., Charlesworth, D., and Monéger, F. (2005). A gradual process of recombination restriction in the evolutionary history of the sex chromosomes in dioecious plants. PLoS Biol. *3*, e4.
14. Rautenberg, A., Hathaway, L., Oxelman, B., and Prentice, H.C. (2010). Geographic and phylogenetic patterns in *Silene* section *Melandrium* (Caryophyllaceae) as inferred from chloroplast and nuclear DNA sequences. Mol. Phylogenet. Evol. *57*, 978–991.
15. Filatov, D.A., Monéger, F., Negrutiu, I., and Charlesworth, D. (2000). Low variability in a Y-linked plant gene and its implications for Y-chromosome evolution. Nature *404*, 388–390.
16. Qiu, S., Bergero, R., Forrest, A., Kaiser, V.B., and Charlesworth, D. (2010). Nucleotide diversity in *Silene latifolia* autosomal and sex-linked genes. Proc. Biol. Sci. *277*, 3283–3290.
17. Charlesworth, B., and Charlesworth, D. (2000). The degeneration of Y chromosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *355*, 1563–1572.
18. Barrett, S.C., Yakimowski, S.B., Field, D.L., and Pickup, M. (2010). Ecological genetics of sex ratios in plant populations. Philos. Trans. R. Soc. Lond. B Biol. Sci. *365*, 2549–2557.
19. Bergero, R., Qiu, S., Forrest, A., Borthwick, H., and Charlesworth, D. (2013). Expansion of the pseudo-autosomal region and ongoing recombination suppression in the *Silene latifolia* sex chromosomes. Genetics *194*, 673–686.
20. Gschwend, A.R., Yu, Q., Tong, E.J., Zeng, F., Han, J., VanBuren, R., Aryal, R., Charlesworth, D., Moore, P.H., Paterson, A.H., and Ming, R. (2012). Rapid divergence and expansion of the X chromosome in papaya. Proc. Natl. Acad. Sci. USA *109*, 13716–13721.
21. Lahn, B.T., and Page, D.C. (1999). Four evolutionary strata on the human X chromosome. Science *286*, 964–967.
22. Bergero, R., Forrest, A., Kamau, E., and Charlesworth, D. (2007). Evolutionary strata on the X chromosomes of the dioecious plant *Silene latifolia*: evidence from new sex-linked genes. Genetics *175*, 1945–1954.
23. Makino, T., and McLysaght, A. (2010). Ohnologs in the human genome are dosage balanced and frequently associated with disease. Proc. Natl. Acad. Sci. USA *107*, 9270–9274.
24. Veitia, R.A., Bottani, S., and Birchler, J.A. (2013). Gene dosage effects: nonlinearities, genetic interactions, and dosage compensation. Trends Genet. *29*, 385–393.
25. Muyle, A., Zemp, N., Deschamps, C., Mousset, S., Widmer, A., and Marais, G.A.B. (2012). Rapid de novo evolution of X chromosome dosage compensation in *Silene latifolia*, a plant with young sex chromosomes. PLoS Biol. *10*, e1001308.
26. Marín, I., Franke, A., Bashaw, G.J., and Baker, B.S. (1996). The dosage compensation system of *Drosophila* is co-opted by newly evolved X chromosomes. Nature *383*, 160–163.
27. Torres, E.M., Williams, B.R., and Amon, A. (2008). Aneuploidy: cells losing their balance. Genetics *179*, 737–746.
28. Sari Gorla, M., Frova, C., Binelli, G., and Ottaviano, E. (1986). The extent of gametophytic-sporophytic gene expression in maize. Theor. Appl. Genet. *72*, 42–47.
29. Honys, D., and Twell, D. (2004). Transcriptome analysis of haploid male gametophyte development in *Arabidopsis*. Genome Biol. *5*, R85.
30. Tanksley, S.D., Zamir, D., and Rick, C.M. (1981). Evidence for extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicon Esculentum*. Science *213*, 453–455.
31. Vicoso, B., and Charlesworth, B. (2009). The deficit of male-biased genes on the *D. melanogaster* X chromosome is expression-dependent: a consequence of dosage compensation? J. Mol. Evol. *68*, 576–583.
32. Melamed, E., and Arnold, A.P. (2007). Regional differences in dosage compensation on the chicken Z chromosome. Genome Biol. *8*, R202.
33. Uebbing, S., Künstner, A., Mäkinen, H., and Ellegren, H. (2013). Transcriptome sequencing reveals the character of incomplete dosage

compensation across multiple tissues in flycatchers. Genome Biol. Evol. *5*, 1555–1566.

34. Carrel, L., and Willard, H.F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature *434*, 400–404.
35. Matsunaga, S., Kawano, S., Michimoto, T., Higashiyama, T., Nakao, S., Sakai, A., and Kuroiwa, T. (1999). Semi-automatic laser beam microdissection of the Y chromosome and analysis of Y chromosome DNA in a dioecious plant, *Silene latifolia*. Plant Cell Physiol. *40*, 60–68.
36. Cermak, T., Kubat, Z., Hobza, R., Koblizkova, A., Widmer, A., Macas, J., Vyskot, B., and Kejnovsky, E. (2008). Survey of repetitive sequences in *Silene latifolia* with respect to their distribution on sex chromosomes. Chromosome Res. *16*, 961–976.
37. Bennetzen, J.L., and Wang, H. (2014). The contributions of transposable elements to the structure, function, and evolution of plant genomes. Annu. Rev. Plant Biol. *65*, 505–530.
38. Montgomery, E.A., Huang, S.M., Langley, C.H., and Judd, B.H. (1991). Chromosome rearrangement by ectopic recombination in *Drosophila melanogaster*: genome structure and evolution. Genetics *129*, 1085–1098.
39. Grabowska-Joachimiak, A., and Joachimiak, A. (2002). C-banded karyotypes of two Silene species with heteromorphic sex chromosomes. Genome *45*, 243–252.
40. Bühler, M., and Moazed, D. (2007). Transcription and RNAi in heterochromatic gene silencing. Nat. Struct. Mol. Biol. *14*, 1041–1048.
41. Bachtrog, D., and Charlesworth, B. (2002). Reduced adaptation of a non-recombining neo-Y chromosome. Nature *416*, 323–326.

