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# Genomics of sex chromosomes

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Sex chromosomes in plants and animals are distinctive, not only because of their gender-determining role but also for genomic features that reflect their evolutionary history. The genomic sequences in the ancient sex chromosomes of humans and in the incipient sex chromosomes of medaka, stickleback, papaya, and poplar exhibit unusual features as consequences of their evolution. These include the enormous palindrome structure in human MSY, a duplicated genomic fragment that evolved into a Y chromosome in medaka, and a 700 kb extra telomeric sequence of the W chromosome in poplar. Comparative genomic analysis of ancient and incipient sex chromosomes highlights common features that implicate the selection forces that shaped them, even though evolutionary origin, pace, and fate vary widely among individual sex-determining systems.

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**Current Opinion in Plant Biology** 2007, **10**:123–130

This review comes from a themed issue on
Genome studies and molecular genetics
Edited by Stefan Jansson and Edward S Buckler

Available online 14th February 2007

1369-5266/$ – see front matter


**DOI** 10.1016/j.pbi.2007.01.013

## Introduction

Dioecious plant species are reported to occur in 38% (167) of angiosperm families [1]. Among the estimated 250 000 angiosperm species, 6% (14 620) are dioecious. Sex determination in plants is controlled by genetic factors, and in some species, sex is influenced by growth hormones and environmental factors. The male genotype often includes a dominant female suppressor whereas the female genotype includes homozygous recessive alleles that result in the abortion of stamens. The most extreme form of sex determination systems involves specialized sex chromosomes that enforce dioecy.

Sex chromosomes of plants and animals share strikingly similar evolutionary processes and have evolved under the same selection forces. The initiation of sex chromosome evolution probably began from a male-sterile or female-sterile mutation in the nuclear genome where genetic recombination was suppressed, leading to subsequent degeneration, differentiation, and extension of male-specific (or female-specific) sequences. The theory that this process drives sex chromosome evolution has become generally accepted as genomic data have accumulated from the sex chromosomes of different organisms.

Sex chromosomes play a pivotal role in sex determination in some crop plants. The heterozygous nature of male or hermaphrodite individuals with XY (or ZW if the female is heterogametic) chromosomes prevents the generation of true breeding male or hermaphrodite lines, which can be an obstacle in breeding programs and in crop production. Molecular dissection of plant sex chromosomes will help in understanding the sex determination mechanism and in cloning sex determination genes for crop improvement. Much of the knowledge gained about the genomics of animal sex chromosomes is applicable to the genomics of sex chromosomes in plants. In this review, we focus on recent advances on the genomics of sex chromosomes, because sex chromosomes are the most 'extreme' form of various sex determination system, and the one with which we, as humans, are the most familiar. Because only a limited number of plant species have sex chromosomes, however, we include genomic analyses of sex chromosomes from both plants and animals when they can add new meaning to concepts of sex chromosome evolution.

## From sex to sex chromosomes

Sex is a universal phenomenon that assures new genetic combinations in all life forms. The predominantly asexual bacteria achieve sexual diversity either by recombination or by the introduction of novel sequences from external DNA sources [2]. The advantages of sexual reproduction were demonstrated in two recent reports. The first report contrasted the growth of a pair of sexual and asexual strains of yeast in a harsh environment. It showed faster evolutionary adaptation in the sexual strain, which achieves a 94% increase in growth rate after 100 generations, whereas only an 80% increase was reached by the asexual strain [3•]. The second study compared the deleterious mutation rates of sexual and asexual parthenogenic populations of water flea. This study showed a dramatic reduction of deleterious mutation rate in the sexually reproducing lineages to just 25% of the rate in the asexually reproducing lineages [4•].

A recent study on the evolution of fungal mating-type loci revealed a surprisingly common feature of sex-determining regions of chromosomes from animals, plants, and fungi [5]. Convergent genomic evolution of sex chromosomes across

kingdoms reflects similar selection forces that drive their formation [6••]. The genomic regions that flank the mating-type locus in *Cryptococcus* are recombination hotspots, as are the small pseudo-autosomal regions (PARs) (recombining regions between X and Y chromosomes) flanking the male-specific region of the Y chromosome (MSY) (non-recombining region between X and Y chromosomes) in humans [7].

Genomic research on sex determination in dioecious species has mostly focused on mapping the sex determination genes of economically important crop plants, such as spinach [8]. Significant progress has been made in cloning the sex determination genes of asparagus and papaya [9,10], and the physical mapping of the sex determination regions is near completion in papaya (Q Yu, P Moore, J Jiang, A Paterson, R Ming, unpublished).

Of the 14 620 dioecious species, only a tiny fraction has evolved sex chromosomes. Specifically, about a dozen species in four families exhibit heteromorphic sex chromosomes (X and Y or Z and W chromosomes are distinguishable microscopically in size and shape from autosomes and from each other), and another couple of dozen species in 13 families exhibit homomorphic sex chromosomes (X and Y or Z and W chromosomes are not distinguishable microscopically) [11,12]. As in mammals, some dioecious flowering species, such as white campion (*Silene latifolia*), papaya (*Carica papaya*), and asparagus (*Asparagus officianalis*), have an active-Y system of sex determination with heterogametic males (XY) and homogametic females (XX). Several dioecious species, such as sorrel (*Rumex*) and hops (*Humulus*), have an X:A ratio system for sex determination (e.g. where a X:autosome ratio of 1.0 results in females and a ratio of 0.5 in males).

Theoretical models have been developed which show that a nuclear male-sterile allele could be advantageous because it prevents inbreeding and it reallocates resources from male to female functions [12]. Suppression of recombination around such a male-sterile locus would lead to the accumulation of deleterious mutations and transposable element insertions that disrupt the function of genes in its neighboring regions [13]. Support for this hypothesis is provided by the recent discovery of an incipient sex chromosome in papaya that showed recombination suppression and degeneration of a 6–7 Mbp small MSY, which accounts for 10–15% of the Y chromosome ([9,14]; Q Yu, P Moore, J Jiang, A Paterson, R Ming, unpublished).

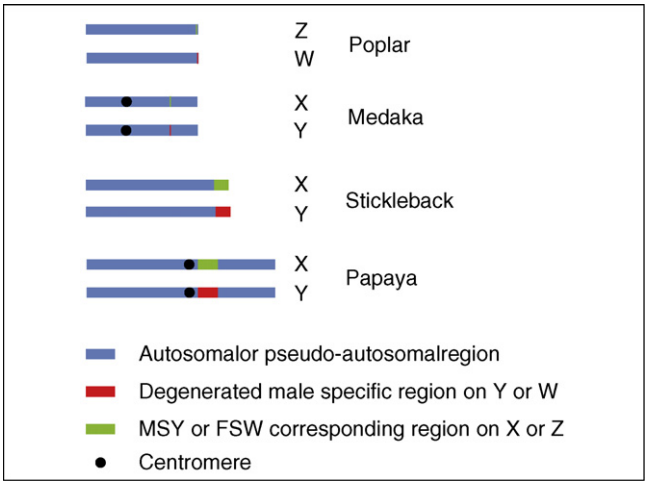
## Suppression of recombination and the rise of sex chromosomes

The male-sterile and/or female-sterile mutation that triggered the initiation of sex chromosome evolution could potentially be located on any part of any chromosome. The original sex determination gene(s) of ancient mammalian sex chromosomes have been lost, but they would have been in the region of the first stratum that covers most of the long arm of the X chromosome [15]. The human sex determination gene *Sry* is in the fourth stratum on the long arm near the border of the PAR. It seems to have evolved relatively recently because the estimated time of divergence between the X-Y gene pairs in this stratum is about 30–50 million years.

The recently evolved homomorphic sex chromosomes in the two fish, medaka and stickleback, and in the plants papaya and poplar allow us to examine the chromosomal origin of the sex determination gene(s) that triggered sex chromosome evolution. The medaka sex determination gene, *dmrt1bY*, was the second sex determination gene identified in vertebrates, after the mammalian *Sry*, and is required for testicular development [16,17]. This gene's autosomal ancestor, *dmart1a*, is conserved across a wide range of animals, and its human ortholog *DMART1* is a candidate downstream sex determination gene in mammals [18]. The MSY of medaka originated from an insertion on linkage group 1 (LG1) of a duplicated fragment from LG9 that contained *dmart1a* [16,17]. This small 258 kb MSY has been mapped to the distal part of the long arm of the chromosome corresponding to LG1 ([19,20••]; Figure 1).

Although heteromorphic sex chromosomes are not visible in the stickleback, accumulated genetic and genomic data support the hypothesis that sex chromosomes are in early stages of evolution in this species. Supportive evidence includes consistent heterogametic males, recombination suppression of loci that are closely linked to the sex determination locus, extensive sequence divergence of the X- and Y-specific bacterial artificial chromosomes (BACs), and the accumulation of transposable elements

**Figure 1**




The chromosomal origin of the MSY region in poplar, medaka, sticklebacks, and papaya (chromosome sizes are estimated averages based on the genome size and chromosome number of each species).

and chromosomal rearrangements in the MSY [21]. Linkage mapping of the stickleback genome placed the male-specific sex determination region at the end of LG19 [21].

High-density linkage mapping of the papaya genome revealed severe suppression of recombination around the sex determination locus, with 225 of the 347 markers on LG1 co-segregating with sex [14]. Physical mapping of this non-recombining region and sample sequencing of the male-specific BACs led to the conclusion that there is an incipient Y chromosome in papaya [10]. Sequencing of X- and Y-specific BACs revealed extensive sequence divergence and chromosomal rearrangements (Q Yu, P Moore, J Jiang, A Paterson, R Ming, unpublished). The current physical map of the papaya MSY covers 6 Mbp, about 10–15% of the Y chromosome, with three gaps remaining. Chromosome florescent *in situ* hybridization placed the papaya MSY near the centromere (Q Yu, P Moore, J Jiang, A Paterson, R Ming unpublished).

The most recently discovered ZW sex chromosomes in poplar again exhibited suppression of recombination around the sex determination locus. The sex-determination region was on a proximal end (telomeric region) of chromosome 19. The female-specific region of the W chromosome (FSW) was 706 kb that showed complete suppression of recombination (0 cM). In the immediate neighboring region, a 6.8 cM segment of the W chromosome consists of 257 kb (37.8kb/cM) genomic sequence, whereas a 15.8 cM segment of the corresponding region on the Z chromosome consists of 231 kb (14.6kb/cM), a 155% reduction of recombination rate on the W chromosome (G Tuscan, pers. comm.).

The chromosomal origin of the MSY and FSW in these four species for incipient sex chromosome evolution reinforces the concept that the suppression of recombination has been essential for the origin of sex chromosomes from autosomes. All three MSYs and the FSW originated in regions where recombination is reduced or restricted. The insertion of duplicated sequences to another chromosome creates a hemizygous state of the inserted sequences that prevents recombination. Recombination is known to be dramatically reduced in pericentromeric and telemetric regions [22]. If chromosomal rearrangements do occur in the region containing the sex-determination genes, as observed in the MSY of these young Y chromosomes, recombination would be further restricted and the MSY would extend along the Y chromosome.

The emergence of sex chromosomes from a bisexual organism appears to be accidental. In flowering plants, male-sterile or female-sterile mutations occur fairly frequently. Only those mutations involving nuclear genes in a region restricted for recombination had any chance to initiate the evolution of sex chromosomes, and a reverse mutation could abruptly cease this process.

## Do Y chromosomes have to shrink?

Because of their relevance to human health, human sex chromosomes are the most intensively studied of all sex chromosomes. The human X and Y chromosomes are the first pair of sex chromosomes to have been sequenced, and have thus provided the first complete picture of the structure and organizations of a pair of highly evolved and specialized sex chromosomes [23,24••]. The human Y chromosome is about 58 Mbp with a 23 Mbp euchromatic region, a 32 Mbp heterochromatic region, and a 2.6 Mbp PAR on the short arm and 0.4 Mbp PAR on the long arm. Eight pairs of giant palindrome structures in the euchromatic region are prominent features revealed by sequence data. These palindromes protect essential genes on the Y chromosome from erosion through gene conversion [25]. By contrast, the X chromosome is nearly three times as large (154 Mbp) and contains 1098 genes [24••].

On the basis of observations of the human Y chromosome, loss of genetic information and shrinking in size were once accepted as elements common to Y chromosome degeneration. Indeed, these characteristics exemplify the degeneration of mammalian sex chromosomes that are ancient (about 300 million years of evolution). However, smaller size is not a genomic feature of young sex chromosomes in plants. The *Silene* sex chromosomes are estimated to be only 5–10 million years old [26••] and the *Silene* Y chromosome is the largest chromosome in its genome at about 570 Mbp, whereas the X chromosome is the second largest chromosome at about 400 Mbp (B Vyskot, pers. comm.). Although sex chromosomes in *Silene* apparently evolved from the largest pair of autosomes, the Y chromosome expanded 42.5% in its first 5–10 million years of existence [26••].

The 258 kb MSY of medaka, along with its corresponding region of the X chromosome, is the second completely sequenced MSY [20••]. The Y core region of 72.1 kb contains the sex-determination gene *dmrt1bY* and is aligned with 42.9 kb of LG9 that contains the ancestral *dmrt1a* gene. This alignment reveals 68% sequence expansion of the MSY. Two bordering regions of the MSY could be aligned with the X chromosome sequence: one 12.2 kb region corresponds to 12.2 kb of the X sequence whereas the other 63.4 kb region corresponds to 58.3 kb of the X sequence, showing 8.7% MSY sequence expansion. The MSY of medaka has remained small despite its estimated age of 10 million years [27]. Its size might have been restricted by the duplicated genes *OlaflnkL* and *OlaflnkR* on flanking borders that can recombine with the single copy X counterpart *OlaflnkX* [20••].

Similar Y sequence expansion is observed in other incipient sex chromosome systems. Two overlapping BACs, both on the stickleback X and Y BACs, were sequenced to show that 316.7 kb of the MSY sequence aligned with 229.0 kb of the X sequence, indicating a 38.3%

sequence expansion [21]. Two pairs of papaya X and Y BACs were also sequenced and revealed MSY sequence expansion (Q Yu, P Moore, J Jiang, A Paterson, R Ming, unpublished).

The W chromosome of poplar has 706 kb of extra sequence in the proximal region of the W chromosome. It is not yet clear whether this sequence expansion was from the accumulation of repetitive sequences, as in sticklebacks and papaya, or from translocation of an autosomal fragment, as in medaka (G Tuscan, pers. comm.).

Do Y or W chromosomes have to shrink? Not necessarily for all sex chromosome systems. Although most ancient XY (or ZW) systems in animals have small degenerated Y chromosomes, the young sex chromosomes in plants have larger Y and W chromosomes resulting from transposable element insertions and local duplications. It is also possible that the degenerated large Y and W chromosome could disappear without going through the phase of shrinking in size and sex determination could be replaced by a X:A or Z:A ratio system.

## Loss and gain of genes on the Y chromosome

A consequence of suppression of recombination is the degeneration of the suppressed region by selection forces such as Muller's ratchet and 'hitchhiking' [28,29]. The best-known example of these processes is the highly degenerated human Y chromosome. The human MSY contains 78 protein-coding genes, including two X-transposed, 16 X-degenerated, and 60 ampliconic (embedded in eight palindromes) genes [23,25]. It is clear that the vast majority of homologs to the 1098 genes on the X chromosomes have been degenerated or lost at a rate of 3–4 genes per million years.

The chimpanzee Y chromosome was recently sequenced and provides a second well-characterized mammalian Y chromosome for comparative analysis [30•,31•]. Detailed sequence comparison revealed specific chromosomal inversions and deletions, and the loss of four genes on the chimpanzee MSY. Greater sequence divergence was detected between the human and chimpanzee Y chromosomes (1.78%) than between their respective whole genomes (1.23%). On the other hand, four new genes were found on the cat MSY, two having X chromosome homologs and the other two from putative autosomal progenitors [32•]. Two of the new genes were later also found in the dog genome. The mammalian Y chromosome appears to be a dynamic system with lineage-specific gene gains and losses that have the potential to change the fate of the Y chromosomes in different lineages.

Recently evolved sex chromosomes allow one to explore the details of Y chromosome degeneration at the beginning stages of evolution. The MSY of medaka is 258 kb and represents only 1% of its chromosome. The ancestral 43 kb sequence on LG9 contains four functional genes and one pseudogene. The sex determination gene *dmrt1bY* is the only functional gene remaining on the MSY [20••].

Plant Y chromosomes at early and advanced stages of sex chromosome evolution show signs of degeneration. Abundant repetitive sequences were detected on plant Y chromosomes, including the recent addition of a novel family of gypsy-like retrotransposons, a tandem repeat TRAYC in *S. latifolia* [33,34], and dispersed repetitive sequences from *Rumex acetosa* [35]. Y-specific elements were found in these two species as well as from *Cannabis sativa* (hemp) and *Marchantia polymorpha* (liverwort) [36–39]. The accumulation of plastid sequences on the Y chromosome could also contribute to the degeneration process [40]. In addition to repetitive and plastid sequence insertions, an inversion event was detected on the Y chromosome of *S. latifolia* that is usually detrimental in the euchromatic region [41].

The lethal effect of the papaya YY genotype suggests the degeneration of the incipient Y chromosome [42]. Comparing the functional genes from the sequences of two pairs of papaya X and Y BACs, one gene on the X appeared to have been deleted from the MSY, but this gene is not related to sex determination because it shows no differential expression among sex types and it is not in the deleted region of a sex reversal mutant (Q Yu, P Moore, J Jiang, A Paterson, R Ming, unpublished).

Gain of genes is also documented on plant Y chromosomes. A duplicated MADS-box gene, *APETALA3* (*AP3*), was transferred to the Y chromosome of *S. latifolia* and was strongly expressed in stamens [43]. A gene family on the Y chromosome of liverwort, without X counterparts, was expressed specifically in male sex organs [39]. The interspecific hybrid from dioecious *S. latifolia* and hermaphrodite *Silene viscosa* revealed both loss and gain of genes on the Y chromosome [44]. The hermaphrodite phenotype of the hybrid indicated that the loss-of-function sex determination gene(s) was replaced by gene(s) from *S. viscose*. On the other hand, two anther development defects, loss of adhesion of the tapetum to the endothecium and precocious endothecium maturation, demonstrated the gain of new dominant genes that promote anther development [44].

The neo-sex chromosomes of *Drosophila* show an accelerated rate of degeneration. In *Drosophila pseudoobscura*, the X chromosome fused to an autosome 18 mya and the neo-Y chromosome lost all but a few dozen of its originally more than 2000 genes [45•]. The neo-Y chromosome in *Drosophila miranda* was formed by fusion of an autosome to the ancestral Y chromosome. In merely one million years, one third of the 2000 genes became non-functional owing to transposable element insertions and the accumulation of deleterious mutations [46•,47]. Of the

four contiguous genomic regions of neo-sex chromosomes investigated, the neo-X showed heterogeneity in levels of dosage compensation, whereas the protein-coding genes on the neo-Y showed similar patterns and degrees of degeneration. This indicated that dosage compensation, a mechanism that equalizes the expression levels of X-linked genes in XY males and XX females, might not have significant impact in the early stage of Y-chromosome degeneration [47]. It is not yet clear whether dosage compensation has evolved on young X or Z chromosomes of plants, or whether events observed on the young neo-X chromosomes of *Drosophila* could happen on young X or Z chromosomes of dioecious plants.

## Divergence of X and Y gene pairs

Analysis of functional X and Y gene pairs provides crucial information on the history of Y chromosome evolution. Sequence divergence of medaka MSY pseudogenes from their homologous genes on LG9 confirmed that *dmrt3* is a pseudogene: the ratio of nonsynonymous and synonymous substitution rates was close to zero. Interestingly, the ratio for *KIAA0712* and its Y chromosome copy *KIAA0172p* was less than 1, indicating that *KIAA0172p* remained functional until very recently [20••].

DNA sequence analysis of 19 X and Y human gene pairs revealed four evolutionary strata (a stratum represents a group of X and Y gene pairs that have a similar level of nucleotide divergence) that caused suppression of recombination in a stepwise fashion [15]. Sequencing of the human X chromosome identified a new, fifth stratum in the 1.0 Mbp of the fourth stratum [24••]. Similarly, sequence divergence analysis of four gene pairs in *Silene* revealed three evolutionary strata, indicating progressive restriction of recombination on its sex chromosomes [26••,48–50]. A comparative genetic map for these four X-linked genes placed the least diverged (*SlX1/SlY1*) and the most diverged (*SlX4/SlY4*) genes at opposite ends of the map, whereas the other two genes, *DD44X/Y* and *SlssX/Y*, which exhibited intermediate divergence, were in between [51]. The *SlX1/SlY1* gene is the closest to the PAR. All amino-acid replacements between *SlssX* and *SlssY* occurred in the Y-linked gene [52]. The *SlssY* gene has an elevated synonymous substitution rate compared with *SlssX*, indicating that the Y chromosome has a greater mutation rate than the X chromosome [52].

Expression profiling of 58 gene pairs from the neo-X and neo-Y chromosomes of *D. miranda* revealed differential expression of all but three genes, with about 80% of all genes from the neo-Y being expressed at lower levels [53•]. Many of the 58 neo-Y genes contain frame-shift and nonsense mutations, but they were still transcribed. This experiment provide useful insights for studies of young sex chromosomes in plants where mildly degenerated Y-linked genes might still be expressed at a reduced level.

## The genomics of sex

Unlike in animals, where the germ line diverges from somatic cells early in embryogenesis, plant cells proceed through a long period of vegetative development, often for months or years, without sexual differentiation. Hermaphrodite and monoecious (male and female flowers on the same individual) plants account for about 94% of angiosperm species and have no sexual polymorphism. The dioecious small fraction (6%) of flowering plants achieve sexual differentiation, primarily involving two whorls of flower organs (i.e. stamens and carpels), when they flower. There is little or no somatic sexual dimorphism in dioecious flowering plants [54].

The sex determination role of the floral homeotic genes that control stamen and carpel development, *AP3*, *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*), has been investigated in dioecious species. None of these genes proved to be the master switch [55–57]. Orthologs of *Arabidopsis SHOOTMERISTEMLESS* (*STM*) and *CUP SHAPED COTYLEDON* (*CUC*), which define the boundaries of flower organs, were cloned in *S. latifolia* [58•]. Expression analysis revealed male-specific patterns before morphological differentiation, making *SlSTM* and *SlCUC* the first strong candidate genes to be associated with sex determination in dioecious plants.

Microarray analyses of 22 746 genes in *Arabidopsis* revealed 105 genes that were preferentially expressed in reproductive structures, including young inflorescences, stage 12 floral buds, and developing siliques [59]. Stamen- or carpel-specific genes would be much fewer. Global expression analysis of target genes that are regulated by *AP3* and *PI*, using 9216 *Arabidopsis* expressed sequence tags (ESTs) concluded that only 47 genes from this collection are likely to be regulated by *AP3* and *PI*, implying that most of the genes that are expressed in petals and stamens are not tissue specific [60].

Why do so few plant species have sex chromosomes? One reason could be the recent origin of angiosperms, the most successful linage in plant kingdom, from a hermaphrodite progenitor about 140–200 mya, whereas the mammalian sex chromosomes originated about 300 mya. Another possible reason could be the fact that plants don't move and hence hermaphrodite plants have an advantage in ensuring successful reproduction, although wind, birds, and insects assist the pollination of dioecious plants. However, the recent discovery of sex chromosomes by the poplar genome sequencing project indicated that there are more homomorphic incipient sex chromosomes to be uncovered in plants.

## Conclusions

Recent genomic information from ancient and incipient sex chromosomes has improved our understanding of the evolutionary origin and processes of this unique pair of

chromosomes in plants. Different types of chromosomal rearrangements and the insertion of specialized chromosome regions appear to have provided the initial suppression of recombination. This fact demonstrates that there are diverse mechanisms for the initiation of sex chromosome evolution. The MSY of young sex chromosomes all share a phase of sequence expansion because of the accumulation of inserted transposable elements and genomic sequences. Thus, these cytologically homomorphic incipient sex chromosomes are seen to be heteromorphic at the molecular level.

Although additional genomic information is coming from the sex chromosomes of model plant species such as *Silene*, sorrel, papaya, and poplar, cloning of the sex determination genes will provide information that is crucial to deciphering sex chromosome systems. Orthologous genes that are involved in establishing floral organ boundaries appear to be good candidates for the sex determination pathway in *S. latifolia*. Sex determination genes in dioecious plants are likely to be species specific, because the abortion of sex organs occurs at different developmental stages, ranging from the early stages of inception of organ primordia to the late stage of formation of microspores. Cloning of sex determination genes from sex chromosomes is a challenging task because genetic recombination is restricted to the MSY, making map-based cloning approaches ineffective. The generation of sex reversal mutants might be a good alternative approach toward identifying the sex-determining genes once MSY and FSW sequences become available.

## Acknowledgements

This work was supported by a grant from the National Science Foundation (NSF) to RM, QY, PHM, JJ, and AHP (Award No. DBI-0553417), and by start-up funds from the University of Illinois at Urbana-Champaign to RM.

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