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SEX CHROMOSOMES IN FLOWERING PLANTS[1]

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Sex chromosomes in dioecious and polygamous plants evolved as a mechanism for ensuring outcrossing to increase genetic variation in the offspring. Sex specificity has evolved in 75% of plant families by male sterile or female sterile mutations, but well-defined heteromorphic sex chromosomes are known in only four plant families. A pivotal event in sex chromosome evolution, suppression of recombination at the sex determination locus and its neighboring regions, might be lacking in most dioecious species. However, once recombination is suppressed around the sex determination region, an incipient Y chromosome starts to differentiate by accumulating deleterious mutations, transposable element insertions, chromosomal rearrangements, and selection for male-specific alleles. Some plant species have recently evolved homomorphic sex chromosomes near the inception of this evolutionary process, while a few other species have sufficiently diverged heteromorphic sex chromosomes. Comparative analysis of carefully selected plant species together with some fish species promises new insights into the origins of sex chromosomes and the selective forces driving their evolution.

Key words: angiosperm; sex chromosome; sex determination; suppression of recombination; Y chromosome degeneration.

Sexual reproduction is a prominent feature of the life cycle in most animals and plants. The enormous diversity of life forms and their wide range of genome complexity, from the single, circular chromosome of prokaryotes to the 640 chromosomes of the angiosperm species *Sedum suaveolens*, are made possible partly by sex. Weismann, (1889) proposed that sexual reproduction increases genetic diversity and provides various combinations of alleles for natural selection. However, several difficulties with this explanation have arisen, such as recombination breaking up assemblies of favorable genes and the two-fold cost of sex (only one of the two parents of sexual reproductive system is capable of bearing offspring) (Williams, 1975; Smith, 1978). Two recent reports support Weismann's hypothesis; faster evolutionary adaptation was shown in a sexual strain than in an asexual strain of yeast (Goddard et al., 2005), and a reduction of deleterious mutations was detected in a sexually reproducing species compared to an asexually reproducing species of water fleas (Paland and Lynch, 2006).

Dioecism has arisen independently from hermaphroditic ancestors in many plant families and genera. Flowering plants began appearing in fossil records at a minimum of 124.6 million year ago (MYA) (Sun et al., 2002), and the crown-group angiosperms were estimated to have evolved 158–179 MYA based on DNA sequence data (Wikström et al., 2001). The ancestor of flowering plants was likely hermaphroditic (Takhtajan, 1969). Although the vast majority of extant flowering plants are hermaphrodites, monoecious and dioecious species occur in 75% of angiosperm families, including all six dicotyledonous and all five monocotyledonous subclasses (Yampolsky and Yampolsky, 1922; Renner and Ricklefs, 1995). Dioecious species accounted for 6% (14 620) of the species in 7.1% (959) of genera and 38% (167) of families of flowering plants (Renner and Ricklefs, 1995). Interestingly, early flower development is similar in many unisexual and hermaphroditic species (reviewed by Dellaporta and Calderon-Urrea, 1993; Matsunaga and Kawano, 2001). Flower ontogeny and the broad phylogenetic distribution of unisexual species suggest that dioecism has arisen independently from hermaphroditic ancestors on many occasions in different plant families and genera. In the majority of cases, the evolution of dioecism has taken place at the species level (Westergaard, 1958).

Dioecism accompanied by sex chromosome dimorphism is common in animals but less prevalent in plants. Appearance of heteromorphic sex chromosomes is the consequence of the evolution of sex to ensure dioecy and to enforce a 1:1 segregation ratio of male and female individuals. Sex chromosomes have evolved in a limited number of dioecious angiosperms, and only a few have been characterized at the cytological and/or molecular levels (Charlesworth, 2002). The intensively studied heteromorphic sex chromosomes in *Silene* might have originated about 5–10 MYA based on the molecular clock calibration from the nuclear genes *Chs* and *Adh* in the family Brassicaceae (Koch et al., 2000; Nicolas et al., 2005), although substitution rates for some plant *Adh* genes could be 10 times slower (Gaut et al., 1996). The homomorphic papaya sex chromosomes evolved more recently as revealed by a study of X and Y gene pairs (Q. Yu, P. Moore, J, Jiang, A. Paterson, R. Ming, unpublished data). Their recent origin is consistent with the primitive nature of most plant sex chromosomes compared with the 300 million-year-old sex chromosomes in mammals (Lahn and Page, 1999).

Dioecious plants provide a particularly interesting system in which to study the genetics and evolution of sex chromosomes (Westergaard, 1958). First, plants with unisexual flowers have evolved independently and multiple times from bisexual

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progenitors, which provides the opportunity to study different mechanisms of sex determination. Second, many dioecious plant species have fertile bisexual relatives, which makes possible comparative studies of sex determination mechanisms and the origins of sex chromosomes from autosomes. Third, in plants it is possible to follow both forward and reverse evolution between dioecism and hermaphroditism because dioecious mutants from hermaphrodites and hermaphrodite mutants from the dioecious plants are each occasionally found. Finally, different plant species at various stages of sex chromosome evolution provide a time series for examining the forces driving Y chromosome degeneration. The recent origin of sex chromosomes in *Silene* and papaya offers the opportunity to access the very early stages of sex chromosome evolution (Westergaard, 1958; Filatov et al., 2000; Negrutiu et al., 2001; Filatov and Charlesworth, 2002; Liu et al., 2004).

Sex chromosomes were discovered more than a century ago (McClung, 1901). Theoretical, cytogenetic, and classical genetic studies of sex chromosome evolution dominated the decades following this discovery. In the past two decades, genomic and molecular biology techniques have made it possible to clone the sex determination genes and to sequence sex chromosomes, as done for mammals (Sinclair et al., 1990; Skaletsky et al., 2003; Ross et al., 2005). In plants, sex determination genes were cloned for monoecious maize (DeLong et al., 1993; Bensen et al., 1995). Genetic maps were constructed for sex chromosomes of asparagus (Reamon-Büttner and Jung, 2000), hops (Seefelder et al., 2000), and papaya (Ma et al., 2004). Sex chromosome-specific genes were cloned and analyzed in *Silene* (Delichère et al., 1999; Atanassov et al., 2001; Moore et al., 2003; Nicolas et al., 2005). Sequencing of the male-specific region of the Y chromosome and corresponding region of the X chromosome is underway in papaya. These recently available genomic, molecular, and genetic data are shedding light on sex chromosome evolution and will allow us to look into how sex chromosomes evolve from autosomes. In this review, we will summarize our current understanding on the evolutionary origin and consequence of sex chromosomes in angiosperm with an emphasis on early stages of sex chromosome evolution.

SEX DETERMINATION IN FLOWERING PLANTS

The hypothesis that angiosperms originated from a hermaphroditic ancestor is supported by the prevalence of hermaphrodites among about 72% of all species (Takhtajan, 1969). Diverse mechanisms that evolved to promote outcrossing include temporal separation of the maturation of male or female organs, gametophytic or sporophytic self-incompatibility, structural avoidance, monoecy, and ultimately dioecy. The first step toward sex chromosome evolution is the occurrence of male sterile or female sterile mutations leading to the development of unisexual flowers. Such mutations occur frequently and repeatedly in plant species, as demonstrated by the presence of unisexual species in 75% of angiosperm families and numerous male sterile mutants in domesticated crop plants (Yampolsky and Yampolsky, 1922; Renner and Ricklefs, 1995). Also, stamen and carpel development involve large numbers of genes at various developmental stages, and mutations of any of the many regulatory genes could trigger abortion or loss of function of male and/or female organs (Wellmer et al., 2004). In dioecious flowers, male sterile mutation can occur from the early stages of the first appearance of the stamen primordia until the late stages of microspore formation; likewise, female sterility can also occur from early stages of inception of the carpel until the late stage of the formation of microspores (Matsunaga and Kawano, 2001).

Unisexual species have sex determination mechanisms ranging from genetic to environmental, but genetic factors play far greater roles. Genetic mechanisms range from a single locus on an autosome to heteromorphic sex chromosomes containing multiple genes involved in sex determination. In some unisexual species, such as hemp, cucumber, and maize, male or female sterility is influenced by environmental factors such as light intensity, day length, temperature, mineral nutrition, and plant hormones (Frankel and Galun, 1977; Chailakhyan, 1979). Cytoplasmic male sterility caused by mutations on the mitochondrial genome is widely used in production of crops that benefit greatly from heterosis, but only nuclear male sterility can trigger sex chromosome evolution.

Chromosomal sex determination systems may be an evolutionary consequence of natural selection in favor of dioecy. Sex chromosomes do not appear suddenly in animals or plants. Rather, it is the pair of autosomes bearing the sex determination genes that have evolved specialized features, i.e., the degeneration of the Y chromosome, resulting in heteromorphy that became the hallmark of sex chromosomes. Two major sex chromosome systems have evolved. One is the active Y chromosome system, or the XY system (or ZW in a heterogametic female system), in which females have two of the same kind of sex chromosome (XX), while males have two distinct sex chromosomes (XY). The other is the X-to-autosome (A) balance system, in which the ratio of X:A chromosomes determines sex by an X chromosome counting system and the Y chromosome is dispensable.

Among the many dioecious plant species, only a few have evolved sex chromosomes (Westergaard, 1958; Renner and Ricklefs, 1995; Charlesworth and Guttman, 1999) (Table 1). As in mammals, some dioecious flowering species have an active-Y system of sex determination with heterogametic males (XY) and homogametic females (XX), such as white campion (*Silene latifolia*), papaya (*Carica papaya*), and asparagus (*Asparagus officianalis*). Several dioecious species have the X:A dosage compensation system for sex determination, for example, sorrel (*Rumex*) and hops (*Humulus*). An X-to-autosome ratio of 1.0 or higher results in a female and 0.5 or lower defines a male (Ono, 1935; Westergaard, 1958), which is similar to the situation in *Drosophila* and *Caenorhabditis elegans* (reviewed by Mittwoch, 1996). These two sex chromosome systems may reflect two different stages of sex chromosome evolution that will be discussed further in the following sections.

In some species, sex expression is under epigenetic control, mediated by chromatin modifications of the sex determining regions, including DNA methylation and nucleosomal histone acetylation (reviewed by Vyskot, 1999). In white campion, hypomethylation induced sex reversal of genetically male plants (XY) to form a perfect flower (Finnegan et al., 2000). Sex determination is not the simple hierarchical chain reaction once envisioned but is the result of interactions among a complex network of genes as learned from the study of the sex-determining region Y (*Sry*) gene pathway that controls human sex determination (Koopman, 1999). The epigenetic influence

TABLE 1. List of plant species with homomorphic or heteromorphic sex chromosomes.

Family	Species	Female sex chromosome	Male sex chromosome	Viability of YY genotype	Sex determination	Reference
Heteromorphic sex chromosomes						
Cannabidaceae	*Cannabis sativa*	XX	XY	Yes	X to autosome ratio	Yamada, 1943
	Humulus lupulus	XX	XY	Yes	X to autosome ratio	Jacobsen, 1957
	H. lupulus subsp. *cordifolius*	$X_1X_1X_2X_2$	$X_1Y_1X_2Y_2$	—	X to autosome ratio	Ono, 1937
	H. japonicus	XX	XY_1Y_2	No	X to autosome ratio	Kihara, 1929
Caryophyllaceae	*Silene latifolia, S. dioica, S. diclinis*	XX	XY	No	Active Y system	Ono, 1939; Nicolas et al., 2005
Cucurbitaceae	*Coccinia indica*	XX	XY	—	Active Y system	Kumar and Vishveshwaralah, 1952
Polygonaceae	*Rumex angiocarpus*	XX	XY	—	—	Löve, 1943
	R. tenuifolius	(XX)XX	(XX)XY	—	—	Löve, 1943
	R. acetosella	(XXXX)XX	(XXXX)XY	—	Active Y system	Löve, 1943
	R. graminifolius	(XXXXXX)XX	(XXXXXX)XY	—	—	Löve, 1943
	R. hastatulus	XX	XY or + XY_1Y_2	—	X to autosome ratio	Smith, 1955
	R. acetosa	XX	XY_1Y_2	—	X to autosome ratio	Ono, 1930; 1935
	R. paucifolius	(XX)XX	(XX)XY	—	—	Löve and Sarkar, 1956
Homomorphic sex chromosomes						
Actinidiaceae	*Actinidia deliciosa*	—		—	Active Y system	Ferguson et al., 1997
	A. chinensis	Male heterozygous		Yes	Active Y system	Testolin et al., 1995
Amaranthaceae	*Acnida* species	Male heterozygous		—	Active Y system	Charlesworth and Guttman, 1999
Asparagaceae	*Asparagus officinalis*	Male heterozygous		Yes	Active Y system	Loptien, 1979
Asteraceae	*Antennaria dioica*	Male heterozygous		No	—	Charlesworth and Guttman, 1999
Caricaceae	*Carica papaya*	Male heterozygous		No	Active Y system	Horovitz and Jiménez, 1967
	Vasconcellea species	Male heterozygous		No	Active Y system	Horovitz and Jiménez, 1967
Caryophyllaceae	*Silene otites*	Uncertain		—	—	Charlesworth and Guttman, 1999
Chenopodiaceae	*Spinacia oleracea*	Male heterozygous		Yes	Active Y system	Warmke and Blackslee, 1939
Cucurbitaceae	*Bryonia multiflora*	Male heterozygous		—	—	Charlesworth and Guttman, 1999
	Ecballium elaterium	Male heterozygous		—	Active Y system	Charlesworth and Guttman, 1999
Dioscoreaceae	*Dioscorea tokoro*	Male heterozygous		—	Active Y system	Martin and Oritz, 1963
Euphorbiaceae	*Mercurialis annua*	Male heterozygous		Yes	Active Y system	Charlesworth and Guttman, 1999
Ranunculaceae	*Thalictrum* species	Male heterozygous		Yes	—	Charlesworth and Guttman, 1999
Rosaceae	*Fragaria* species	Female heterozygous		—	—	Kihara, 1930
Vitaceae	*Vitis* species	Male heterozygous		Yes	Active Y system	Westergaard, 1958

Compiled mostly from Westergaard (1958), Charlesworth and Guttman (1999), and Matsunaga and Kawano (2001). "—" indicates missing data.

over sex determination might be the consequence of the regulation of the expression of a gene or genes in this network.

SUPPRESSION OF RECOMBINATION AT THE SEX DETERMINATION LOCUS

Suppression of recombination is a pivotal event in sex chromosome evolution. Without suppression of recombination, the male sterile or female sterile mutations could revert to hermaphroditism. The typical features of young sex chromosomes are suppression of recombination at and around the sex determination locus and moderate degeneration of the male-specific region.

Suppression of recombination in specific chromosomal regions is a widespread phenomenon in sexually reproducing organisms. It is often triggered by rearrangement or modification of DNA sequences. Direct evidence was documented for suppression of recombination within two pericentric inversions on human chromosomes 1 and 8 (Jaarola et al., 1998). Inversions were also the cause of suppression of recombination that led to the degeneration of the Y chromosome in human (Lahn and Page, 1999). Although the contents of recombination-suppressed regions evolve rapidly, the regions themselves appear to remain in the same location over long periods (Bowers et al., 2005). Gradual reduction of recombination rates might account in part for reducing recombination of the X and Y chromosomes in *Silene* (Nicolas et al., 2005). DNA methylation is another mechanism for suppression of recombination as reported in the fungus *Ascobolus immersus* (Maloisel and Rossignol, 2006).

Suppression of genetic recombination has been documented for primitive and advanced sex chromosomes (Westergaard, 1958; Ohno, 1967; Ma et al., 2004). The papaya sex locus has been genetically mapped to linkage group 1 (Sondur et al., 1996). In a recently constructed high density map, a total of 225 markers, which is 66% of the 342 markers on linkage group 1, co-segregated with the sex locus, indicating severe suppression of recombination at this region (Ma et al., 2004). A similar result has been shown on a linkage map of the medaka fish. Two large clusters of loci consisting of 19 and 13 markers, respectively, flanked a region of 2.6 cM containing the male-determining region (Naruse et al., 2000). In asparagus, the sex locus is located on linkage group 5 where 20 random markers have been mapped to a total of 55.3 cM, averaging one marker in 2.6 cM of this linkage group (Reamon-Büttner and Jung, 2000; Jamsari et al., 2004). However, nearer the sex locus, four markers, excluding the newly integrated sex-linked STS markers, were mapped in a region of 0.5 cM. Recombination suppression at and near the sex locus was further confirmed by physical mapping of the male-specific region in papaya (Liu et al., 2004) and by genetic mapping using male and female meioses in medaka (Kondo et al., 2001).

In the XY system, suppression of recombination and DNA

sequence degeneration occur on the Y chromosome, while X chromosomes in females recombine normally. In addition to the large clusters of sex co-segregating markers, suppression of recombination in the male-specific region of the Y chromosome can be evaluated by comparing X and Y linkage maps. In hops (*Humulus lupulus*), the two markers flanking the male sex determination locus were 3.7 cM apart on the Y chromosome linkage map, while the genetic distance between the same two markers was 14.3 cM on the X chromosome linkage map, an almost four-fold reduction of recombination on the Y chromosome (Seefelder et al., 2000). A similar reduction of genetic distance is found in the male meiotic map of the three-spined stickleback, a fish species with an emerging Y chromosome (Peichel et al, 2004). The sex determination gene of sticklebacks is located at the end of linkage group 19. A pair of markers next to the sex determination gene was 6.4 cM apart based on male meiosis (i.e., the primitive Y chromosome) but 25.7 cM apart based on female meiosis (the X chromosome); again recombination on the Y chromosome was reduced approximately four-fold.

Multiple genes in the suppressed male-specific region of the Y chromosome may be specialized in male-specific functions. In white campion, three dispersed male determining loci were located on the Y chromosome, with two on one arm containing genes controlling carpel suppression and early stamen promoting and one on the other arm conferring late anther fertility (Westergaard, 1958; Farbos et al., 1999; Lardon et al., 1999; Lebel-Hardenack et al., 2002). Because these genes are physically located on distant segments of the Y chromosome, they could potentially segregate, resulting in hermaphroditic and sterile individuals unless recombination between them was suppressed.

EMERGENCE OF PRIMITIVE SEX CHROMOSOMES

Sex chromosomes are usually heteromorphic. However, a number of dioecious flowering plant species appear to have an active Y system for sex determination without any cytological evidence of heteromorphism. For example, in papaya, it has been controversial for several decades whether sex determination is controlled by a single gene, a complex of genes, or sex chromosomes (Storey, 1953, 1976). Recently developed genomic and molecular techniques make it possible to examine the molecular basis of sex determination in papaya and other dioecious plant species to resolve such issues.

Sex determination in papaya was first thought to be controlled by a single gene with three alleles, M, M^h, and m, with classical Mendelian segregation in populations generated from crosses among three sex types (Hofmeyr, 1938; Storey, 1938). However, male individuals (Mm) and hermaphrodite individuals (M^hm) are heterozygous, whereas female individuals (mm) are homozygous recessive. The genotypes with homozygous dominant alleles, MM, M^hM^h, and MM^h, are lethal, resulting in a 2:1 segregation of hermaphrodite to female from self-pollinated hermaphroditic seeds and a 1:1 segregation of male to female or hermaphrodite to female from cross-pollinated female seeds. On the basis of co-segregation of long peduncles of flowers with males and the lethal factor with hermaphrodites and males, Storey (1953) proposed that sex determination in papaya is controlled by a group of closely linked genes that are confined to a small region on the sex chromosome within which recombination is suppressed. Influenced by the sex determination system in *Drosophila*, Hofmeyr (1939, 1967) suggested that the symbols M_1 (M) and M_2 (M^h) represent inactivated regions of slightly different lengths from which vital genes are missing. On the basis of interspecific hybridization in Caricaceae and the fact that homozygous dominant genotypes were not viable, Horovitz and Jiménez (1967) proposed that sex determination in papaya is the XX-XY type. A more recent modification of the model proposed to explain papaya sex expression is that the three alleles encode different *trans*-acting factors to activate or inhibit stamen and carpel development (Sondur et al., 1996).

As a step toward cloning the sex determination gene in papaya, a high-density genetic mapping of the papaya genome was constructed using 1494 amplified fragment length polymorphism (AFLP) markers, five sequence-characterized amplified region (SCAR) markers, and two morphological markers. It is clear from this high-density map that recombination was suppressed in the region containing the sex determination locus as 225 markers co-segregated with sex (Ma et al., 2004). The initial physical map of the non-recombining region resulted in an estimated size of 4–5 Mbp or 10% of the chromosome (Liu et al., 2004). Our most current physical map consists of five contigs spanning a combined 6 Mbp length, suggesting that the non-recombining region is about 10–15% of the chromosome (Q. Yu, P. Moore, J, Jiang, A. Paterson, R. Ming, unpublished data). Considering that 225 (66%) of the 342 markers on linkage group 1 were in the non-recombining region, the polymorphism rate in this region was increased 14-fold compared with the markers on the remainder of the chromosome. The differences in recombination indicate a high sequence divergence between the X and Y homologs in this region. Selective sequencing of the BACs (bacterial artificial clones) identified by male-specific markers has revealed a decreased gene density and an increased transposable element density in the non-recombinant Y region. These findings led to the conclusion that a pair of incipient sex chromosomes has formed in papaya (Liu et al., 2004). Two additional lines of evidence supported the existence of sex chromosomes in papaya: (1) Embryo development stops in 20–50 days after pollination on genotypes with two Y chromosomes (or homozygous dominant alleles as called previously) (Chiu et al., 2000). (2) A pair of chromosomes separated precociously in 60–70% of pollen mother cells (Kumar et al., 1945; Storey, 1953), which is likely due to the lack of homology over the 10–15% of the male specific (non-recombining) region (Liu et al., 2004).

Papaya is a major fruit crop in tropical and subtropical regions. The lethal effect of two Y chromosomes has precluded the development of true-breeding hermaphroditic cultivars. The lack of true-breeding hermaphrodites creates a problem of segregating sex types, which requires growing multiple seedlings per hill and then manually thinning out undesirable sex types or excess trees when the plants are old enough to flower. By that time, the plants have undergone considerable resource competition with their undesirable neighbors. Because of this situation and its economic significance, papaya has received considerable efforts to discover DNA markers to clone the sex determination genes that might be used to identify and eliminate undesirable sex types at the seed or early seedling stage. It is conceivable that similar incipient sex chromosomes exist in other angiosperm species that have not undergone intensive research.

A primitive Y chromosome, recently reported in stickleback

fish (*Gasterosteus aculeatus*), is morphologically indistinguishable from the X chromosome (Peichel et al., 2004). It is evident that the sex-determining genes in some species are not located on classical heteromorphic sex chromosomes but rather within non-recombining regions on apparently homomorphic sex chromosomes. These primitive Y chromosomes share common features with degenerated Y chromosomes in plants and animals: heterozygosity in males or females, suppression of recombination, accumulation of repetitive sequences, and substantial X–Y nucleotide divergence. These common properties reveal similar, if not the same, evolutionary processes affecting the emergence and progress of sex chromosomes across life kingdoms.

DEGENERATION OF THE Y CHROMOSOME

The forces driving Y chromosome degeneration were proposed by evolutionary biologists and validated by molecular evidence obtained from model species. Once recombination has been suppressed in a chromosomal region, the prototype Y chromosome will gradually accumulate deleterious mutations by a process known as Muller's ratchet (Muller, 1964; Felsenstein, 1974). Selection for favorable alleles may drag along some of these deleterious mutations, and thus "hitchhiking" may add to the degeneration of the suppressed region (Rice, 1987). Other factors involved in Y chromosome degeneration may include background selection (Charlesworth et al., 1993; Charlesworth, 1994), which accelerates the fixation of mildly deleterious alleles and delays the fixation of mildly advantageous alleles, and the Hill–Robertson effect (McVean and Charlesworth, 2000), which inhibits the spread of favorable alleles and the elimination of deleterious ones from the interference of closely linked alleles under selection. Accumulated deleterious mutations may cause the Y chromosome to degenerate in both size and gene content and to diverge from the X chromosome (Charlesworth and Charlesworth, 2000), as a result of recombination suppression around the male-specific region of the Y chromosome (MSY).

The precocious separation of the primitive sex chromosomes in pollen mother cells (Storey, 1953) and the lethal effect of the YY genotype are clear indications of Y chromosome degeneration in papaya. Despite its small MSY, a mosaic arrangement of X- and Y-like sequences was detected in this small region (Liu et al., 2004). Evidence of Muller's ratchet is abundant and extremely low gene density was detected from the MSY sequence (Q. Yu, P. Moore, J, Jiang, A. Paterson, R. Ming, unpublished data). Most frequently, these deleterious mutations are caused by transposable element insertions because over-abundant retroelements were found in the papaya MSY as in the *Drosophila* and human Y chromosomes (Steinmann and Steinmann, 1992; Skaletsky et al., 2003; Liu et al., 2004). Duplications, frequently detected by DNA markers and direct DNA sequencing in papaya MSY (Liu et al., 2004; Q. Yu, P. Moore, J, Jiang, A. Paterson, R. Ming, unpublished data), could potentially play a role in protecting essential genes from degeneration, as is the case of the nine giant palindrome structures of the human MSY (Skaletsky et al., 2003). Extensive sequence divergence between the primitive X and Y chromosomes has been detected (Liu et al., 2004). Furthermore, the hermaphrodite Y^h and male Y in papaya share nearly identical DNA sequences in most parts of the MSY, yet sequence divergence did occur on these two Y chromosomes. These results indicate that the MSY in papaya hermaphrodites and males is derived from a common ancestral chromosome much more recently than the divergence of the X and Y chromosomes. This observation supports the hypothesis that males are modified hermaphrodites (or vice versa) in papaya (Storey, 1976).

In sticklebacks, extensive sequence divergence has been found between the X and Y chromosomes with an average of only 64% identity based on comparison of 250-kb sequences. As in papaya, duplicated sequences are present, and repetitive DNA has accumulated in the evolving Y chromosome. Interestingly, the Y region is 87 kb longer than its corresponding region on X and had large gaps in the sequence alignment because of insertions on the Y chromosome. This result may indicate that the sex determination region on the stickleback Y chromosome has been slightly enlarged compared to X, even though there are no cytologically visible differences between these two sex chromosomes. In other words, the primitive stickleback Y chromosome may be on an evolutionary path toward becoming significantly larger, like the enormous Y chromosome of the white campion, most likely through transposable element insertions and intrachromosomal duplication.

The medaka male-specific region is about 260 kb and represents only 1% of the chromosome. This region has accumulated repetitive sequences, and some genes have degenerated (Nanda et al., 2002). A male determination gene, *MDY*, in the medaka male-specific region has been characterized in detail. *MDY* is required and sufficient for male sex expression (Matsuda et al., 2002). No homologue has been found on the X chromosome, but there is an autosomal homologue, *dmrt1*. The *MDY* gene seems to have originated by a recent duplication of the autosomal gene, *dmrt1* (Nanda et al., 2002), which is present only in close relatives of medaka (Kondo et al., 2004).

Evidence for genetic degeneration of the Y chromosome has emerged with the accumulation of DNA sequence data of the classic flowering plant model for sex chromosome research, the white campion. The Y chromosome of white campion is largely non-recombining with the X chromosome. However, the Y chromosome looks euchromatic. Microdissected Y chromosome DNA of white campion competes with female genomic DNA to hybridize to the Y chromosome by fluorescent in situ hybridization (FISH) resulting in similar signal patterns on the X and Y chromosomes and autosomes (Matsunaga et al., 1999). To date, only two lines of evidence suggest genetic degeneration of its Y chromosome. One is that the YY genotype is not viable (Ye et al., 1990), and the other is that a functional X-linked male reproductive organ-specific gene 3 (*MROS3*) has a degenerated Y-linked copy (Guttman and Charlesworth, 1998). Five additional sex-linked housekeeping genes with intact X- and Y-linked copies have been isolated in white campion, including *Silene latifolia* X-gene 1/Y-gene 1 (*SlX1*/*SlY1*) (Delechère et al., 1999), *SlX3*/*SlY3* (Nicolas et al., 2005), *SlX4*/*SlY4* (Atanassov et al., 2001), Differential Display 44 X- and Y-linked allele (*DD44X*/*Y*) (Moore et al., 2003), and *Silene latifolia* spermidine synthase X- and Y-linked allele (*SlssX*/*Y*) (Filatov, 2005a), all with functional Y-linked genes. Collectively, this suggests that the degeneration of genes on the white campion Y is at a very early stage. Gene sequences have somehow diverged from their respective X homologs. The synonymous (silent mutation) divergence between the gene copies on the X and Y

chromosome is 1.7%, 16%, 8%, and 7% for *SlX1*/*SlY1*, *SlX4*/*SlY4*, *DD44X*/*Y*, and *SlssX*/*Y*, respectively.

A comparative genetic map for these X-linked genes was recently constructed (Filatov, 2005b). The gene order corresponds to what would be expected from the evolutionary strata model proposed for human sex chromosomes in that the least diverged sequence is closest to the pseudo-autosomal region (PAR) (Lahn and Page, 1999). The least diverged (*SlX1*/*SlY1*) and the most diverged (*SlX4*/*SlY4*) genes are at opposite ends of the map, while the other two genes (*DD44X*/*Y* and *SlssX*/*Y*) with intermediate divergence are in between. The *SlX1*/*SlY1* gene is the closest to the PAR. According to Filatov (2005a), all amino acid replacements between *SlssX* and *SlssY* occurred in the Y-linked gene. Some of these mutations affect highly conserved amino acid residues and are likely to disrupt the function of the *SlssY* gene, even though it is actively transcribed. The *SlssY* gene has an elevated synonymous substitution rate, compared with *SlssX*, suggesting that the Y chromosome has a higher mutation rate than the X chromosome (Filatov, 2005a).

The sequence of the human MSY provided new insights into our understanding of the structure and evolution of the Y chromosome (Skaletsky et al., 2003). The human MSY is made up of three classes of sequences, X-transposed, X-degenerate, and ampliconic (that exist within multiple, repeated palindromic segments) sequences. In addition to the genes in the ampliconic regions, most other genes in the MSY are found in the X-degenerate regions, which were once identical to the X sequence but have now diverged extensively from it. These genes display 60–96% sequence identity to their X-linked homologues and seem to be remnants of their ancient autosomes. Half of the genes in X-degenerate regions are pseudogenes, with sequence similarity to functional X homologues, while hundreds of other X-homologous genes were lost in the process of Y chromosome degeneration. The Y chromosome has acquired male fertility genes and lost many other genes, whereas the X chromosome has maintained its ancestral genes. Sequence divergence between X and Y chromosomes shows a clear pattern in relation to their chromosomal positions. The sequences of genes closest to the PAR have the least synonymous site divergence, while loci further away have extensively diverged (Lahn and Page, 1999; Skaletsky et al., 2003).

These data demonstrate that degenerative processes have occurred in the non-recombining region of the primitive Y chromosome, even though sex chromosomes of papaya, white campion, and stickleback fish have originated recently. As evolution proceeds, the Y chromosome may be predicted to change as a result of transposable element insertions, duplications, inversions, and translocations. The ancient human Y chromosome shrank to about one-sixth the size of the corresponding X chromosome during its 300 million years of evolutionary history. The human Y chromosome is an excellent case to reveal what could happen during the degeneration process. Conversely, the primitive Y chromosomes in plants are ideal models to study the mechanisms underlying the initial stages of sex chromosome evolution.

STAGES OF SEX CHROMOSOME EVOLUTION

Westergaard (1958) grouped plant sex chromosome into three types illustrating different evolutionary stages. In the first, the most primitive sex chromosome was characterized by the viability of the YY genotype, in which Y differs from X only in the sex determination genes; this condition is represented by *Ecballium*. The second type is that the YY genotype is inviable, while the Y chromosome plays a decisive role in sex determination; this condition is represented by papaya and white campion. The third type is that the Y chromosome is irrelevant to sex determination and sex is determined through the X–autosome balance; this condition is represented by sorrel (*Rumex acetosa*) and Japanese hop (*Humulus japonicus*).

Recent extensive genetic and genomic studies on the male-specific region in these species have led to refined models (Jablonka and Lamb, 1990; Charlesworth, 1996; Charlesworth and Charlesworth, 2000; Charlesworth et al., 2005). Based on the most recent data, sex chromosome evolution might be divided into five stages (Fig. 1). (1) A male sterile or a female sterile mutation occurs on a chromosome and recombination is suppressed at this locus and its immediate neighboring region leading to initiation of the degeneration process, but the YY genotype is viable. Asparagus is a good example for this stage. (2) Suppression of recombination spreads to additional linked loci that lead to the degeneration of a small chromosomal region and the formation of a male-specific region on the primitive Y chromosome. The loss of gene content is sufficiently extensive to cause lethality of the YY genotype even though the primitive sex chromosomes still appear to be homomorphic at the cytological level. The papaya sex chromosomes are at this early stage. (3) The accumulation of transposable elements and the duplication within the male-specific region cause the expansion of DNA content on the Y chromosome. The non-recombining region spreads to the majority of the Y chromosome and further degeneration occurs. At this stage, the X and Y chromosomes are heteromorphic, and the Y chromosome is physically larger than the X chromosome. The *Silene* sex chromosomes possess these properties. (4) Severe degeneration of the Y chromosome causes the loss of function for most genes and this enables deletions of the non-functioning Y chromosome sequences to result in shrinking the Y chromosome in size. There are no known plant sex chromosomes at this stage, but the sex chromosomes in mammals are good examples. It is also possible that some sex chromosome systems would not have this phase of shrinking in size but would continue to expand and degenerate until stage 5 when the Y chromosome got lost. In either case, a small portion of the sex chromosomes is still recombining to keep the X and Y chromosome pair together. (5) Suppression of recombination spreads to the entire Y chromosome. Further reduction in size of the Y chromosome and complete loss of the recombining pseudo-autosomal region occur. The Y chromosome is totally lost and sex determination is controlled by X to autosome ratio. A new Y chromosome might evolve, but it would have no effect on sex determination. Sorrel, Japanese hop, *Drosophila*, and nematode sex chromosomes are at this stage.

Sex chromosomes and maternal inheritance of organelle genomes are two evolutionary processes that are comparable between animals and plants. Plant sex chromosomes are mostly at early stages of evolution, but sorrel and Japanese hop appear to be exceptions. Although the sorrel and Japanese hop sex chromosomes evolved much more recently (angiosperm evolved about 158–179 MYA) than the 300 million years old mammal sex chromosomes, they appear to be at a later stage of sex chromosome evolution because the two Y chromosomes do

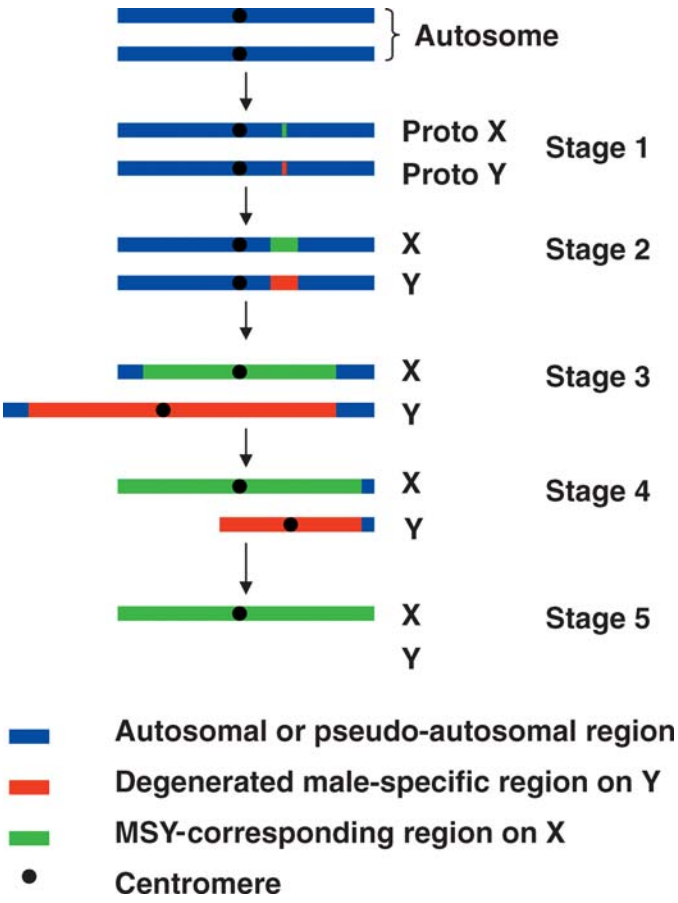

Fig. 1. The five stages of sex chromosome evolution based on the size of the nonrecombining region, degree of degeneration, and size of Y chromosome. Stage 1: Suppression of recombination at the sex determination locus and its neighboring regions led to mild degeneration of the suppressed region. YY genotype is viable. Stage 2: Suppression of recombination continues to spread, and a small MSY region evolved. YY genotype is not viable. Stage 3: The MSY expands in size and degenerates in gene content by accumulation of transposable element insertions and intrachromosomal rearrangements. The X and Y chromosomes become heteromorphic. Stage 4: Severe degeneration of the Y chromosome causes loss of function for most genes. Deletion of nonfunctional DNA sequences results in shrinking of the Y chromosome in size. Stage 5: Suppression of recombination spreads to the entire Y chromosome. The Y chromosome is lost, and X-to-autosome ratio sex determination system has evolved.

not contain sex determination genes. This might imply that the rate of Y chromosome degeneration varies among species or that some species-specific chromosomal events could dramatically alter the course of sex chromosome evolution.

MODEL SPECIES FOR THE STUDY OF PLANT SEX CHROMOSOMES

Sex chromosomes of plants were first described in white campion and hop (Blackburn, 1923; Winge, 1923), sorrel (Kihara and Ono, 1923), and *Elodea* (Santos, 1923). Heteromorphic sex chromosomes have been convincingly demonstrated in species of four families (Table 1) (Westergaard, 1958; Parker, 1990; Charlesworth and Guttman, 1999; Matsunaga and Kawano, 2001). Among the species with heteromorphic sex chromosomes, white campion has distinctive X and Y chromosomes, which are the largest and second largest chromosomes, respectively, in the male plants. Sorrel has one large X and a pair of different small Y chromosomes in male plants.

Among the species with homomorphic sex chromosomes, papaya has a clearly defined MSY region, while asparagus has an *M* locus with evidence of suppression of recombination (Reamon-Büttner and Jung, 2000; Jamsari et al., 2004; Liu et al., 2004). A female heterozygous sex chromosome system might exist among *Fragaria* species in Rosaceae (Kihara, 1930).

Sex determination of asparagus is under the control of an active-Y chromosome system. The genome size of diploid *Asparagus officinalis* is 1323 Mbp/1C for 10 chromosomes, 3.6× the papaya genome of 372 Mbp/1C for nine chromosomes (Arumuganathan and Earle, 1991). The large genome of asparagus allows the separation of its 10 pairs of chromosomes by size and morphology. The chromosome pair 5 of *Asparagus officinalis* was identification as the sex chromosomes by trisomic analysis, although the X and Y chromosomes are homomorphic and contain only a small amount of constitutive heterochromatin (Loptien, 1979). The Y chromosome contains two tightly linked genes, a male activator (*M*) and a female suppressor (*F*) supported by the appearance of rare hermaphrodite and sterile plants in the asparagus population (Marks, 1973). The viability of the YY genotype indicates that the Y chromosome degeneration is mild and at an early stage of sex chromosome evolution.

Papaya is trioecious with three sex types: male, female, and hermaphrodite. Classical genetic and cytogenetic studies led to several hypotheses for papaya sex determination (Hofmeyr, 1938, 1939; Storey, 1953; Horovitz and Jiménez, 1967). Based on high density genetic mapping, physical mapping, and DNA sequence data, sex determination in papaya is controlled by a pair of homomorphic primitive sex chromosomes with a small MSY that account for 10–15% of the Y chromosome (Ma et al., 2004; Liu et al., 2004; Q. Yu, P. Moore, J, Jiang, A. Paterson, R. Ming, unpublished data). The available genomic resources and the small MSY region opened the door for sequencing the MSY and the corresponding region of the X chromosome and the eventual cloning of the sex determination genes.

In white campion, the Y chromosome is decisive in determining sex as shown by three observations: (1) application of hormones does not convert the sex, (2) the presence of a single Y chromosome can suppress female development when three X chromosomes are present, and (3) autosome ratios have no effect on the sex determining factors on the Y chromosome (Westergaard, 1958). In other words, as is true in mammals, the sex of the individual is determined entirely by the presence or absence of the Y chromosome, which is called the active-Y system.

In sorrel, male plants have one X and two different Y chromosomes ($2n = 15$, XY_1Y_2), and females have two X chromosomes ($2n = 14$, XX). The two Y chromosomes are highly heterochromatic. Both Y chromosomes in sorrel are required for pollen fertility but not for stamen development. In contrast to white campion, Y chromosomes in sorrel do not suppress female gynoecium development and do not contain male determining genes, because plants with $2A + 2X + 1$ or 2Y were female. Instead of an active-Y system, sorrel sex is determined by an X-to-autosome balance system (Ono, 1935;

Westergaard, 1958). The Y chromosome deletion caused the merger of the pollen mother cells and sterile pollen. The centromeres of the two Y chromosomes are variable, which may involve the expression of the sequences on Y chromosome (Ainsworth et al., 1999).

PROSPECTS

To understand the origin and the process of sex chromosome evolution, it is necessary to study a series of organisms at various stages in the evolutionary process. Flowering plants provide such a series of incipient, early stage, and late stage sex chromosomes. Cloning of the sex determination genes from multiple species is a critical step toward elucidating the sex determination pathways and unraveling the mysteries of sex chromosome evolution in flowering plants. However, the Y chromosomes have evolved from the suppression of recombination at the sex determination locus, and map-based cloning is not an option. Sequencing the MSY region from Y chromosomes at different evolutionary stages would facilitate the cloning of the sex determination genes in addition to revealing the sequence features and evolutionary history of the Y chromosomes. Specifically, the complete sequence of the MSY provides the necessary genomic resources to locate the deleted region(s) of induced sex-reversal Y deletion lines generated via irradiation (Farbos et al., 1999) and subsequently the candidate genes for sex determination. The small MSY region of papaya is being sequenced, and whole-genome shotgun sequencing of the female papaya genome is well underway. Knowing the genomic sequences of the sex chromosomes and/or the entire genome will remedy difficulties with drawing conclusions based on fragmented DNA markers and/or sequence data and will naturally expedite identifying the sex determining genes. The resulting genomic tools and resources would also foster investigations of interactions between genotype and environment in sex determination and sex chromosome evolution.

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