

# Gender in plants: sex chromosomes are emerging from the fog

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Although most plants have flowers with both male and female sex organs, there are several thousands of plant species where male or female flowers form on different individuals. Surprisingly, the presence of well-established sex chromosomes in these dioecious plants is rare. The best-described example is white campion, for which large sex chromosomes have been identified and mapped partially. A recent study presented a comprehensive genetic and physical mapping of the genome of dioecious papaya. It revealed a short male specific region on the Y chromosome (MSY) that does not recombine with the X chromosome, providing strong evidence that the sex chromosomes originated from a regular pair of autosomes. The primitive papaya Y chromosome thus represents an early event in sex chromosome evolution. In this article, we review the current status of plant sex-chromosome research and discuss the advantages of different dioecious models.

Although in animals, the germ line is differentiated early in development, plants have no distinct germ line [1]. Instead, totipotent meristematic cells proceed through a long period of vegetative development before they eventually form complex sexual organs – the flowers [2]. The majority of plants are cosexuals, possessing either the bisexual flowers (hermaphrodite species) or the unisexual flowers of both sexes on one individual, MONOECY (see Glossary). In others, individuals form either exclusively male or female flowers (DIOECY). Plant dioecy is accompanied by little or no somatic sexual dimorphism [3].

## What do we call sex chromosomes?

Sex chromosomes have evolved in both animal and plant kingdoms independently and repeatedly. The first plant sex chromosomes were revealed in the dioecious plants white campion [4] and sorrel [5], approximately at the same time as mammalian sex chromosomes were discovered [6]. Although we have some understanding of sex chromosomes in humans, mouse, fruit fly and *C. elegans*, our knowledge of the basis of sex determination or sex chromosome structure in the majority of other organisms is poor. There are even large groups of evolutionarily advanced vertebrates, for example, certain fish, in which sex chromosomes are not apparent and where sex changes during the life cycle are due to the environment [7], or

reptiles, where gender is controlled by temperature during embryo development.

A definition of sex chromosomes is that they carry sex-determining genes (i.e. which control female and male fertility), and that they are heteromorphic [distinguishable microscopically in the two sexes by size or shape (e.g. arm ratios), both from autosomes and from each other]. Sex chromosomes – at least partially – do not recombine during meiosis in the heterogametic gender (i.e. in XY males in a majority of animal and plant species or in ZW females in birds and butterflies). When the sex-determining genes are mapped to chromosome pairs that are not morphologically different they are called homomorphic, whereas sex chromosomes (X and Y or Z and W) that can be distinguished by microscopy are classified as heteromorphic (historically called accessory chromosomes, heterochromosomes or allosomes). When more than two types of sex chromosomes are found in one individual, they

## Glossary

**Monoecy:** plant species in which individuals form unisexual male and female flowers, often physically separated, on the same individual.

**Dioecy:** plant species that form unisexual flowers on two types of individuals, staminate flowers on males and pistillate flowers on females. The word dioecious is from Greek and means 'two households'.

**Gynoeceum:** the female ovule-bearing part of a flower composed of ovary, style and stigma.

**Heterogamety:** individuals forming two types of gametes; the sex segregates from the heterozygous parent, its gametes possess either X or Y (in other species either Z or W) chromosomes.

**Homogamety:** individuals forming only one type of gamete, usually females possessing the X chromosome in mammalian and fruit fly types of sex determination or males possessing the Z chromosome in birds and butterflies.

**Epigenetics:** a non-mendelian type of genetics involving heritable changes of gene expression that occur without a change of DNA sequence. Epigenetic processes are usually based on transcriptional silencing linked to cytosine methylation and/or chemical modifications of nucleosomal histones.

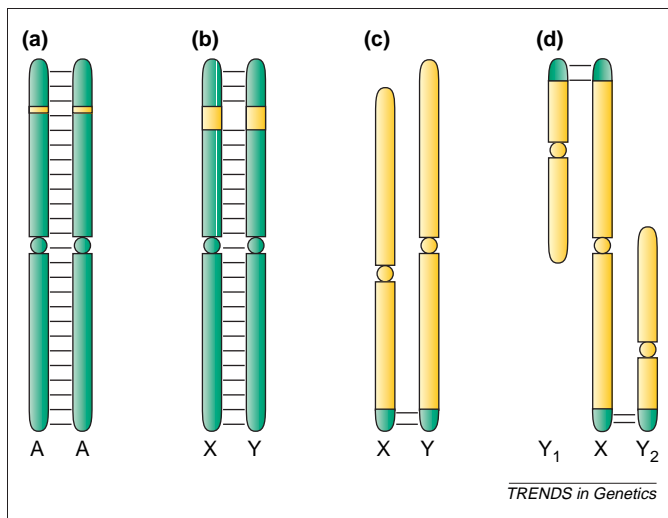
**Dosage compensation:** developmental mechanisms by which some species equalize the levels of gene expression of X-chromosome linked genes in XY-males and XX-females. These mechanisms can be either negative (down-regulation, suppression of one of the two X chromosomes in females, e.g. mammals) or positive (up-regulation, enhancement of activity of the single X chromosome in males, for example, fruit fly).

**Homosporous:** a non-flowering plant species producing only one type of spores that form the gametophyte harbouring both types of sex organs (analogous to monoecious flowering plants).

**Gynodioecy:** plant species in which individuals are either female (pistillate flowers) or hermaphrodite (bisexual flowers). Gynodioecy is often regarded as an evolutionarily intermediate stage between bisexuality and dioecy.

**MADS box genes:** a conserved group of homeotic genes encoding transcriptional factors, which control identity of floral whorls.

**MSY:** the male specific region of the Y chromosome, which does not recombine with the X chromosome and as a result of this loss is often genetically degenerate.



**Figure 1.** A sketch of the four main steps in the evolution of plant sex chromosomes, from the most primitive (left) to evolutionarily advanced and degenerate (right). (a) Squirting cucumber (*Ecballium elaterium*) with single locus-based sex determination and no observed blockage of genetic recombination observed. 'A' stands for autosomes. (b) Papaya (*Carica papaya*) possesses homomorphic sex chromosomes X and Y, with a short non-recombining region on the Y chromosome, MSY. (c) White campion (*Silene latifolia*) has large sex chromosomes with a Y that is largely non-recombining but looks euchromatic. (d) Sorrel (*Rumex acetosa*) has polymorphic sex chromosomes with two different Y chromosomes, both are constitutively heterochromatic.

are termed polymorphic. Good examples of this polymorphism in plants include sorrel (*Rumex acetosa*: male  $XY_1Y_2$ , female XX), and hop (*Humulus lupulus* var. *cordiflorus*: male  $X_1X_2Y_1Y_2$ , female  $X_1X_1X_2X_2$ ). From an evolutionary point of view we expect that relatively young, simple genetic systems of sex determination (e.g. mercury, spinach and papaya) should fall into the category of homomorphic sex chromosomes (Figure 1). Inversions, duplications and deletions, which might be part of degenerative processes that occur on the Y chromosome, could lead to the next evolutionary step, X-Y heteromorphy (e.g. white campion or liverwort). Translocations or chromosome fission can result in the sex chromosome polymorphism, as mentioned previously, sometimes accompanied by a globally heterochromatic character of the Y chromosomes (e.g. sorrel).

### Genetic determination of gender

Many dioecious species are known (~5% of species in the plant kingdom, spread over 75% of families), including white campion, sorrel, hop, hemp, pepper, spinach and ginkgo [8]. They include many taxonomic groups (Table 1), from mosses (e.g. *Marchantia polymorpha*) and gymnosperms (e.g. *Ginkgo biloba*), to dicotyledonous (e.g. *Silene latifolia*) and monocotyledonous angiosperms (e.g. *Asparagus officinalis*). Our knowledge of the genetic basis for sex determination in these plants is rather limited. In some plants, sex is determined by a simple mendelian genetic

**Table 1.** Well-studied dioecious plant species

Systematic group	Family	Species	Characteristics of sex determination (and sex chromosomes)	
Bryophyta, mosses	Hepaticeae	<i>Marchantia polymorpha</i> , liverwort	Either X (female) or Y (male) chromosome in haploid gametophytes	
Coniferophyta, gymnosperms	Ginkgoaceae	<i>Ginkgo biloba</i> , ginkgo tree	Heterogametic males	
Magnoliopsida, angiosperm dicotyledonous	Caryophyllaceae	<i>Silene latifolia</i> , white campion	Dominant Y, heterogametic XY males	
		<i>Silene dioica</i> , red campion	Dominant Y, heterogametic XY males	
		<i>Silene otites</i> , Spanish catchfly	Heterogametic males	
		<i>Silene diclinis</i>	Heterogametic females	
	Polygonaceae	<i>Rumex acetosa</i> , common sorrel	X-A polymorphic system, heterogametic $XY_1Y_2$ males	
		<i>Rumex thyriflorus</i>	X-A polymorphic system, heterogametic $XY_1Y_2$ males	
		<i>Rumex rothschildianus</i>	X-A polymorphic system, heterogametic $XY_1Y_2$ males	
		<i>Rumex hastatulus</i>	X-A system, XY heterogametic males	
		<i>Rumex acetosa</i>	X-A system, XY heterogametic males	
	Cannabinaceae	<i>Humulus lupulus</i> , hop	X-A system, XY heterogametic males	
		<i>Humulus lupulus</i> var. <i>cordiflorus</i>	X-A polymorphic system, heterogametic $X_1X_2Y_1Y_2$ males	
			<i>Humulus japonicus</i>	X-A polymorphic system, heterogametic $XY_1Y_2$ males
			<i>Cannabis sativa</i> , hemp	X-A system, heterogametic XY males
	Cucurbitaceae	<i>Bryonia dioica</i> , white bryony	Heterogametic males	
		<i>Coccinea indica</i>	Dominant Y, heterogametic XY males	
			<i>Ecballium elaterium</i> , squirting cucumber	Heterogametic males
Euphorbiaceae		<i>Mercurialis annua</i> , annual mercury	Heterogametic males	
Chenopodiaceae		<i>Atriplex garrettii</i> , orache	Heterogametic males	
		<i>Spinacia oleracea</i> , spinach	Heterogametic males	
Actinidiaceae		<i>Actinidia deliciosa</i> , kiwi fruit	Heterogametic males	
Caricaceae		<i>Carica papaya</i> , papaya tree	Dominant Y, heterogametic XY (homomorphic) males	
Loranthaceae		<i>Viscum fischeri</i> , mistletoe	Heterogametic males	
Rosaceae		<i>Fragaria elatior</i> , wild strawberry	Heterogametic females	
Salicaceae		<i>Salix viminalis</i> , osier (willow)	Heterogametic females	
		<i>Populus tremula</i> , European aspen	Heterogametic males	
Liliopsida, angiosperm monocotyledonous	Asparagaceae	<i>Asparagus officinalis</i> , asparagus	Heterogametic males	
	Dioscoreaceae	<i>Dioscorea tokoro</i> , wild yam	Heterogametic males	

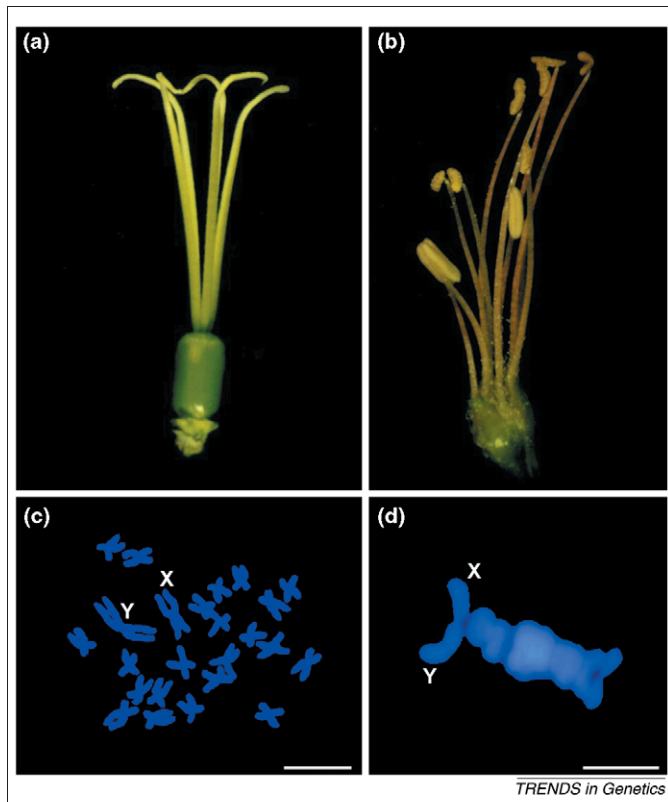
system based on the segregation of one locus, as in squirting cucumber (*Ecballium elaterium*), or a few loci, as in annual mercury (*Mercurialis annua*). Although there are thousands of dioecious species, only in a few of them have heteromorphic sex chromosomes been unambiguously demonstrated [9,10]. The most well-known species are white campion (*Silene latifolia*, previously known as *Melandrium album*) and common sorrel (*Rumex acetosa*).

White campion possesses clearly different, large heteromorphic sex chromosomes, X and Y in males and two Xs in females. The heterogametic male gender with the genetically dominant Y chromosome in this system is analogous to the mammalian system (Figure 2). However, we do not know much about this genetic system. There are three regions on the (male) Y chromosome that control sexual development. They include genes encoding GYNOECIUM suppression, stamen promotion and male fertility [11,12]. Theoretical predictions about the existence and organization of these three Y chromosome regions have been confirmed recently by the molecular mapping of deletion mutants [13–15]. Little is known about the function of the X chromosome [16]. The X and Y chromosomes are easily distinguished by size, the Y being the largest chromosome, a property that has facilitated the construction of chromosome libraries from manual or laser microdissected chromosomes [17,18]. Recently, a modified fluorescence *in situ* hybridization protocol with laser beam microdissected and degenerate oligonucleotide primed (DOP) amplified X or Y chromosomes has revealed

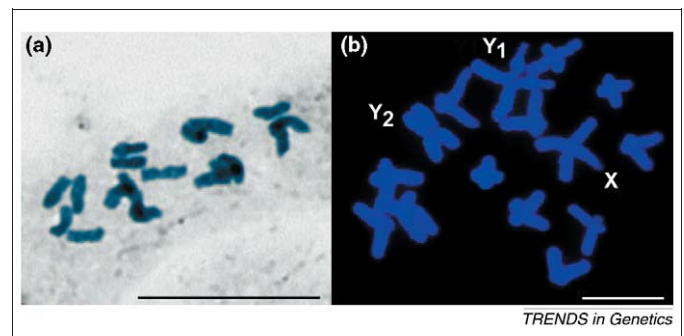
differences in structure between these sex chromosomes [19]. It is also possible to localize gene sequences by PCR on laser-sorted chromosomes [20]. The large genus *Silene* is sexually highly varied, with at least four dioecious and many hermaphrodite (e.g. *S. conica*, sand catchfly) or gynodioecious (e.g. *S. vulgaris*, bladder campion) species [21]. Comparisons of certain nucleotide sequences of the X and Y chromosomes have revealed that the Y chromosome of *S. latifolia* (white campion), although mostly euchromatic, might be genetically degenerate, at least to some extent [22]. Currently, several laboratories in Europe, USA and Japan are trying to shed light on the molecular mechanisms of sex-chromosome structure and determination in this species [23–27].

*Rumex acetosa* and related sorrel species have an X–A system, similar to the fruit fly, with two Y chromosomes, which are necessary for male fertility but not for the development of male flowers (Figure 3). The X is required in both sexes. A multiple Y chromosome system is usually due to the translocation of an autosome to the X chromosome followed by a co-segregation of the normal autosomal partner with the original Y chromosome to maintain a balanced karyotype. This results in two Y chromosomes [28]. It is unclear whether such a translocation event occurred in sorrel because the same types of abundant repetitive sequences cover both the Y chromosomes, Y<sub>1</sub> and Y<sub>2</sub>, indicating a common origin (Figure 4) [29,30]. The two heterochromatic Y chromosomes in sorrel persist condensed during interphase and these ‘sex-bodies’ are thus unique examples of epigenetic modification of plant constitutive heterochromatin [31].

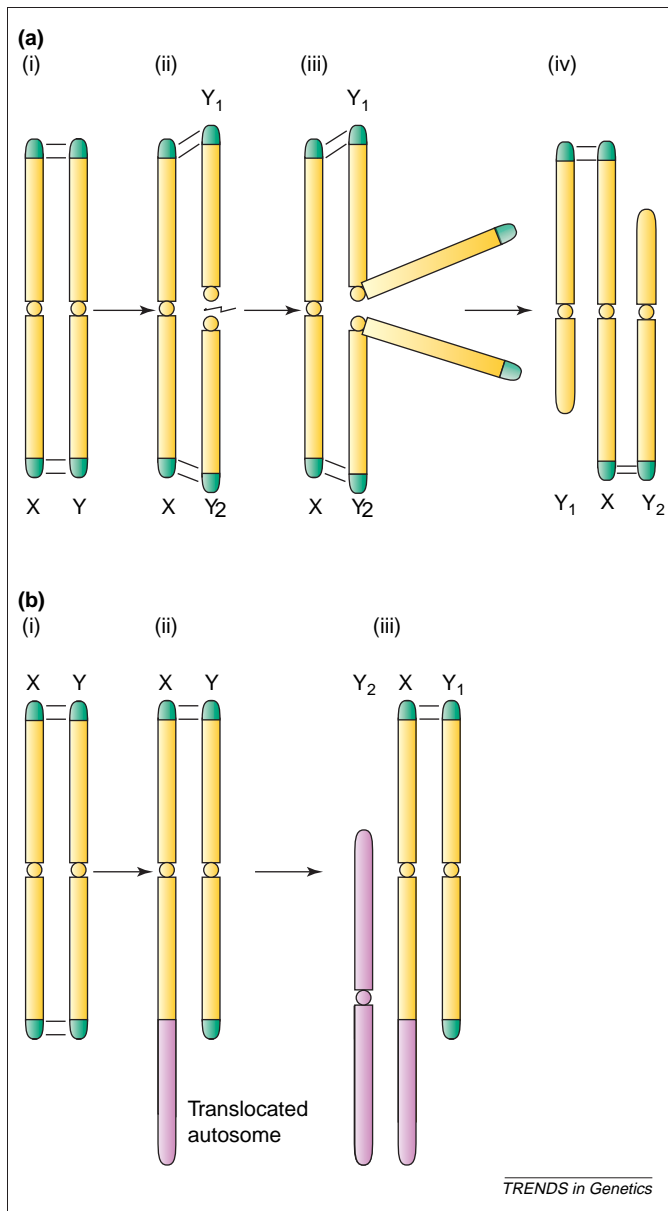
During the past decade, a classical example of plant dioecy has been revisited: the liverwort (*Marchantia polymorpha*). Individual liverwort plantlets (gametophytes) are haploid and possess either an X or a Y chromosome. The Y chromosome is much smaller than the X chromosome and has accumulated numerous repetitive DNA sequences that do not occur on any other chromosome. A novel Y-specific gene family was found to be embedded among these repeat sequences. This gene family encodes a protein that is expressed exclusively in male sexual organs. Another five putative genes that are also present in the Y chromosome-specific repeat region have



**Figure 2.** The dioecious model plant *Silene latifolia*. (a) Female and (b) male flowers; the calyx and corolla were removed to display pistils and stamens, respectively. (c) Mitotic root tip male metaphase with  $2n = 22 + XY$ . (d) Pollen mother cells in metaphase I of meiotic division indicating a restricted pairing region of the X and Y chromosomes. Bars represent 10  $\mu\text{m}$ .



**Figure 3.** The mitotic metaphase plates of two model dioecious plants, illustrating early and late steps of sex chromosome evolution in papaya and sorrel, respectively. (a) *Carica papaya*,  $2n = 18$ . In this case, the homomorphic pair of sex chromosomes could not be distinguished. (b) *Rumex acetosa* male,  $2n = 12 + XY_1Y_2$ . Note a deeper 4'-6-diamidino-2-phenylindole (DAPI) staining of the two Y chromosomes indicating their constitutive heterochromatin structure. Bars represent 10  $\mu\text{m}$ .



**Figure 4.** Two possible ways the multiple  $XX-XY_1Y_2$  chromosome system might have evolved. **(a)** The evolution of the  $XX-XY_1Y_2$  system through a fission event [29]. (i) In the beginning there are  $XY$  sex chromosomes. (ii) During centric fission of the  $Y$  chromosome, two telocentric sex chromosomes are newly established ( $Y_1$  and  $Y_2$ ). (iii) Both telocentric  $Y$  chromosomes form isochromosomes. (iv) Deletion of the pairing region at one end of both new  $Y$  chromosomes. **(b)** The evolution of  $XX-XY_1Y_2$  system through autosomal translocation to the original  $X$  chromosome [28]. (i) As in the fission model in the beginning there are  $XY$  sex chromosomes. (ii) Translocation of autosome to original  $X$  chromosome. (iii) The homologue of translocated autosome is segregated with the original  $Y$  to maintain a balanced karyotype and the  $XY_1Y_2$  system is established.

been described [32]. These five genes are amplified on the  $Y$ , which suggests that the  $Y$  evolved by co-amplifying genes with repeat sequences.

There might also be some examples of female HETEROGAMETY in plants, such as in natural polyploid strawberry (*Fragaria elatior*), willow (*Salix viminalis*) and Spanish catchfly (*Silene otites*). Formally, this system would resemble female heterogamety ( $ZW$  chromosomes) and male HOMOGAMETY ( $ZZ$ ) in birds and butterflies, however, this remains to be confirmed.

### Other mechanisms involved in sex expression

EPIGENETICS has a controlling role in animal and plant reproductive development [33]. One of the best examples of an epigenetic process is the inactivation of one of the two  $X$  chromosomes in mammalian females, a phenomenon known as Barr body formation or lyonization. DOSAGE COMPENSATION of  $X$ -linked genes in mammals is an evolutionary consequence of  $Y$  chromosome degeneration and ensures that both sexes are provided with similar levels of gene products [34]. Some data indicate that this phenomenon also occurs in dioecious plants but clear molecular evidence is still missing [35]. Epigenetic mechanisms, especially DNA methylation, are known to have an important role both in flower setting and in sex organ formation [36]. Genomic imprinting, a reversible process by which a sex-specific modification of some alleles leads to functional differences between paternal and maternal genomes in progeny, has also evolved in flowering plants, although only in the endosperm line [37]. Experimental hypomethylation of the dioecious *Silene latifolia* genome induces sex reversal: a majority of genotypically male plants ( $XY$ ) form perfect (bisexual) flowers, indicating that the expression of sex-determination genes is under epigenetic control. The bisexual flowers display various degrees of gynoecium development and seed set. These traits are transmitted to several successive generations but only when hermaphrodite plants are self-crossed or used as pollen donors (holandric inheritance). The sex reversal is inherited with incomplete penetrance and varying expressivity, facts that strongly emphasize its epigenetic character [38]. An  $XY$  epimutant plant was also used to demonstrate the genetic erosion of the  $Y$  chromosome. When the epimutant is used as a seed partner in a cross with a hermaphrodite mutant possessing a large deletion on the  $Y$  chromosome, which makes it unable to pass through meiosis, only female progeny appear. These data indicate that the  $Y$  chromosome is not transmitted through the female line, and that the  $Y$  is not able to support development of the female embryo sac [39].

Sex expression in plants is often influenced by hormones, as has been well described in the monoecious models such as the fern *Ceratopteris richardii* and maize *Zea mays*. *C. richardii* is a HOMOSPOROUS fern, and the gender (either hermaphrodite or male) of its gametophyte is determined by the pheromone antheridiogen, probably a gibberellin-like substance [40]. At least one antheridiogen-induced gene has been cloned and characterized recently [41]. Maize is probably the best-described monoecious plant, with male tassels at the apex and female ear inflorescences along the main shoot. As in *C. richardii*, the gender of maize flowers is realized through gibberellin action. A pair of antagonistic genes, *Silkless1* and *Tassel-seed2*, seems to be responsible for feminization and masculinization of flowers, respectively. The female active *Silkless1* produces a gibberellin-like, pistil-specific factor that inhibits stamen formation and promotes pistillate florets [42]. Other plant hormones, especially cytokinins and auxins, can modulate the gender of many dioecious and monoecious plants in a variety of ways in several different species [43]. In many

plant species dioecy or GYNODIOECY is unstable and/or influenced by environment.

### Molecular analysis of the sex chromosomes

Recent studies of plant sex chromosomes have characterized successfully the first active genes that are linked to *Silene latifolia* sex chromosomes – *MROS3* [26,22], *SIX1* (or its homologue *Y1*) [23], *SIX4* (or its homologue *Y4*) [44], and *DD44* [27]. None of these genes, however, have a direct relation to sex determination. In contrast to mammalian sex chromosomes, which have been evolving for at least 250–300 million years (My), the sex chromosomes of *S. latifolia* are ~10 times younger. Nevertheless, the sequence analysis of some of these genes shows that the degeneration of the Y chromosome is already in progress. The comparisons of X and Y alleles of *MROS3* [22], *SIX1* (*Y1*) [24] and *SIX4* (*Y4*) [44] reveal different stages of their sequence diversification. Although the silent-site divergence between *SIX4* and *SIY4* is 18% and between *MROS3-X* and *MROS3-Y* is ~19%, the value for *SIX1* and *SIY1* is much lower, only 4%. Similar data are also obtained for amino acid differences. These findings can be explained by the fact that these genes are localized in distinct parts of the sex chromosomes where recombination blockage has occurred at different times during evolution. Another hypothesis is that during the evolution of sex chromosomes some genes were translocated from autosomes after the diversification of sex chromosomes had begun [45]. The potential ‘strata’ on *S. latifolia* Y chromosome corresponding to individual evolutionary events (inversion, translocation) have to be determined more precisely by analyzing a larger amount of genes.

The lack of recombination between proto-X and proto-Y chromosomes eventually leads to the accumulation of slightly deleterious mutations and degeneration [46]. These changes can be accelerated because genes on the Y chromosomes are present only in one copy, in one of the two sexes, and thus the effective population size is four times reduced compared with autosomes. Thus the Y chromosome is more susceptible to random processes, leading to the fixation of deleterious alleles by genetic drift [47]. There are several other mechanisms acting on a non-recombining chromosome. One of them is Muller’s ratchet, a stochastic loss of the least mutated class of chromosomes. Closely related to Muller’s ratchet is the Hill-Robertson effect, in which weakly selected closely linked alleles that interfere with each other inhibit the spreading of advantageous alleles and the elimination of deleterious alleles. However, experiments on *Drosophila miranda* show that the Hill-Robertson effect is of minor importance for the degeneration of the Y chromosome [46]. Another process that impacts the evolution of the Y chromosome is background selection, which reduces the effective population size by selecting against deleterious mutations at linked regions. Finally, genetic hitchhiking can lead to the fixation of deleterious alleles that are linked to a favorable mutation on the Y chromosome [10].

Probably the clearest criterion for characterizing Y chromosome degeneration is a test of whether the Y chromosome is sufficient to enable the survival of individuals without at least one copy of ‘the ordinary’ sex

chromosome (i.e. the X chromosome) [11]. To our knowledge, this criterion is fulfilled only in the liverwort. Another rare example of the Y sufficiency is *Asparagus officinalis*, in which YY ‘supermales’ are viable [48]. In other plant dioecious models (e.g. white campion), at least one copy of the X chromosome is necessary for somatic survival, which means that the Y is at least partly degenerate and not able to provide some basic gene products.

Recent studies have also revealed some genes on plant Y chromosomes that have no homologues on the X chromosome. The Y chromosome of *Marchantia polymorpha* contains a gene family expressed specifically in male sexual organs [32]. Another example of such a sequence is a duplicated MADS BOX GENE on *S. latifolia* Y chromosome that is strongly expressed in developing stamens [45]. This result indicates that the evolution of the Y chromosome can involve duplications of autosomal regions. Recent studies of an advanced phase of Y chromosome degeneration have revealed several types of repetitive DNA sequences. For example, LINE-like retrotransposons are accumulated (to several hundred copies) at the termini of the long arm of the hemp Y chromosome [49]. In sorrel, accumulation of sequences that are repeated in tandem has occurred predominantly on the Y chromosomes [30]. The pattern of this repetitive DNA is characteristic for both  $Y_1$  and  $Y_2$  and has no homology to known sequences in the EMBL Data Library (GenBank accession nos. AB020591 and AB020601).

### So young but degenerate: the papaya primitive Y chromosome

The papaya sex determination system has long been considered to be clear and simple. A single locus *M* with three alleles has a key role in sex determination ( $M_1$ , male;  $M_2$ , hermaphrodite; *m*, female). Males and hermaphrodites are heterozygous of the constitution  $M_1m$  and  $M_2m$ , respectively, whereas the recessive combination *mm* stands for females. Any combination of two dominant *M* alleles is embryonically lethal [50]. Because males are heterozygous and females are homozygous, sex determination in papaya has been regarded as the XY type (males are heterogametic, the male Y is dominant). Papaya is an important crop plant in the Pacific region and, due to its small genome (372 Mb, comparable with *Arabidopsis* or rice), could provide excellent opportunities to study sex chromosomes and their evolution. Ming and colleagues [51] constructed a high-density genetic map of papaya based predominantly on amplified fragment length polymorphism (AFLP) and found a high level of DNA polymorphism in a genomic region surrounding the mapped sex locus. The observation that this region reveals no recombination activity led the authors to conclude that they had localized the MSY region of the proto-sex chromosome system of papaya [52]. The subsequent physical mapping of the non-recombining region using a BAC library revealed that the MSY is short, only ~4–5 Mb. However, this region is strictly male specific and harbours the male (or hermaphrodite) gene, which is dominant and crucial for floral sex expression. This region forms only ~10% of the chromosome length, which is

certainly negligible compared with the 95% of the human Y that does not recombine with the X chromosome. Preliminary chromosome analyses show that the papaya sex chromosomes are homomorphic.

Ray Ming and colleagues demonstrate that degenerative processes in the non-recombining region of the papaya Y chromosome have occurred, indicating that the first steps of sex chromosome evolution and diversification are under way [52]. The fact that the erosion of Y chromosome genes in papaya has already begun is clear from the fact that homozygous YY embryos abort 25–50 days after pollination. Sequencing of some parts of the non-recombining region revealed that in comparison with a genome-wide sample of papaya DNA the MSY has ~38% lower gene density, 28% higher retroelement density, and 189% higher inverted repeat density. In addition, just as has been recently shown in the euchromatic region of the human Y chromosome, degenerated and abundant inverted repeats have also been found in the Y of papaya. These repeats can help to explain how a non-recombining chromosome can preserve essential genes from degeneration. As recombination between X and Y chromosomal regions stops, the Y chromosome is unable to check its partner by crossing-over. This situation finally leads to an accumulation of repetitive sequences and a loss of gene function in the male specific region of the Y chromosome. The need to maintain the expression of genes responsible for male characters forces the Y chromosome to maintain important genes in the MSY by some other means. In the human Y chromosome, an MSY region that carries genes indispensable for male functions is arranged in duplicated segments and most of these duplications reveal palindromic character [53]. Y–Y gene conversion probably helps the Y chromosome avoid the irrecoverable deletion of genes. A recent study on human and ape Y chromosome palindromic regions shows low divergence within these sequences, which can be maintained only by intra-chromosomal transfer of DNA information – gene conversion [54]. A duplicated arrangement of Y-linked sequences and the existence of gene families on the Y chromosome have recently been observed in papaya. It is not yet clear whether the duplicated Y-linked sequences have a similar role in maintaining Y-chromosome function in plants as they do in animals. Although papaya represents a early step in sex chromosome evolution, its MSY region surprisingly shows signs of genetic degeneration similar to the evolutionarily much older human Y chromosome. The similarity of DNA sequences between the MSY region of the male and hermaphrodite papaya plants, in contrast to their divergence from the corresponding X-linked region, is consistent with much other evidence that dioecious plants might evolve via gynodioecy, and that males are modified hermaphrodites. Future comparative studies of papaya relatives (both monoecious and dioecious) can help to clarify the mechanisms that led to the evolution of sex chromosomes and dioecy.

### Concluding remarks

Plant sex chromosomes in dioecious species, although rare, are highly variable and evolutionarily young, which make them excellent models to study the structure and evolution of sex chromosomes. Plant sex chromosomes can be

grouped into at least four classes illustrating different stages of sex chromosome evolution (Figure 1). The simplest single gene-based system in squirting cucumber (and similar simple determination as described previously, for example, in annual mercury) presents the first step before the establishment of sex chromosomes. Although papaya possesses homomorphic sex chromosomes with a short MSY, the degenerative processes on the Y chromosome have already been observed. A gradual degeneration of the Y chromosome in white campion led to the evolution of large heteromorphic sex chromosomes. Sorrel and liverwort represent the most advanced step in sex chromosome diversification. The Y chromosomes in these species have accumulated large amounts of specific DNA repeats and appear globally heterochromatic. The characterization and comparison of these plant models enable us to study the stages leading to the evolution of non-recombining sex chromosomes in both the plant and the animal kingdoms.

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