

Sex Chromosomes in Land Plants

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Abstract

Sex chromosomes in land plants can evolve as a consequence of close linkage between the two sex determination genes with complementary dominance required to establish stable dioecious populations, and they are found in at least 48 species across 20 families. The sex chromosomes in hepatics, mosses, and gymnosperms are morphologically heteromorphic. In angiosperms, heteromorphic sex chromosomes are found in at least 19 species from 4 families, while homomorphic sex chromosomes occur in 20 species from 13 families. The prevalence of the XY system found in 44 out of 48 species may reflect the predominance of the evolutionary pathway from gynodioecy towards dioecy. All dioecious species have the potential to evolve sex chromosomes, and reversions back from dioecy to various forms of monoecy, gynodioecy, or androdioecy have also occurred. Such reversals may occur especially during the early stages of sex chromosome evolution before the lethality of the YY (or WW) genotype is established.

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INTRODUCTION

Sexual systems in plants are almost overwhelmingly diverse, and understanding their origin and evolution requires data from many different fields, including ecology, developmental biology, and genetics. Sexual system here refers to the distribution and function of gamete-producing morphological structures (136). The open architecture and modular growth of plants have resulted in numerous gamete production patterns, which in turn are reflected in the large number of sexual systems (9, 36, 180). The sexual system that predominates in animals, namely the restriction of male and female function onto different individuals (gonochory), is strikingly rare among plants: Among land plants, strict separation of the sexes (dioecy) occurs in only 9–10%, i.e., 29,000 out of the 300,000 species with an uneven distribution (for details, see Phylogenetic Perspective section below). Of the flowering plants (angiosperms), approximately 6% or 14,600 species in 960 genera and 200 families

are dioecious (129). Although the circumscription of angiosperm families has changed dramatically since 1995, the numbers of recognized dioecious genera and species have hardly changed. The patchy phylogenetic distribution of dioecy, which is the precondition for the evolution of sex chromosomes, has long been taken to indicate that sex chromosomes in plants have evolved many times independently (24, 30, 57, 58, 77, 92, 100, 156, 166).

Here we review the occurrence of sex chromosomes in land plants, focusing on the molecular data regarding their evolution and function. Although sex chromosomes are currently known only from a limited number of species, progress in model systems, mainly *Silene* and *Carica*, has been rapid and molecular data are permitting the testing of classic predictions of the steps expected to lead to the origin of sex chromosomes. To set the stage, we begin with the mechanisms of sex determination. Theory predicts that sex chromosomes (gonosomes) evolve from autosomes and that heteromorphy of sex chromosomes is one step in their evolution, occurring well after the differentiation into XY or WZ functionality (13, 99, 171). As the sex chromosomes diverge from their autosomal ancestor and from each other, they can increase or decrease in size. Our review summarizes the evidence for this happening in groups with sex chromosomes of different evolutionary ages (cf. Evolution of Sex Chromosomes section below). We also bring in phylogenetic and molecular clock-based evidence. Our review ends by considering reasons for why sex chromosomes in plants are rare and, where they have evolved, XY systems appear to predominate.

MECHANISMS OF SEX DETERMINATION

Hermaphroditism is the norm in land plants, and the separation of sexual function into different individuals evolved independently many times from a hermaphroditic ancestral state (156). The arisal of genes determining the presence of male and female structures therefore

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”

sets the stage for the evolution of sex chromosomes. The first step toward sex chromosome evolution is the occurrence of male and female sterile mutations, leading to the development of unisexual reproductive structures. 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 (129). Stamen and carpel development involve large numbers of specialized genetic functions that are required at various developmental stages, and mutations in any of the many regulatory genes could trigger abortion or loss of function of male and/or female organs (170, 189). Growth hormones affect sex expression in some species, but not others. The recently cloned sex determination genes in the Cucurbitaceae family provide a good example of the underlying genetics.

Several species in the Cucurbitaceae, including cucumber (*Cucumis sativus*) and honey melon or cantaloupe (*Cucumis melo*), show intraspecific polymorphism in their sexual systems. In these species, floral primordia are initially bisexual with sex determination occurring by the selective developmental arrest of either the stamen or the carpel, resulting in unisexual flowers (2, 61). In melon, sex determination is governed by the genes *andromonoecious* (*a*) and *gynoecious* (*g*), and the interactions of their different alleles can result in a range of sexual phenotypes (63, 126). Monoecious (*A-G-*) and andromonoecious (*aaG-*) individuals bear male flowers on the main stem and female or hermaphrodite flowers, respectively, on axillary branches. In addition, gynoecious (*Aagg*) and hermaphrodite (*aaagg*) individuals bear only female or hermaphrodite flowers, respectively (63). In cucumber, three major genes account for most sex phenotypes. The female (*F*) gene is partially dominant to the recessive *f* allele and controls femaleness. The *F* allele causes the female phase to start early in plant growth and *FF* plants are gynoecious. Androecious (*a*) increases maleness, and plants of the *aaff* genotype are androecious, bearing only male flowers. The monoecious (*M*) gene, like the *A* gene

in melon, acts as a stamen suppressor in buds determined to develop a carpel (178).

In both *Cucumis* species, sex expression patterns can be modified by hormonal and environmental factors, with ethylene playing a major role (20, 183). For example, treatment of monoecious melon plants with ethylene leads to plants with only female flowers. In contrast, treatment of gynoecious melon plants with ethylene inhibitors leads to hermaphrodite flowers. Consistent with ethylene being a feminizing agent, the *A* gene in melon and the *M* gene in cucumber are orthologs and code for the rate-limiting step in ethylene biosynthesis, the 1-aminocyclopropane-1-carboxylic acid synthase (*ACS*) gene. In both plants, the *ACS* genes, referred to as *CmACS-7* in melon (17) and *CsACS-2* in cucumber (18), are expressed in carpel primordia, and loss of enzymatic activity leads to stamen development. The observation that the ethylene signal required to inhibit the stamens is generated in a different tissue, the carpel primordium, and must be translocated to the target tissue, is puzzling. Ethylene has a dual role and is also required at the same time for carpel development; applying exogenous hormone, or engineering melons to overexpress *ACS*, results in increased femaleness (118). The *F* gene in cucumber is likely to encode an *ACS* enzyme (161). Nevertheless, melon and cucumber plants expressing inactive *CmACS-7* and *CsACS-2* isoforms develop normal carpels, suggesting that other *ACS* isoforms provide the carpel-promoting function.

The isolation of the gynoecy gene in melon has solved another piece in the puzzle, explaining how hermaphrodite and gynoecious lines develop in melon (88, 89). The transition from male to female flowers in gynoecious lines results from epigenetic changes in the promoter of a zinc-finger-type transcription factor, *CmWIP1*. This natural and heritable epigenetic mutation resulted from the insertion of a transposon, which is required for initiation and maintenance of the spreading of DNA methylation to the gene promoter. Expression of *CmWIP1* leads to carpel abortion, resulting in the development of unisexual male flowers.

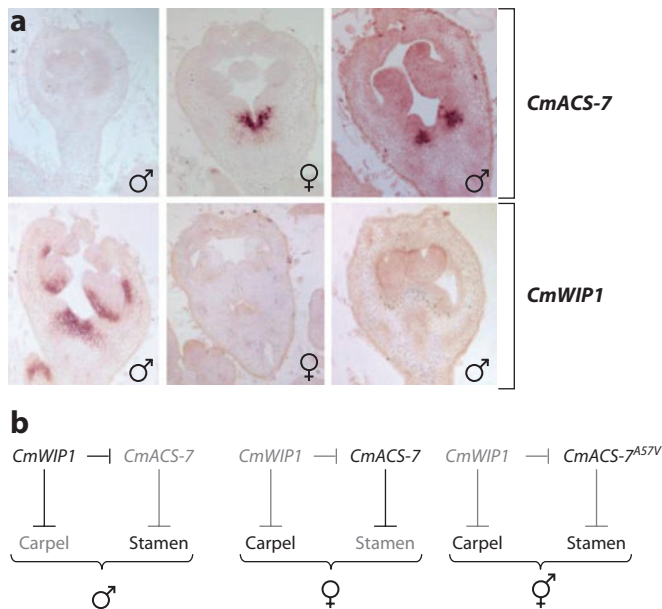


Figure 1

Sex determination model in melon. (a) In situ analysis of *CmACS-7* and *CmWIP1* expression in the early stages of flower development. *CmACS-7* expression is detected in carpel primordia of female and hermaphrodite flowers, and no expression is detected in the male flower. By contrast, *CmWIP1* is expressed in carpel primordia of male flower and not expressed in female and hermaphrodite flowers. (b) Model for sex determination in melon based on Boualem et al. (17) and Martin et al. (89): *CmACS-7* and *CmWIP1* are repressors of stamen and carpel, respectively. The genes in gray are not expressed.

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

Gynodioecy: dimorphic breeding system in which male sterile individuals (i.e., females) coexist with hermaphroditic individuals in populations

CmWIP1 expression also indirectly represses *CmACS-7* expression, requiring the carpel to be expressed, to allow stamen development. Together the data suggest a model in which the two genes interact to control the development of male, female, and hermaphrodite flowers in melon (Figure 1). To understand how androecy emerges in cucurbits will require the isolation of androecy genes.

Now that the isolation of the sex genes from cucurbits and maize (32) has clearly established links with hormonal pathways, among the next challenges are to decipher how the sex determination signals are perceived and how the information is translated to cause organ-specific abortion. This will likely require integrating knowledge about main floral homeotic regulators with an understanding of local and long-distance hormone function.

THE RISE OF SEX CHROMOSOMES

Genes involved in flower development are randomly distributed in the genome and scattered on every chromosome (170, 189). Mutations in many flower development genes have the potential to cause male or female sterility, thereby leading to monoecy, gynodioecy, androecy, and dioecy. Two types of mutations are required to establish dioecy, one aborting stamens (male sterile) and the other aborting carpels (female sterile). Dioecious species can evolve sex chromosomes only when the two sex determination genes are closely linked on the same chromosome and have complementary dominance (25). The complementary dominance of the two sex determination genes is necessary for sex chromosomes to function. For example, in the XY (male heterogametic) system, the female is controlled by homozygous XX chromosomes and the male sterile mutation has to be a loss-of-function (i.e., recessive) mutation. The Y chromosome contain a functioning male fertility allele as well as a gain-of-function (i.e., dominant) mutation at a different locus that suppresses the development of female sex organs. The prevalence of XY sex determination (male heterogametic) over ZW sex determination (female heterogametic) is discussed in a separate section.

The mapping of the sex determination genes in wild strawberry, *Fragaria virginiana*, illustrates the earliest stage of sex chromosome evolution (151, 152). The two sex determination genes, male sterility and female fertility, were mapped on linkage group 6 with a genetic distance of 5.6 cM. Obviously, recombination occurs between these two genes, resulting in male, female, hermaphrodite, and neuter individuals among sexual progeny.

A pivotal event in sex chromosome evolution is the suppression of recombination between the two sex determination genes. Without suppression of recombination, the male sterile or female sterile mutations could revert to hermaphroditism or neuter individuals as shown in wild strawberry. The haplotype of

the Y chromosome that is suppressed for recombination has to be the wild-type allele of the male sterile locus and a gain-of-function mutant allele of the female abortion locus. In other words, the Y chromosome contains the functioning alleles of the two sex determination genes, whereas the X chromosome contains the recessive alleles of both genes.

Suppression of recombination in specific chromosomal regions is a widespread phenomenon in plants, often protecting the haplotype that provides selective advantage, such as self-incompatibility (21, 168), apomixis (3), and sex determination (80). The mechanisms of recombination suppression include chromosome rearrangements and DNA methylation (55, 83). The most common chromosomal rearrangement that causes recombination suppression in animals is inversion, as shown by the two pericentric inversions on human chromosomes 1 and 8 (55) and by the five evolutionary strata of the human Y chromosome (70, 132). Translocation, deletion, and duplication also trigger recombination suppression because of the lack of homologous sequences in those regions involved in chromosomal rearrangement. DNA methylation as another cause of recombination suppression is reported in the fungus *Ascobolus immerses* (83).

Recombination suppression in the male-specific region of the Y chromosome (MSY) or the female-specific region of the W chromosome (FSW) is a hallmark of sex chromosomes. Recombination suppression occurs between the XY (or ZW) chromosomes, whereas the XX (or ZZ) recombine normally. Comparing the Y linkage map via male meiosis and the X linkage map via female meiosis may reveal suppression of recombination. In hops (*Humulus lupulus*), the two markers flanking the sex-determining region are 14.3 cM apart on the X-chromosome linkage map, but 3.7 cM apart on the Y-chromosome linkage map, showing a fourfold reduction of recombination on the Y chromosome (144). Similar findings are documented in stickleback fish X and Y chromosomes (121).

Sequencing the genome and the male- (or female-)specific region can reveal the molecular basis of recombination suppression in nascent sex chromosomes. The 258 kb of the medaka fish MSY originated from a translocation of 72.1-kb region containing the sex determination gene *dmrt1bY* from linkage group 9 (67). This fragment has no counterpart in the new X chromosome, and there is therefore no recombination. The FSW of poplar is a 706-kb region at the end of chromosome 19 with no counterpart on the Z chromosome (182). Again, the suppression of recombination is due to the lack of homologous sequence on the Z chromosome, which may have been caused by translocation or insertion/deletion. Suppression of recombination has also spread to the neighboring region with 37.8 kb/cM in the W chromosome versus 14.6 kb/cM in the Z chromosome, a 2.6-fold reduction. In papaya, the 8.6-Mb MSY has no recombination with its X counterpart owing to inversions (R. Ming, unpublished data). Once the two sex determination genes are completely suppressed for recombination, a pair of incipient sex chromosomes is formed.

EVOLUTION OF SEX CHROMOSOMES

Westergaard (171) grouped plant sex chromosomes into three types to illustrate different evolutionary stages. The earliest stage of sex chromosome evolution was characterized by having a viable YY genotype where the Y differs from the X only in its sex determination genes. This condition is represented by *Ecballium* and asparagus. The second stage has a YY genotype that is inviable, but the Y chromosome plays a decisive role in sex determination; this condition is represented by papaya and white campion (37, 77). In the third stage the Y chromosome is irrelevant to sex determination and sex is determined by the X:autosome ratio as exemplified by sorrel (*Rumex*).

Recent extensive genetic and genomic studies on the male-specific region in these and other species have led to refined models (27, 57, 99). More genomic and DNA sequence data

Androdioecy: dimorphic breeding system in which female sterile individuals (i.e., males) coexist with hermaphroditic individuals in populations

Complementary dominance: the state of two mutations in a closely linked region in which one is a loss-of-function recessive mutation and the other a gain-of-function dominant mutation

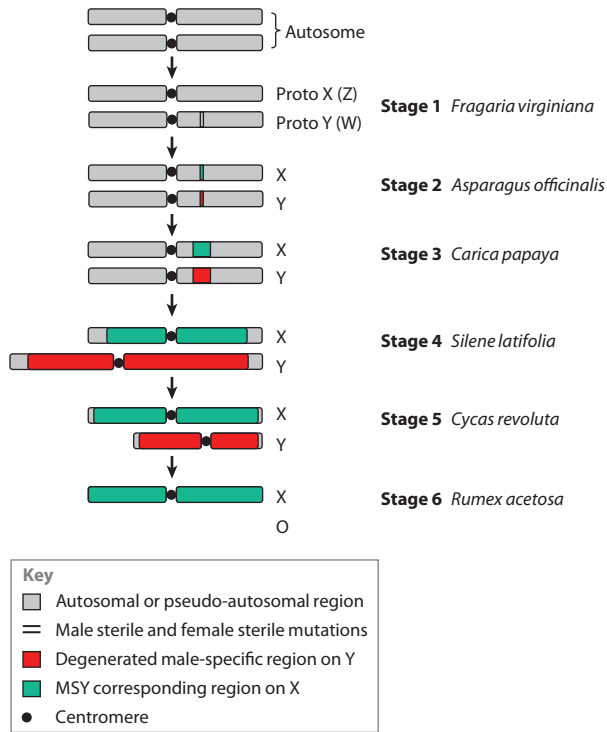


Figure 2

The six stages of sex chromosome evolution. Stage 1: Unisexual mutation of two sex determination genes with complementary dominance. Stage 2: Suppression of recombination between the two sex determination genes and YY genotype is viable. Stage 3: Suppression of recombination spread to neighboring regions and a small male-specific region of the Y chromosome (MSY) region evolved. YY genotype is not viable. Stage 4: The MSY expands in size and degenerates in gene content via accumulation of transposable element insertions and intrachromosomal rearrangements. The X and Y chromosomes become heteromorphic. Stage 5: Severe degeneration of the Y chromosome. Deletion of nonfunctional DNA sequences results in reduction of Y-chromosome size. Stage 6: Suppression of recombination spreads to the entire Y chromosome. The Y chromosome is lost, and X-to-autosome ratio sex determination system has evolved.

have become available in the past few years, and we now recognize six stages of sex chromosome evolution. In **Figure 2** each of the six stages is represented by a species of land plant as follows:

- **Stage 1.** A male and a female sterile mutation with complementary dominance each occur in close proximity on a chromosome. Male sterility is due to a loss-of-function mutation in an XY system while female sterility is due to a dominant Y-linked

allele that causes aborted female gametes. The situation is reversed in a ZW system where females are the heterogametic sex. In this case a Z-linked female sterile allele causes ZZ males to develop, while a W-linked dominant male sterile allele causes ZW females to develop. Recombination between the complementary male and female sterile loci is not suppressed, and hermaphrodite and neuter individuals exist in the population. Wild strawberry is an excellent example of this stage (136).

- **Stage 2.** Recombination is suppressed between the two sex-determining loci and its immediate neighboring regions, leading to initiation of the degeneration process. A small male-specific region on the nascent Y chromosome is formed. The second feature of this stage is that the YY genotype is viable and an all-male population exists. Asparagus sex chromosomes represent this stage.
- **Stage 3.** Suppression of recombination extends to neighboring regions allowing a large number of Y-linked genes to degenerate and form a male-specific region on the nascent Y chromosome. The male-specific region expands through accumulation of retrotransposons and translocation and duplication of genomic fragments. The XY chromosomes appear to be homomorphic at the cytological level, but they are heteromorphic at the molecular level. The loss of gene content is sufficiently extensive to cause lethality of the YY genotype (26). Papaya sex chromosomes illustrate this stage (77).
- **Stage 4.** The male-specific region spreads to the majority of the Y chromosome and further degeneration occurs. Accumulation of transposable elements and duplications within the male-specific region cause significant expansion of DNA content on the Y chromosome. At this stage, the X and Y chromosomes are heteromorphic, and the Y chromosome can be much larger than the

X chromosome. White campion sex chromosomes possess these properties (37).

- **Stage 5.** Severe degeneration of the Y chromosome causes the loss of function for most genes, and loss of nonfunctioning Y chromosome sequences results in a shrinking of the Y chromosome. Some sex chromosome systems may not undergo this phase of shrinking but instead keep expanding and degenerating until a complete loss of the Y chromosome. In either case, a small portion of the Y chromosome continues to meiotically pair with the X chromosome allowing proper disjunction. There are no known angiosperm sex chromosomes at this stage, but the gymnosperm species *Cycas revoluta* has heteromorphic sex chromosomes with a reduced Y chromosome.
- **Stage 6.** Suppression of recombination spreads to the entire Y chromosome. Further reduction of the Y chromosome in size and complete loss of the recombining pseudoautosomal region occur. The Y chromosome is totally lost and a new sex determination system evolves that is based on the X-to-autosome ratio. A new Y chromosome could form but plays no role in sex determination. Sorrel (*Rumex* spp.) sex chromosomes are at this stage.

The two unisex mutations with complementary dominance in a closely linked region set the stage for the appearance of sex chromosomes. Suppression of recombination between the two mutations marks the beginning of sex chromosome evolution. It is often caused by chromosomal rearrangements, mostly inversions as documented in the ancient human Y chromosome and the nascent papaya Y chromosome (70, 185). This is not a one-time event, and chromosomal rearrangements continue to occur, accompanied by recombination suppression expanding into the neighboring regions. Once the first recombination suppression occurs in the sex-determining region, the Y or W chromosome is on its way toward degeneration of its gene content, accumulation or specialization of genes that increase male

fitness, and eventual disappearance, as detailed in Steinemann & Steinemann (153).

The degeneration of the Y (or W) chromosome is a gradual and continuing process until the Y-chromosome has completely degenerated and is lost. The cause of the degeneration process has long been a subject of study. Four major theories were proposed and validated by molecular evidence obtained from model species. Muller's ratchet explains the accumulation of deleterious mutations through random mutation, transposable element insertion, and chromosomal rearrangements that disrupt function without a mechanism to repair or replace the loss-of-function mutations (40, 103). This is perhaps the primary force of Y-chromosome degeneration. "Hitchhiking" happens when favorable mutations are selected and linked deleterious mutations come along because there is no recombination in this region (130). Background selection accelerates the fixation of mildly deleterious alleles and delays the fixation of mildly advantageous alleles (22, 25). The Hill-Robertson effect with weak selection inhibits the spread of favorable alleles and the elimination of deleterious ones as a result of the interference of closely linked alleles under selection (96). Deleterious mutations may accumulate and cause the Y chromosome to degenerate in both size and gene content and finally to diverge from the X chromosome (24).

The precocious separation of the nascent sex chromosomes (155) and the lethal effect of the YY genotype are clear indications of Y-chromosome degeneration in papaya. Evidence for Muller's ratchet in papaya is abundant as extremely low gene density was detected from the MSY sequence, and more pseudogenes of the Y alleles were found in XY gene pairs (186; R. Ming, unpublished data). Most frequently, these deleterious mutations are caused by transposable element insertions because there is an overabundance of retroelements in the papaya MSY, 85.6% versus 52.0% in the rest of the genome (99). The high frequency of repetitive sequences in the MSY hints that Y-chromosome degeneration occurred at an

accelerated pace during the early stages of sex chromosome evolution. Duplications, frequently detected by DNA markers and direct DNA sequencing in papaya MSY (78), 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 (148). Extensive sequence divergence between the incipient X and Y chromosomes has been detected in papaya (78, 185). The hermaphrodite Y and male Y in papaya share nearly identical DNA sequence in most parts of the MSY, and sequence divergence did occur on these two Y chromosomes with an average DNA sequence identity of 98.8% (187). The MSY in papaya hermaphrodites and males is derived from a common ancestral chromosome that split into its two current forms more recently than the split that formed its X and Y chromosomes (for more details see Caricaceae, section below).

The Y chromosome of white campion, *Silene latifolia*, is largely nonrecombining with the X chromosome and is enormous at 570 Mb, 150 Mb larger than the 420-Mb X chromosome. Microdissected Y-chromosome DNA of white campion competes with female genomic DNA during fluorescent in situ hybridization experiments, resulting in similar signal patterns on the X, Y, and autosomes (93). Several lines of evidence suggest genetic degeneration of the *S. latifolia* Y chromosome. The strongest evidence is that the YY genotype is not viable (181). A functional X-linked male reproductive organ-specific gene (MROS3) has a degenerated Y-linked copy (48). Analyses of seven X/Y gene pairs revealed increased intron length, lower expression, and high mutation rates of Y alleles (85).

A liverwort haploid Y chromosome was the first plant Y chromosome to be almost fully sequenced. There are 64 genes in the 10-Mb Y chromosome, and 14 of them are detected only in the male genome and expressed exclusively in reproductive organs (179). Another 40 genes are expressed in both vegetative and reproductive tissues, including six that have diverged X alleles.

These data demonstrate that degenerative processes have occurred in the nonrecombining region of the Y chromosome in papaya and white campion, even though sex chromosomes of the above species have originated recently, approximately 2–3 and 8–24 MY ago, respectively (101, 185). Because they are often at early stages of formation, the incipient Y chromosomes in plants are ideal models to study the mechanisms underlying the initial stages of sex chromosome evolution.

SEX CHROMOSOMES IN LAND PLANTS

Heteromorphic chromosomes in land plants were first described in the liverwort *Sphaerocarpos donnellii* (4), whereas Correns's (33) work on *Bryonia dioica* was the first Mendelian-type evidence for genetic sex determination in any organism, whether animal or plant. Heteromorphic sex chromosomes have been found in all major lineages of land plants except hornworts, lycopods, and ferns (**Table 1**, **Figure 3**), whereas homomorphic sex chromosomes are known only from gymnosperms and angiosperms. We have conducted an exhaustive search of the literature, and **Table 1** summarizes the current data on species with strong cytogenetic or molecular evidence for the presence of sex chromosome. Among 28 species with heteromorphic sex chromosomes, 27 are XY and 1 is ZW. Among the 20 species with homomorphic sex chromosomes, 16 are XY and 4 are ZW. In the following sections we briefly describe the sex chromosomes in each of the 20 families of land plants that show evidence of recombination suppression in the sex-determining region (**Table 1**).

Bryophytes

Bryophytes include the most ancient land plants, and by a historical coincidence, sex chromosomes in plants were first discovered in liverworts, the sister to all other land plants (4). Together, liverworts, mosses, and hornworts comprise 22,600 species, but sex chromosomes

are known in only 5 (0.02%) species from two families in liverworts and leafy mosses. The 100 species of hornworts are not dioecious, thus no sex chromosomes. The known sex chromosomes of liverworts and leafy mosses are heteromorphic.

The primary cause of Y chromosome degeneration and sex chromosome evolution is the suppression of recombination at the male or female specific region of XY or ZW chromosomes, respectively. The forces driving sex chromosome evolution appear to be the same between gametophyte-dominant nonvascular plants and sporophyte-dominant vascular plants, as suggested by the *Marchantia polymorpha* Y chromosome (179).

Hepaticaeae. Liverworts were the first group of plants with cytological evidence for sex chromosomes (4, 5). In *Sphaerocarpos austinii*, *S. donnellii*, and *S. texanus*, each of the four haploid spores germinates and produces a clump of two male and two female plants side by side. In haploid gametophytes, there is usually only one sex-associated chromosome (Table 1). However, in *Frullania* there are two X chromosomes in the female and a single Y in the male (158). Usually, the Y chromosome in hepatics is the largest chromosome, but in *Frullania dilatata*, female plants with their two large sex chromosomes have a 1.35-fold higher C value than do male plants with only one sex chromosome (158). Sex-specific markers for *Sphaerocarpos* and *Marchantia* and an almost complete sequence of the Y chromosome of *Marchantia polymorpha* have been obtained (95, 111, 112, 179).

Ditrichaceae. In leafy mosses, sex chromosomes were first reported in *Ceratodon purpureus*, and this remains the only well-documented case (S. McDaniel, personal communication; Table 1). A linkage map for this species revealed numerous loci with significant segregation distortion, and the pattern of nonrandom associations among loci indicates that this results from interactions between the sex chromosomes (94).

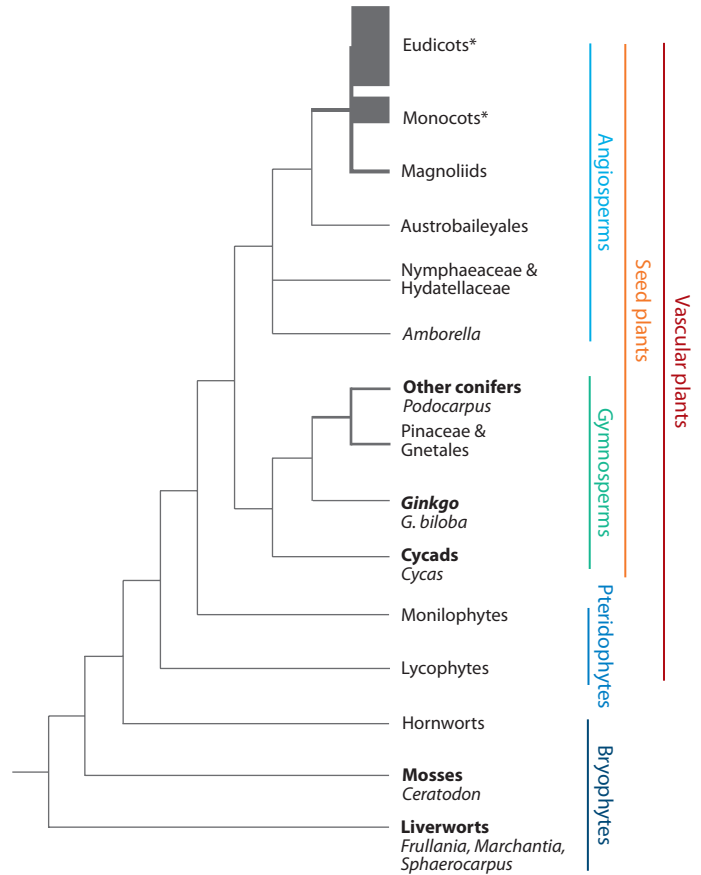


Figure 3

Distribution of sex chromosomes among land plants. The asterisk next to monocots and eudicots refers to the presence of sex chromosomes in these clades, shown in more detail in Figure 4. Phylogeny reproduced with permission from Qiu (127). The thicker lines are roughly proportional to the species numbers in the clades (clades with <500 species are drawn with thin lines).

Gymnosperms

Among the 1010 gymnosperm species, sex chromosomes are known in only 6 (0.6%) from three families, but all of them are heteromorphic sex chromosomes, possibly reflecting a high evolutionary age. *Ephedra foliata* has also been reported as having heteromorphic sex chromosomes (97). This report, however, was based on faint satellites at the end of the proposed XY chromosomes. The X chromosome has a satellite at each end, while the Y chromosome has a satellite at one end only. In *Cycas*

Table 1 Examples of land-plant species with homomorphic or heteromorphic sex chromosomes*

Higher taxon	Species	Female	Male	Viability of YY or WW genotypes	Sex determination mechanism	References and notes
Heteromorphic sex chromosomes (under light microscopy)						
Bryophytes						
Hepaticae	<i>Frullania dilatata</i>	X ₁ X ₂ female plants with two large sex chromosomes ($n = 9$) have a 1.35-fold higher C value than male plants with only one sex chromosome ($n = 8$)	Y	In haploid organisms, the diploid sporophyte is always heterogametic	X (females) and Y (males) in haploid gametophytes	(158)
	<i>Marchantia polymorpha</i>	X	Y	See above	See above	(111, 112, 179)
	<i>Sphaerocarpos donnellii</i> , <i>S. texanus</i>	X	Y	See above	See above	Sex-linked RFLP bands (6, 95)
Ditrichaceae	<i>Ceratodon purpureus</i>	X	Y	See above	See above	(94; S. McDaniel, pers. comm.)
Gymnosperms						
Cycadaceae	<i>Cycas revoluta</i>	XX	XY (X is larger than Y)	Unknown	Active Y system	(145)
Ginkgoaceae	<i>Ginkgo biloba</i>	ZW (W is larger than Z)	ZZ	No	Active W system	(71)
Podocarpaceae	<i>Podocarpus macrophyllus</i>	X ₁ X ₂	YX ₁ X ₂	Unknown	Unknown	Females have $2n = 38$ (all telocentric) and males $2n = 37$ (36 telocentric and 1 large submetacentric chromosome, which probably arose by fusion) (49, 50)
	<i>P. longifoliolatus</i>	X ₁ X ₂	YX ₁ X ₂	Unknown	Unknown	As for <i>P. macrophyllus</i>
	<i>P. elatus</i>	X ₁ X ₂	YX ₁ X ₂	Unknown	Unknown	As for <i>P. macrophyllus</i>

Angiosperms		XX	XY	Yes (fide 8)	Active Y system	(137-139)
Cannabaceae, Rosales	<i>Cannabis sativa</i>	XX	XY		Active Y system	(137-139)
	<i>Humulus lupulus</i>	XX	XY	Probably no	X to autosome ratio, but Y is essential for normal development	(46, 60)
Caryophyllaceae, Caryophyllales	<i>H. lupulus</i> var. <i>cordifolius</i>	X ₁ X ₁ X ₂ X ₂	X ₁ Y ₁ X ₂ Y ₂	Unknown	X to autosome ratio	(113)
	<i>H. japonicus</i>	XX	X ₁ Y ₁ Y ₂	Unknown	X to autosome ratio	(46)
	<i>Silene latifolia</i> , <i>S. dioica</i> ,	XX	XY (Y about 1/3 longer than the X)	No	Active Y system	(110, 164)
	<i>S. dioicinis</i>	XX	X ₁ Y ₁ Y ₂	Unknown	Active Y system	Neo-sex chromosomes originated by reciprocal translocation between the original Y chromosome and an autosome (54)
Cucurbitaceae, Cucurbitales	<i>Coccinia grandis</i> (synonym: <i>C. indica</i>)	XX	XY (Y is 2.5 times longer than the X)	Unknown	Active Y system	(69, 134)
	<i>Trichosanthes dioica</i> <i>T. kirilovii</i> (including <i>T. japonica</i> and <i>T. multiloba</i>), <i>T. ovigera</i> (including <i>T. cucumeroides</i>)	XX	XY	Unknown	Active Y system	(105, 120, 135)

(Continued)

Table 1 (Continued)

Higher taxon	Species	Female	Male	Viability of YY or WW genotypes	Sex determination mechanism	References and notes
Polygonaceae, Caryophyllales	<i>Rumex acetosa</i>	XX	X ₁ Y ₂ ; the X is slightly larger than each of the two Ys	Unknown	X to autosome ratio	This has a table summarizing all <i>Rumex</i> chromosome data (16, 107)
	<i>R. acetosella</i>	XX	XY	Unknown	Active Y system	(34, 66)
	<i>R. gymniifolius</i>	XX	XY	Unknown	Unknown?	(80)
	<i>R. hastatulus</i>	XX	XY or XY ₁ Y ₂	Unknown	Active Y or X to autosome ratio	(149)
	<i>R. papillaris</i>	XX	X ₁ Y ₁ Y ₂	Unknown	Unknown	(107)
	<i>R. paucifolius</i>	XX	XY	Unknown	Active Y system?	(150)
	<i>R. suffruticosus</i>	XX	XY	Unknown	Active Y system?	(34)
Homomorphic sex chromosomes (under light microscopy)						
Angiosperms						
Actinidiaceae, Ericales	<i>Actinidia chinensis</i> (diploid)	XX	XY	Yes	Active Y system	Incipient sex chromosomes were identified in Linkage Group 17 where the sex-determining locus was located in the subtelomeric region (42, 44)
Amaranthaceae, Caryophyllales	<i>Amaranthus tataricus</i>	XX	XY	Unknown	Active Y system	(104)
Areaceae, Arecales	<i>Phoenix dactylifera</i>	XX	XY	Yes	Active Y system	(147, 184)
Asparagaceae, Asparagales	<i>Asparagus officinalis</i>	XX	XY	Yes	Active Y system	(79, 157)
Caricaceae, Brassicales	<i>Carica papaya</i>	XX	XY	No	Active Y system; the Y is 4 Mb longer than the X	(53, 78, 185)
	<i>Vasconcellea cundinamaricensis</i> , <i>V. goudotiana</i> , <i>V. parviflora</i> , <i>V. pulchra</i>	XX	XY	No	Active Y system	(53, 175)

	<i>Silene otites</i>	ZW? XX?	ZZ? XY?	Unknown	Unknown	Suggested female heterogamy (140)
Caryophyllaceae, Caryophyllales						Suggested male heterogamy (169)
	<i>S. colpophylla</i>	XX	XY	Unknown	Active Y system	(101)
Chenopodiaceae, Caryophyllales	<i>Spinacia oleracea</i>	XX	XY	Yes	Active Y system	(71)
Cucurbitaceae, Cucurbitales	<i>Bryonia dioica</i>	XX	XY	Unknown	Active Y system	(33, 116)
	<i>Echallium elaterium</i>	XX	XY	Unknown	Active Y system	(43, 171)
Datisceae, Cucurbitales	<i>Datisca cannabina</i>	ZW	ZZ	Yes	Two loci linked (not recombining?)	(174)
Dioscoreaceae, Dioscoreales	<i>Dioscorea tokoro</i>	XX	XY	Unknown	Active Y system	(159)
Santalaceae, Santalales	<i>Viscum fischeri</i>	X ₁ X ₂ X ₃ X ₄	Y ₁ Y ₂ Y ₃ Y ₄ Y ₅	Unknown	Unknown	A chain of four X and five Y, i.e., multiple sex chroms. due to translocations (8)
Rosaceae, Rosales	<i>Fragaria virginiana</i>	ZW	ZZ	Unknown	Two loci linked and recombining	(152)
	<i>F. moschata</i> (including <i>F. elatior</i>)	ZW	ZZ	Unknown	Two loci linked (not recombining?)	(2)
Salicaceae, Rosales	<i>Populus trichocarpa</i>	ZW	ZZ	Unknown	Active W system	(182)

* Updated from Ming et al. (99) and focusing on molecular evidence or strong genetic and cytogenetic evidence. Species cited as having sex chromosomes in earlier reviews, but excluded here, include the following: 1. *Salix viminalis*: sometimes listed as having homomorphic sex chromosomes. However, based on a first linkage map, Semirikov et al. (146) found that “a single locus governs the sex determination and... nonrecombining sex chromosomes are absent in *S. viminalis*.” Similarly, Gunter et al. (47) concluded, “The fact that more than 2000 primer sequences have been screened in both *Populus* and *Salix*, and only two have exhibited an association with gender, provides evidence for the lack of sex chromosome structure in Salicaceae.” 2. *Mercurialis annua*: “*Mercurialis annua* does not have sex chromosomes” (64). 3. *Pistacia vera*: sometimes listed as having homomorphic sex chromosomes, but Yakubov et al. (177) did not find evidence of sex chromosomes. 4. *Vallisneria spiralis*: Winge (173) suggested that this species has XX (female), XO (male). However, Westergaard (172) showed that this was in error. 5. *Elodea canadensis*: supposedly XY (141, 142) but not substantiated by more recent work. 6. *Dioscorea sinuata*: supposedly XO (98) but not supported by subsequent studies. 7. *Ephedra foliolata*: XY differ in satellites (97), but Owens et al. (114) concluded that “the existence of sex chromosomes has not been established by cytological analysis for any species of Ephedra.”

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

pectinata, the X chromosome has a faint satellite at one end, but the Y chromosome has none (1). A similar observation was reported in a pair of shorter, subterminal regions of chromosomes in *Ginkgo* (75, 124), but this finding was disputed by more recent studies (31, 72). For this reason and for lack of support from molecular and genetic data, we exclude *Ephedra foliata* and *Cycas pectinata* from **Table 1**.

Cycadaceae. The female and male plants of *Cycas revoluta* have distinctive heteromorphic XY chromosomes (145). This species has 10 pairs of autosomes and one pair of sex chromosomes. The X chromosome is the fourth largest chromosome and the Y is the ninth largest chromosome. The Y chromosome is thus small and represents stage 5 in our scheme of chromosome evolution (**Figure 2**).

Ginkgoaceae. Sex chromosome research has focused on *Ginkgo biloba* for more than 50 years. Male and female *Ginkgo* plants have 22 autosomes and a pair of ZW sex chromosomes. Newcomer (109) was the first to recognize that the largest chromosomes are the sex chromosomes, and that they are dimorphic on the basis of their centromere positions, one metacentric and the other submetacentric. However, Newcomer thought *G. biloba* had male XY heterogamety. It was not until 1987 that *G. biloba* was recognized as having ZW female heterogamety (31). Microdissection and chromosome painting validated the ZW system (72). The W chromosome is just slightly larger than the Z chromosome when viewed by light microscopy (it would be millions of base-pair difference at the molecular level), explaining why Newcomer (109) mistakenly classified the system as XY. Despite the ancient history of *G. biloba*, the sex chromosomes appear to be at the early part of stage 4 (**Figure 2**), with the expansion of the W chromosome.

Podocarpaceae. The diploid female and male plants of *Podocarpus macrophyllus* have different numbers of chromosomes with $2n = 38$ in females and $2n = 37$ in males (50). All 38 chro-

somes in females are telocentric, whereas 36 chromosomes in males are telocentric and the 37th is a large submetacentric chromosome. At meiotic metaphase I, 17 bivalent and 1 trivalent are formed in pollen mother cells. The largest chromosome in males pairs with two middle-sized telocentric chromosomes, indicating that the male chromosome originated from a telocentric fusion of two chromosomes. Thus, this species has a unique X_1X_2Y sex chromosome system (50). Earlier, Hair & Beuzenberg (49) had reported trivalent formation and intraspecies chromosome number variation in *P. elatus*, *P. longefoliolatus*, and *P. macrophyllus*. They had proposed chromosome fusion as the cause of the trivalent but did not relate their observation to sex chromosomes. Hizume et al. (50) followed this lead and proposed the presence of sex chromosomes in *P. macrophyllus*. Conceivably, *P. longefoliolatus* and *P. elatus* share the same ancestral autosomes from which the Y chromosome of *P. macrophyllus* evolved.

Angiosperms

Angiosperms are the most successful land plants in terms of species numbers. Among their extant 250,000 species, only 37 (0.01%) in 16 families have cytogenetic and/or molecular evidence for the presence of sex chromosomes (**Table 1**), and many more species with sex chromosomes remain to be discovered. Among the 37 species, 17 species in 4 families have heteromorphic sex chromosomes, whereas the other 20 species in 12 families have homomorphic sex chromosomes. All known homomorphic sex chromosomes in land plants are angiosperms, perhaps reflecting a recent origin.

Actinidiaceae. *Actinidia chinensis*, the kiwi fruit, has an XX/XY sex determination system and homomorphic chromosomes (42, 44, 160). Linkage group 17 was identified as an incipient sex chromosome where the sex-determining locus was located in a recombination-suppressed subtelomeric region (42). The genus *Actinidia* contains 30 species, but besides *A. chinensis* few have been analyzed in terms of the karyotype.

Asparagaceae. Crosses between females and males of *Asparagus officinalis* result either in progeny with a 1:1 male to female segregation ratio or in all-male progeny (131). Sex determination in this species is under the control of an active-Y chromosome system. The all-male populations consist of super males with a YY genotype. Chromosome 5 in *A. officinalis* was identified as the sex chromosome by trisomic analysis, although the X and Y chromosomes are homomorphic and not distinguishable under light microscopy (79). The Y chromosome contains two tightly linked genes, a male activator (*M*) and a female suppressor (*F*). Rare recombinants between these loci can produce hermaphrodite and sterile progeny (87). Sex determination in asparagus has been mapped to a single locus *M* (128), indicating recombination suppression between the two sex determination genes. Physical mapping has narrowed the gap between the flanking markers of the *M* locus, but the gap has not been filled yet owing to the highly repetitive nature of the sequence (157). The viability of the YY genotype and occurrence of rare recombination between the two sex determination genes indicated that asparagus sex chromosomes are between stages 1 and 2, and close to stage 2 (**Figure 2**). The *Asparagus* genus consists of some 300 species, and the evolution of sex chromosomes from other species in the genus is not well studied.

Areaceae. The sex chromosomes of date palm (*Phoenix dactylifera*) are indistinguishable by light microscopy. However, the fluorescent intensities of heterochromatin spots are larger on the Y chromosome (147). The identification of sex-specific DNA markers provides further support for the existence of sex chromosomes in date palm (184). *Phoenix* comprises 12 other species besides *P. dactylifera*, most of which are grown as ornamentals, potentially making this a promising system for the study of sex chromosome evolution.

Cannabaceae. The Cannabaceae comprise just six species, *Humulus lupulus*, *H. japonicus*, *H. yunnanensis*, *Cannabis sativa*, *C. indica*,

and *C. ruderalis*. Sex determination is similar to *Rumex* and involves an X-to-autosome balance (X/A) system or multiple sex chromosomes (XX/X₁Y₁Y₂) (**Table 1**). Genetic analyses have focused mainly on common hop, *H. lupulus*, and hemp, *C. sativa*, for which male-specific markers have been described (35, 56, 84, 122, 123, 139, 144). No molecular studies have yet been carried out on *H. japonicus*, but the sex chromosomes can easily be identified after C-banding/DAPI staining, suggesting enrichment in repetitive sequences (45). Indeed, several hundred non-long terminal repeat retrotransposons appear to have accumulated in the terminal region of the long arm of the hemp Y chromosome (138, 139). In *H. lupulus* sex chromosomes are heteromorphic and can be distinguished by the slightly smaller size of the Y as well as by differential banding with DAPI staining (60). The analysis of markers around the sex-determining locus indicates that the suppression of recombination is restricted to a very small region (144).

Caricaceae. Crosses between females and males of *Carica papaya* produce 1:1 male to female offspring (51, 154). The same ratio is obtained when crossing females with hermaphrodites. Crosses between hermaphrodite and male produce 1:1:1 hermaphrodite:male:female ratio. However, selfed hermaphrodite trees and the occasional male flowers that produce carpels always segregate into hermaphrodites and females, or males and females at the ratio 2:1. We now know that these deviations from 3:1 Mendelian segregation ratio are due to lethality of the YY genotype in the latter crosses. However, no distinctive heteromorphic sex chromosomes have been found. Nevertheless, a presumptive pair of sex chromosomes has been identified in pollen mother cells based on their precocious meiotic separation (68, 155). High-density genetic mapping places the sex determination locus within a large cluster of cosegregating markers that comprises 67% of the linkage group, thus demonstrating suppression of recombination in this sex-determining region

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 (downregulation, suppression of one of the two X chromosomes in females, e.g., mammals) or positive (upregulation, enhancement of activity of the single X chromosome in males, for example, fruit fly)

(81). Fine mapping, physical mapping, and survey sequencing of male-specific bacterial artificial chromosomes led to the conclusion that papaya has a pair of incipient sex chromosomes (78). The X and Y chromosomes diverged approximately 2–3 mya (185), and the two different Y chromosomes, Y for male and Y^h for hermaphrodite, diverged from each other approximately 73,000 years ago (187). Papaya sex chromosomes are the second largest pair, but within error range of the largest pair of chromosomes (167, 188). In our scheme (**Figure 2**), the papaya sex chromosome represents stage 3, and Y chromosome expansion is evident at the molecular level (185).

Sex chromosomes in the genus *Vasconcellea* have been investigated recently (175), and X and Y allele-specific sequences have been cloned in the dioecious species *V. goudotiana*, *V. parviflora*, and *V. pulchra* as well as the trioecious species *V. cundinamaricensis*. Sex chromosomes in *Vasconcellea* species appear to have evolved at the species level, on the basis of phylogenetic analyses of X and Y alleles among these species, monoecious *V. monoica*, and papaya. Among the 35 species in the Caricaceae, 32 are dioecious, two trioecious, and one monoecious. Our hypothesis is that except for the monoecious species, the other species all have sex chromosomes; however, only five species in two genera have been examined so far.

Caryophyllaceae. *Silene* remains the best-studied plant model system for the evolution of sex chromosomes, although the large size of the genus (750 species) and poorly understood phylogeny hamper the inference of evolutionary directions. Dioecy and sex chromosomes evolved several times in the genus: Some species have heteromorphic and others homomorphic sex chromosomes (**Table 1**). One dioecious clade consists of the species *S. diclinis*, *S. dioica*, *S. beuffelii*, *S. latifolia*, and *S. marizii*, all formerly in section *Elisanthe* and previously considered to have similar sex chromosomes (110). Recent data, however, reveal that *S. diclinis* has neo-sex chromosomes that originated by reciprocal translocation between the original

Y chromosome (homologous to the Y chromosome of *S. latifolia*) and an autosome (54, 58). Another dioecious clade includes *S. colpophylla*, *S. otites*, and *S. acaulis*. Genetic mapping performed in *S. colpophylla*, with homomorphic sex chromosomes, has shown that its sex chromosomes evolved from a different pair of autosomes than did those in section *Elisanthe*, although there remains an XX/XY system (102). The sex-determining system of *S. otites* is still unclear (**Table 1**) (58). Taken together, these findings illustrate the evolutionary plasticity of plant sex chromosomes (58).

The *S. latifolia* sex chromosomes are composed of two distinct domains: the male-specific region of the Y chromosome and possibly two pseudoautosomal regions that recombine (37, 143) (the finding of two pseudoautosomal regions contradicts cytogenetic data and needs further confirmation). Studies of Y-chromosome deletion mutants have indicated that at least three sex-determining loci are in the male specific part of the Y chromosome: one that suppresses femaleness and two that promote maleness (38, 58, 73, 74, 190).

The first isolated X-linked gene in *S. latifolia*, *MROS3X*, was reported to have a degenerate Y-linked homolog (48). However, *MROS3X* is a member of a multicopy gene family (62), and the degenerate Y copy may simply be a defective copy translocated from elsewhere. Indeed, the divergence between the *MROS3X* and defective *MROS3Y* is too high (>30%) to fit the current model of recent sex chromosome evolution in *Silene* (7). Of 11 X-linked genes isolated from the *S. latifolia* X chromosome, several have expressed Y-linked homologs (14, 57, 91), but only one of these homologs shows signs of genetic degeneration (41). Thus, there is little evidence so far for active genetic degeneration of the *S. latifolia* Y (7). The data also show that in *Silene* inversions occurred after recombination had stopped, so the inversions could not have played a significant role in suppressing recombination between the X and Y chromosomes (7, 14, 191).

Experimental evidence for X chromosome inactivation (dosage compensation) based on

the timing of replication between the two X chromosomes, methylation, and histone H4 acetylation patterns in *S. latifolia* remains ambiguous (59, 108, 165). For a recent review of the role of DNA hypermethylation *S. latifolia* sex determination see Reference 58.

Chenopodiaceae. Most spinach (*Spinacia oleracea*) varieties segregate clearly into males and females at a 1:1 ratio, although there is a low frequency of various intersex types, not just hermaphrodites (12). Heteromorphic sex chromosomes have not been found, and trisomic analysis from a cross between diploid and triploid plants identified the largest chromosome as the XY chromosome (39). Linkage mapping placed the sex determination region at a single locus of the largest linkage group with a noticeable cluster of closely linked markers (65), a hallmark of recently evolved sex chromosomes. There is no report of supermale spinach, suggesting that the YY genotype is not viable. Florescent in situ hybridization mapping of 45S rDNA detected four loci in females, but three loci in males (71), indicating the loss of a 45S-rDNA locus and possible evidence of Y-chromosome degeneration. The spinach sex chromosome is at stage 3 in our scheme (Figure 2).

Cucurbitaceae. In Cucurbitaceae, specialized sex chromosomes have evolved in *Bryonia* and *Ecballium*, where they are homomorphic, and in *Coccinia* and *Trichosanthes*, where they are heteromorphic (Table 1). *Bryonia dioica* was the first organism for which experimental evidence for an XY sex determination system was provided via crosses of the dioecious *B. dioica* and the monoecious *B. alba* (33). Phylogenetic study of the ten species of *Bryonia* indicates that dioecy re-evolved in the lineage leading to *B. dioica* and that the sex chromosomes of *B. dioica* are young, i.e., a few million years old. There is so far no evidence of polyploidization or other major chromosomal restructuring, and the chromosome number of *B. dioica* [$2n = 2x = 20$ ($n =$ number of chromosomes in a haploid gamete and $x =$ number of mono-

ecious chromosomes)] is the same as that of most other monoecious and dioecious species of *Bryonia* (163). Molecular evidence from two male-linked SCAR (sequence-characterized amplified regions) markers and chromosome walking have revealed a complex arrangement of *Copia*-like transposable elements on the Y chromosome (115, 116). The sister group of *Bryonia*, *Ecballium elaterium*, has dioecious and monoecious populations, both occurring throughout the Mediterranean region. The two forms are interfertile, and crosses between monoecious and dioecious *Ecballium* demonstrated male heterogamety (43, 171). As in *Bryonia*, the male is the heterogametic sex.

Cytogenetic evidence for *Coccinia grandis* shows that during meiosis, the X and the Y, which is approximately 2.5 times longer than the X (69; N. Holstein & S. Renner, unpublished data), form a heteromorphic bivalent with a very short pairing contact. The Y chromosome is fairly strong in determining maleness given that plants with two or three X chromosomes and one Y chromosome are all males (133). The sex chromosomes of *C. grandis* may be 3 million years old because that is the time when *C. grandis* diverged from its sister species, the Ethiopian endemic *C. ogadensis* (52). However, the chromosomes of *C. ogadensis* have not been studied, and all 29 species of *Coccinia* are dioecious, raising the possibility that sex chromosomes evolved at the base of the genus, but without heteromorphy (none of the other five species whose chromosomes have been studied have heteromorphic chromosomes).

Datisceae. The evidence of incipient sex chromosomes in dioecious *Datisca cannabina* is mainly from linkage mapping of the sex-determining region to a single locus on linkage group 7 (174). The designation of the ZW system in this species has two lines of evidence: (a) Among four reported crosses in *D. cannabina*, two produced 1:1 male to female segregation ratios, but the other two produced almost entirely female progeny, a sign of having superfemales with a WW genotype. (b) The sister species *D. glomerata* is androdioecious, and

it has been suggested (174) that it arose from a reverse mutation in a dioecious population. This would only be possible in a ZW system where males originate from a homozygous recessive mutation on the Z chromosome while females arise from a gain-of-function dominant mutation. The hermaphrodite allele is recessive to the female allele, supporting this hypothesis (174). If *D. cannabina* had an XY system, a reverse mutation would lead to gynodioecy, not androdioecy.

Dioscoreaceae. The incipient sex chromosomes of the dioecious wild yam, *Dioscorea tokoro*, are inferred on the basis of linkage mapping of sex determination to a single locus on male linkage group 10 (Y), which shows suppression of recombination when compared with the homologous linkage group (X) from the female parent (159). Intraspecific crosses in the tetraploid species *D. floribunda* produced progenies segregating with 1:1 or 3:1 males to females, fitting with the XXYY system proposed by Martin (90). The genus *Dioscorea* comprises 600–800 species, making it difficult for evolutionary-phylogenetic investigations of sex determination.

Polygonaceae. The reproductive systems in *Rumex*, a genus of 200 species, include hermaphroditism, polygamy, gynodioecy, monoecy, and dioecy. In dioecious *Rumex* species, two sex-chromosomal systems and sex-determining mechanisms have been described (Table 1): XX/XY with an active Y chromosome (e.g., *R. acetosella*) and XX/XY₁Y₂ with sex determination based on the X/A ratio (e.g., *R. acetosa*). The sex chromosomes are the largest of the karyotype, with the X slightly larger than each of the Ys. At least one species, *R. hastatulus* has two chromosomal races, a Texas race with XX/XY and a North Carolina race with XX/XY₁Y₂ (104). In *R. acetosa*, the similarity of repetitive sequences on the two Y chromosomes suggests that they probably originated from a Y chromosome that underwent centromere fission and gave rise to a pair of metacentric Y chromosomes possessing iden-

tical arms that subsequently underwent deletions; they are both heterochromatic and highly differentiated from the euchromatic X (76). The role of the X/A ratio in the sex determination of *R. acetosa* resembles the sex-determining system of *Drosophila*, where the primary genetic sex-determining signal is provided by the ratio of X-linked genes to autosomal genes (125). Diploids or polyploids with an X/A ratio of 6 to 1 are females, whereas those with an X/A ratio of 0.5 are males. The Y is not involved in sex determination, but it is required for meiosis in pollen mother cells (119). Comparative study of the chromosomal organization of satellite-DNA sequences in XX/XY and XX/XY₁Y₂ *Rumex* species demonstrates that active heterochromatin amplification occurred and was accompanied by chromosomal rearrangements (107). The XX/X Y₁Y₂ sex chromosome systems appear to have evolved from XX/XY systems. Furthermore, the distribution of Y satellite DNA in hermaphrodite and dioecious species of the genus *Rumex* suggests that the amplification of tandem repeats in the Ys is not necessary to suppress recombination but accelerates the differentiation of the Y from earlier stages in its evolution (86).

Rosaceae. The ZW chromosomes of the wild strawberry, *Fragaria virginiana*, are at the earliest stage of sex chromosome evolution (151, 152). The two sex determination genes, causing recessive male sterility and dominant female fertility, respectively, have been mapped to linkage group 6 and are still recombining with a genetic distance of 5.6 cM. Recombinant progeny are either hermaphrodites or neuters. A sister species, *F. moschata*, showed strictly 1:1 female to male segregation in two intraspecific crosses of the variety “Capron” (no hermaphrodite or neuter plants were found in the small populations). *Fragaria virginiana* represents the ZW chromosome at stage 1 in our scheme (Figure 2).

Salicaceae. Genome sequencing of *Populus trichocarpa* (poplar) has revealed incipient sex chromosomes (162, 182). The ZW

chromosomes exhibit suppression of recombination around the sex-determining region that is on one end (telomeric region) of chromosome 19. The FSW comprises 706 kb and shows complete suppression of recombination. In the immediate neighboring region, a 6.8-cM segment of the W chromosome comprises of 257-kb (37.8kb/cM) 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.

Santalaceae. The mistletoe genus *Viscum* comprises 65–100 species of which only *V. fischeri* is known to have sex chromosomes. Diploid male plants have $2n = 23$ and produce seven meiotic bivalents plus a multivalent chain of nine chromosomes. The multivalent chain in the males is a consequence of reciprocal translocations, one of which involved the chromosome carrying the sex determination factors (8). Regular meiotic assortment in pollen results in transmission of 11 or 12 chromosomes. Female plants have the chromosome number $2n = 22$ and are homozygous for the 11-chromosome genome.

PHYLOGENETIC PERSPECTIVE AND AGES OF PLANT SEX CHROMOSOMES

As discussed in the Introduction and illustrated in **Figures 3** and **4**, the phylogenetic distribution and the sizes of dioecious clades are extremely uneven: Of the liverworts, 75% or 6000 species are dioecious (176); of the leafy mosses, 50% or ~7250 species (176); of the hornworts, lycophytes, and true ferns next to none; and of the gymnosperms, 36%, namely all 300 species of cycads, *Ginkgo biloba*, and the approximately 80 Gnetales. Of the flowering plants (angiosperms), ~6% or 14,600 species in 960 genera and 200 families are dioecious (129). The circumscription of dioecious angiosperm families continues to change; there are currently 415 families.

Approximately 40 species in 21 genera from 15 families of flowering plants are currently known to have sex chromosomes (**Table 1**, **Figure 4**), and of these, only ~20 species in 6 genera (*Cannabis*, *Humulus*, *Silene*, *Coccinia*, *Trichosanthes*, and *Rumex*) have heteromorphic sex chromosomes. These numbers are almost unchanged since Westergaard's (171) review in the 1950s, probably because botanical cytology is a shrinking discipline. As a result, few new plant species are investigated in terms of the chromosome complements. Molecular-cytological and molecular-genetic methods, however, are now revealing sex chromosomes (i.e., recombination-suppressed regions with linked male- and female-determining genes) in species where they had previously only been suspected on the basis of progeny sex ratios or weak cytological evidence (see **Table 1** for examples). Whether all dioecious species are on an evolutionary trajectory toward sex chromosomes, however, remains uncertain.

As is evident from **Figures 3** and **4**, there is no clear pattern in the distribution of sex chromosomes among land plants or among flowering plants. They are found in annual as well as perennial plants, and in tropical as well as temperate species. This is in contrast with the distribution of dioecy, which is over-represented among trees and climbing species, compared with herbaceous or shrubby species (129).

Ages of plant sex chromosomes have been inferred from molecular clock-based divergence times of sister species with and without sex chromosomes. In *S. latifolia*, the sex chromosomes may be between 8 and 24 million years old (101), an estimate that will become more precise once the precise relationship of *S. latifolia* to its four closest relatives, all of which have sex chromosomes (cf. *Silene*, section above), is resolved. In *C. papaya*, 0.5–2.2 million years of divergence were estimated for four pairs of X/Y genes distributed across more than half the MSY indicating that the sex chromosomes probably evolved at species level, long after the divergence of Caricaceae from their closest relatives, the Moringaceae

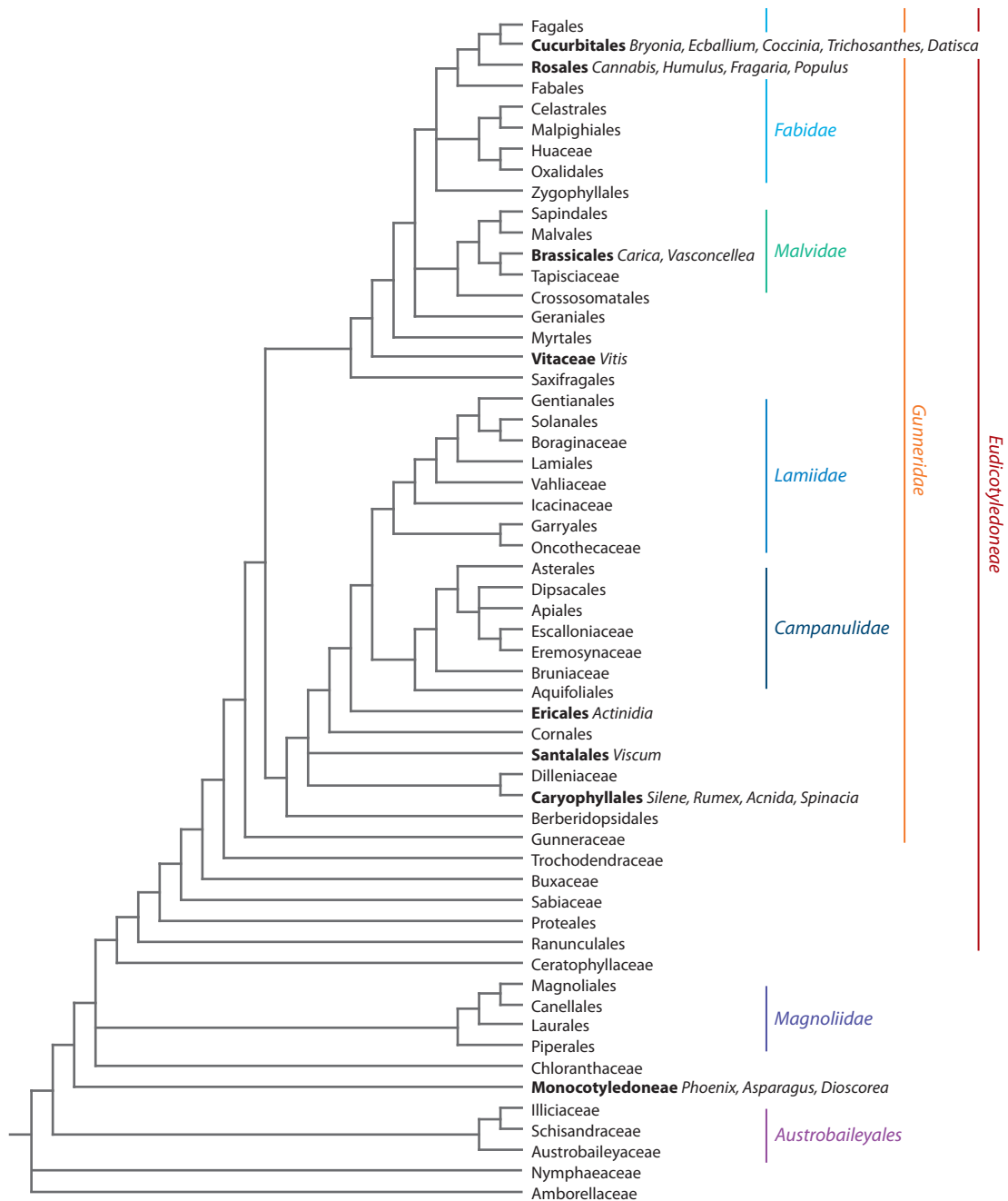


Figure 4

Distribution of sex chromosomes among flowering plants. Phylogeny reproduced with permission from Bell et al. (11).

(185, 187). For *B. dioica*, an age of less than 10 million years has been inferred (163), and sex chromosomes in *Rumex* may have originated between 15–16 mya (106).

WHY THE XY SYSTEM IS PREVALENT

Although the evolution of bisexual flowers from the unisexual cones of gymnosperms is still an unsolved problem (10), it is widely agreed that bisexual flowers are an early or even ancestral trait in the angiosperms. Unisexual flowers evolve from bisexual flowers via random mutations affecting stamen and carpel development. In addition, where such mutants are picked up by selection, populations will begin to include individuals with unisexual flowers as well as individuals with bisexual flowers, leading to gynodioecy or androdioecy. The vast majority of random mutations are deleterious loss-of-function recessives. However, a few mutations are gain-of-function and dominant. Because a large number of genes are involved in the development of stamens and carpels, it follows that many genes, likely regulatory genes, have the potential to cause male or female sterility after loss-of-function mutations. The abortion may occur at any developmental stage, from the induction of stamen primordia to the maturation of pollen grains in female flowers or from the induction of carpel primordia to the development of styles and stigmas in male flowers (92). The relevant genes are randomly distributed on different chromosomes, and a mutation causing male or female sterility could occur on any chromosome. When a recessive mutation occurs, it has to become homozygous to show a phenotype, with fixation being a low frequency outcome. The mutation rate of male sterility or female sterility should be more or less the same, depending on the number of potential genes that could trigger stamen or carpel abortion. However, male and female sterile mutations have different probabilities of becoming fixed in a population. Male sterile individuals with female flowers will easily be

pollinated and achieve full seed set through pollination from individuals with bisexual flowers. As pollen is abundant and typically mobile, male sterile mutants can achieve a high frequency in the population before strong selection against this phenotype occurs. In contrast, female sterile individuals with male flowers will produce pollen that must compete directly with that produced from bisexual individuals in the population. In a random-mating population, a female-sterile mutant (i.e., a male plant) cannot be established by selection unless it more than doubles pollen output (or survival to maturity). An even greater reproductive advantage is required to establish female sterility in a partially self-fertilizing population, where the pollen that a plant contributes to the pollen pool is worth less than its ovules in terms of genes transmitted to the next generation, because a large fraction of the ovules are self-fertilized (25). Empirically, gynodioecy is much more abundant than androdioecy (25), whereas androdioecy is extremely rare and often appears to be derived from prior dioecy through additional mutations (117). Because of these theoretical considerations and empirical data, it is thought that dioecy most commonly evolves through the intermediate state of gynodioecy.

The second mutation, causing female abortion, will usually be recessive (as explained above) and will most likely occur on a different chromosome. Two recessive mutations on different chromosomes may result in similar frequencies of female plants, increasing frequencies of hermaphrodite plants, and decreasing frequencies of male plants and neuters, eventually causing a reversal back to gynodioecy. If a second recessive mutation occurred on the same chromosome and became homozygous (to have a phenotype), the respective individuals would be neuters regardless of the genetic distance. If a second gain-of-function dominant mutation occurred on a different chromosome, it would produce 75% male plants and 25% female or hermaphrodite plants when heterozygous, or entirely male plants when homozygous, thus

driving the population to extinction. However, if a second gain-of-function dominant mutation causing carpel abortion occurred on the same chromosome, the outcome could be more stable: If the two mutations were more than 50 cM apart, they would behave independently as described above. If they were closely linked, they would produce 1:1 female to male ratios plus low-frequency hermaphrodites and neuters, depending on the genetic distance. This is the theory of two linked genes with complementary dominance for establishing dioecy proposed by Charlesworth & Charlesworth (25), and it is the first stage of sex chromosome evolution. It could be said that all well-established dioecious species should have sex chromosomes.

Sex chromosomes in dioecious species that evolved via the gynodioecy pathway for the above reasons are male heterogametic, which is the XY system. Conversely, sex chromosomes in dioecious species that evolved via the androdioecy pathway are female heterogametic, the ZW system. Gynodioecy is the more common pathway as discussed above, hence the prevalence of the XY system.

WHY SEX CHROMOSOMES ARE RARE IN PLANTS

Sex chromosomes are common in animals, but rare in plants. The primary function of sex chromosomes is to reinforce dioecy, a sexual system that may increase outcrossing in species lacking self-incompatibility (23). The following reasons may explain why sex chromosomes are rare in plants: Plants are sedentary, and their open architecture (bauplan) with numerous meristems allows them to grow sexual organs in varying numbers, depending on intrinsic and extrinsic factors. Self-pollination and neighbor-flower pollination (geitonogamy) are therefore common and provide reproductive assurance. Unisexual individuals, by contrast, face a higher risk of pollination failure, especially when population densities are low.

Perhaps for this reason, dioecy is rare in plants (for absolute numbers, see the Introduction and Phylogenetic Perspective sections above), and the rarity of sex chromosomes follows.

Another reason for the rarity of plant sex chromosomes may lie in the frequency of polyploidy in plants (171). Polyploidy may prevent sex chromosomes from evolving, because the frequency of homozygous recessive alleles in four or eight homologous chromosomes is much lower than in two homologous chromosomes. The wild strawberry, *Fragaria virginiana*, with sex chromosomes at stage 1 is an octoploid, but it is allopolyploid (AAA'A'BBB'B') with disomic inheritance (19). Conversely, the prevalence of diploidy in animals could be the consequence of sex chromosomes, because polyploids would disturb the sex determination system and thus produce less fit (or sterile) individuals.

CONCLUSIONS

Sex chromosomes in land plants are more widespread than previously thought, although the list of plants with sex chromosomes (Table 1) has grown only slightly since Westergaard's (171) review. Molecular and genetic studies in recent years have revealed a number of new species with homomorphic sex chromosomes, and more sex chromosomes will be discovered in dioecious species when genome sequencing becomes a routine practice. If we define sex chromosomes as having two closely linked sex determination genes as we did here, then all established dioecious species have sex chromosomes, but not all of them have heteromorphic sex chromosomes. It is also clear now that dioecious species with sex chromosomes at stages 1 and 2 (Figure 1), without or with recombination suppression between the two sex determination genes, will sometimes revert back to hermaphroditism if that sexual strategy is selectively advantageous in a particular population and environment.

FUTURE ISSUES

1. The 48 species with sex chromosomes are likely a small portion of the dioecious species that have sex chromosomes. Investigation of additional dioecious species using high-throughput genomic technologies, such as genetic mapping by sequencing and whole-genome shotgun sequencing, will uncover more sex chromosomes in land plants.
2. Sex determination genes have been cloned in monoecious maize and gyno/androdioecious melons, but they have not been cloned in dioecious species with sex chromosomes. Cloning of these genes in multiple species will help to understand the gene network involved in sex chromosome evolution and floral sex phenotypes.
3. Functional analyses of plant sex chromosomes have lagged behind their studies in metazoans. As sex chromosomes and genome sequences become available, such as in poplar and papaya, detailed analyses of X- and Y-specific genes will lead to the identification of sex determination genes and may reveal dosage compensation in plants.
4. DNA methylation on sex chromosomes is known to affect sex expression in plants, but the underlying molecular mechanism is unknown. Sex reversal under different environmental conditions is likely under epigenetic control and needs to be explored.
5. The often young sex chromosomes in angiosperms provide a unique system compare the nascent sex chromosomes with their homologous autosomes in sister species. In two families, Caryophyllaceae and Cucurbitaceae, it is possible to compare ancestral autosomes with heteromorphic and homomorphic sex chromosomes, as well as a series of sex chromosomes at different stages. These comparisons will help to uncover the evolutionary events and forces that shaped sex chromosome evolution.

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