Plant Sex Chromosomes

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Abstract

Although individuals in most flowering plant species, and in many haploid plants, have both sex functions, dioecious species—in which individuals have either male or female functions only—are scattered across many taxonomic groups, and many species have genetic sex determination. Among these, some have visibly heteromorphic sex chromosomes, and molecular genetic studies are starting to uncover sex-linked markers in others, showing that they too have fully sex-linked regions that are either too small or are located in chromosomes that are too small to be cytologically detectable from lack of pairing, lack of visible crossovers, or accumulation of heterochromatin. Detailed study is revealing that, like animal sex chromosomes, plant sex-linked regions show evidence for accumulation of repetitive sequences and genetic degeneration. Estimating when recombination stopped confirms the view that many plants have young sex-linked regions, making plants of great interest for studying the timescale of these changes.

Contents

INTRODUCTION

Haploid plant: a plant whose meiotic products develop into free-living haploid gametophytes that develop the gamete-producing organs; examples include liverworts, mosses, and hornworts

Monoecy: the characteristic of having separate male and female flowers on the same individual plants

Heteromorphic: having a cytologically distinguishable sex chromosome; microheteromorphism may be detectable by detailed cytological examination, genetic mapping, or genome sequencing

Both animals and plants include species with separate sexes (called dioecy in plants). In most angiosperms, each individual has both sex functions; such species are called cosexual. In diploid plants, some cosexual species have hermaphrodite flowers, and some are monoecious (**Figure 1**). In haploid plants (as in animals), hermaphroditism occurs, but monoecy does not: Such plants' gametophytes may be unisexual like those of angiosperms and gymnosperms, producing only antheridia or archegonia (sometimes called dioicy), but (unlike diploid plants' gametophytes) haploid plants can have cosexual gametophytes.

Control of individuals' gender may be environmental or genetic. For example, the gender developed by a free-living gametophyte may depend on the presence of a nearby female gametophyte (105). However, the mode of control is unknown for most haploid plants other than those with visible chromosome differences between gametophytes of the two sexes (reviewed in 74). Environmental sex determination is also known in dioecious diploid plants (86, 121), but most of the 15,600 angiosperms that are listed as dioecious in the latest compilation (89) (around 5% of all flowering plants) probably have genetic sex determination, although environmental conditions often influence the extent to which individuals function as females (32, 36, 37, 45, 97). However, cytogenetic data are available from fewer than 100 angiosperm species. Among these, approximately half have chromosomes that are cytologically heteromorphic in one sex but not the other; most are XY/male XX/female systems, called male heterogamety, and sex chromosomes are also seen in some gymnosperms and many bryophytes (58, 74).

The crucial feature of sex chromosomes is not heteromorphism, however, but rather suppressed recombination in the sex-determining genome region, which allows heteromorphism to evolve. To cover the diversity of plant sex-linked regions, including recently evolved regions, I therefore

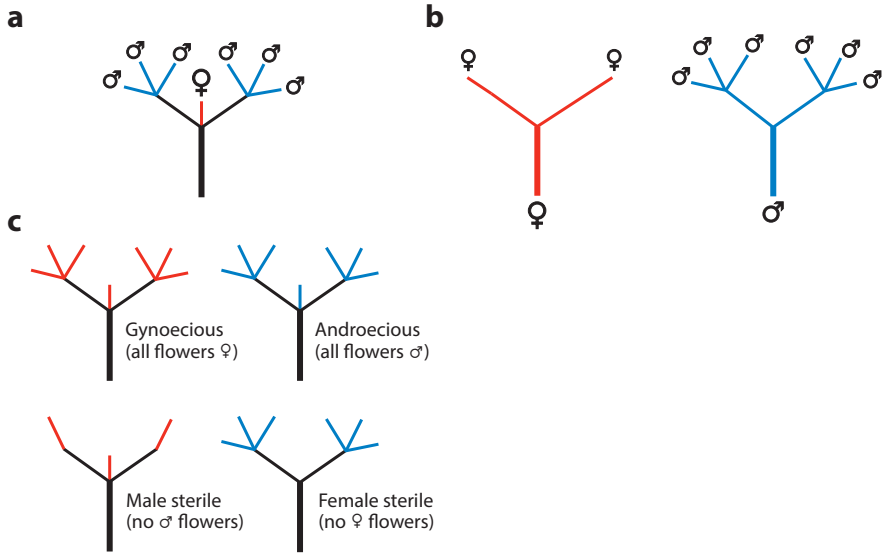

Figure 1

Sex determination in (*a*) monoecious and (*b*) dioecious plants, along with (*c*) unisexual phenotypes derived from monoecy. In monoecious plants (panel *a*), sex is determined late in development, and individual flowers develop as male (*blue stems*) or female (*red stems*) on a plant body that has no gender (*black lines*). In diploid dioecious plants (panel *b*), sex is determined by a plant's genotype at fertilization and is expressed during the development of individual inflorescences. There is a genetic sexual polymorphism, sometimes involving sex chromosomes, and individual plants' genders could potentially be ascertained, even for immature plants, from the genotypes at sex-linked loci or, if sex chromosomes have evolved, by examining their chromosomes cytologically. Panel *c* gives examples of potential mutant unisexual phenotypes in monoecious plants. Note that, if male flowers are abolished, the plant might develop extra female flowers in locations where these do not normally develop (*bottom left diagram*).

review both cytologically recognizable heteromorphic sex chromosomes (implying extensive non-recombining regions, generally including many sex-linked genes other than the sex-determining genes themselves) and fully sex-linked genome regions in plants without chromosomal heteromorphism (**Figure 2**, **Table 1**). When there is a physically small non-recombining region and most of the chromosome is pseudoautosomal, modern cytological methods, such as fluorescence in situ hybridization (FISH) using bacterial artificial chromosomes (BACs) as probes, sometimes reveal microheteromorphism, indicating that the region is non-recombining, as in papaya (*Carica papaya*) and its close relative *Vasconcellea parviflora* (56, 66). When it is unknown whether a non-recombining region exists, the region is called a genetic sex-determining locus (which does not imply that a single sex-determining gene is involved, because sex determination may involve two or more genes, as explained below).

Given the scattered taxonomic distribution of dioecious plants, suggesting that genetic sex determination often evolved recently (74, 89, 115), many species may not yet have evolved extensive sex-linked regions. Dioecious species with sex chromosome heteromorphism occur along with ones without heteromorphism in the genera *Silene*, *Rumex*, and *Spinacia* (see **Table 1**) as well as in *Coccinia* (101), and at least two other angiosperm families, Santalaceae and Cannabaceae, include species exhibiting each state (58, 74), implying fairly recent evolution of heteromorphism. Molecular genetic approaches are now starting to detect sex-linked regions in species with chromosomal heteromorphism and homomorphic dioecious species. **Table 1** summarizes current information.

Heterogamety: heterozygosity for the sex-determining locus in one sex in diploid dioecious species; males are the heterozygous sex in XY systems

Bacterial artificial chromosome (BAC): a DNA molecule used to clone and sequence genome regions of approximately 100–200 kb

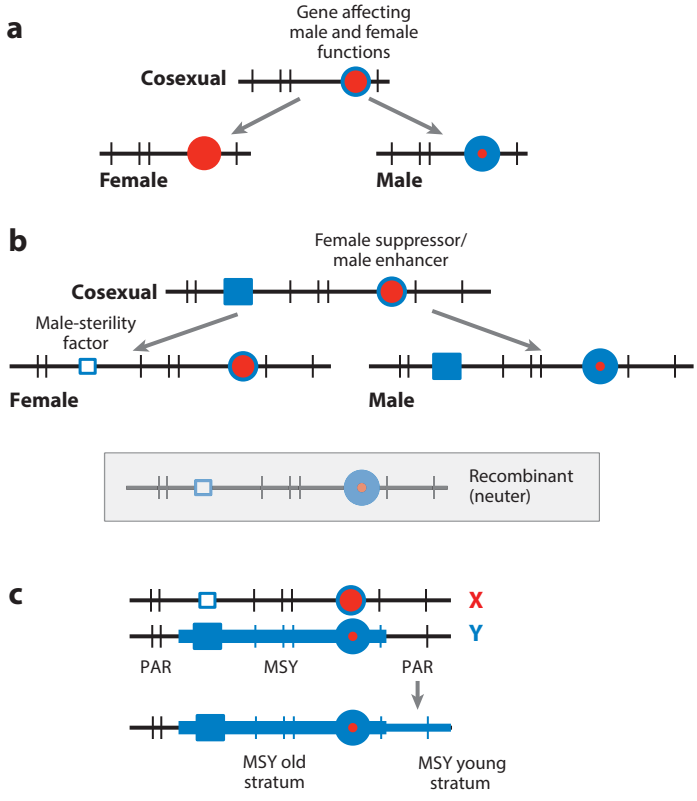

Figure 2

Possible sex-determining loci. (*a*) A single locus in a chromosome region (*black horizontal lines*) that, in the cosexual state, affects the relative allocation of reproductive resources to male and female functions. Females and males could hypothetically evolve by mutations to alleles that allocate all or most resources to one sex function or the other (*circles with more red or blue*). Other genes in the region are indicated by black vertical lines. (*b*) Linked but recombining sex-determining genes. Part of a chromosome is shown (*black horizontal lines*), with two genes organized into different haplotypes that control different sexes. The large blue square is an allele permitting male functions, and the small unfilled square indicates a loss-of-function mutation, creating females (which must be homozygous if the male-sterility mutation is recessive). A second factor (*circles*) controls the levels of female (*more red*) or male (*more blue*) allocation; the haplotype with more male expression determines that the individual is largely male and suppresses female functions. Recombination between the two genes will produce maladaptive individuals (*gray box*) carrying both the male-sterility mutation and the female-suppressing allele at the gene affecting male and female functions. (*c*) Diploid haplotypes of a non-recombining region of a genome in a male after the evolution of recombination suppression between two genes like those shown in panel *b*. The region is labeled Y for the male-specific male-determining version of the region (MSY, shown by a *thick blue horizontal line*) and X for the haplotype in females, and is flanked by recombining pseudoautosomal regions (PARs), as in papaya. The MSY region may include many genes in addition to the sex-determining genes, and these are colored blue to indicate that these alleles may have male-specific sequence variants distinguishing them from their X-linked alleles. This model assumes that the female-suppressing allele at the gene affecting male and female functions acts in a dominant negative manner, so that heterozygotes are male.

Table 1 Sizes and ages of sex-linked regions in plants for which some molecular genetic data are available

Species	Ploidy	Sex chromosomes[a]	Estimated size of non-recombining region and number of protein-coding genes	Approach used to discover variants	Number of genes sequenced	Estimated mean silent site divergence	Estimated time of recombination suppression (Mya)[b]	Reference(s)
Silene latifolia	2*n*	XY heteromorphic	Most of the ~500-Mb Y chromosome; includes many genes	RNA-Seq	12	0.086	5–10 (two strata)	8, 10, 26, 28, 64, 76
Rumex hastatulus XY race	2*n*	XY heteromorphic	—	RNA-Seq	698	0.0188	1.5	53; S.I. Wright, personal communication
Rumex hastatulus XY1Y2 race, Y1-X	2*n*	XY1Y2			510	0.0207 (5 genes with estimates > 0.1)	1.5	
Rumex hastatulus XY1Y2 race, Y2-X	2*n*	XY1Y2			788	0.0057	0.4	
Carica papaya	2*n*	XY microhetero-morphic	8 Mb of ~50-Mb chromosome 1; ~100 genes	BAC sequencing	38	0.024	7.3 (two strata)	112
Pistacia vera	2*n*	ZW small chromosomes	Probably small (<1% of SNPs associated with sex)	RAD-Seq	—	—	—	2, 59
Fragaria virginiana (subdioecious)	8*n*	ZW homomorphic	No non-recombining region	Genetic mapping	—	—	—	103
Fragaria chiloensis	8*n*	ZW	Small					
Diospyros lotus	2*n*	XY homomorphic, small chromosomes	Small	Male-specific *k*-mer search, RNA-Seq	21	0.014	31.4	1
Vitis vinifera	6*n*	XY homomorphic	154.8 kb of ~18-Mb chromosome 2	Genome sequencing	2	0.02	15	39, 57, 85; S. Picq & R. Bacilieri, personal communication

(*Continued*)

Table 1 (*Continued*)

Species	Ploidy	Sex chromosomes[a]	Estimated size of non-recombining region and number of protein-coding genes	Approach used to discover variants	Number of genes sequenced	Estimated mean silent site divergence	Estimated time of recombination suppression (Mya)[b]	Reference(s)
Populus trichocarpa, *P. balsamifera*, and *P. deltoides*[c]	4*n*	XY homomorphic	~100 kb of 19-Mb chromosome 19	Genome sequencing	1	0.012[d]	23	31, 44
Salix viminalis and *S. purpurea*	4*n*	ZW homomorphic	2.5 Mb of *S. purpurea*'s 15-Mb chromosome 15; 48 protein-coding genes	NA	—	—	—	31, 87
Asparagus officinalis	2*n*	XY homomorphic	Probably ~1.7 Mb	AFLP	—	—	—	106
Actinidia chinensis	2*n*	XY homomorphic	No fully sex-linked markers	AFLP	—	—	—	40
Marchantia polymorpha	*n*	UV highly heteromorphic, both sex chromosomes small	Entire small (10-Mb) chromosome;[e] at least 64 genes	Genome sequencing	6	0.84	65	119
Ceratodon purpureus	*n*	UV homomorphic	80 Mb of the largest (100-Mb) chromosome[f]	Genome sequencing	8	0.041	3	72

Abbreviations: AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; NA, not applicable; RNA-Seq, RNA sequencing; SNP, single-nucleotide polymorphism. Dashes indicate that no data are available.

[a]Data are from References 57, 74, and 115. The notation XY is used purely to denote male heterogamety (and ZW to denote female heterogamety), but chromosome heteromorphism is not implied and is noted explicitly when present.

[b]Ages were estimated assuming a neutral substitution rate for plant nuclear genes of 6.5×10^{-9} per generation as well as 1 year per generation for *Silene*, *Rumex*, and the two haploid species; 4 years per generation for papaya; 10 years per generation for grapevine; and 30 years per generation for *Populus* and *Diospyros*.

[c]All three *Populus* species are very closely related; the largest silent site divergence is between *P. trichocarpa*, *P. balsamifera*, and *P. deltoides* (0.74%), and the same markers are sex linked in all three species. The X-Y divergence values are similar to the *P. trichocarpa* value.

[d]Divergence was estimated from sequences described in Reference 43.

[e]No genetic map is available, so the chromosome has no number.

[f]The chromosome carrying the sex-linked region may represent as much as one-third of the genome's total DNA.

Although plant Y chromosomes are often larger than their X counterparts (83), the Y is clearly the homolog of the X, as the chromosomes pair in meiosis [in a region called the pseudoautosomal region (PAR); see **Figure 2**] and segregate regularly, and homologous X- and Y-linked genes have been discovered in sex-linked regions (**Table 1**). Discovery of sex-linked genes also allows tests of whether related dioecious species have the same sex-determining regions.

Recently evolved sex-linked regions in dioecious plants allow detailed study of the initial evolution of separate sexes and of suppressed recombination and its consequences. I therefore first discuss the evolution of dioecy, which explains why close linkage between the sex-determining genes evolves and helps indicate questions that can best be studied in plants now that molecular methods suitable for non-model species have been developed.

Inbreeding depression: low survival or fertility of progeny produced by self-fertilization or other close inbreeding

Gynodioecy: polymorphism for females and individuals with both sex functions (hermaphrodite flowers or monoecious plants)

Androdioecy: polymorphism for males and individuals with both sex functions (hermaphrodite flowers or monoecious plants)

Subdioecy: the presence of individuals with both sex functions (plants with hermaphrodite flowers, or monoecious plants) together with unisexual females and males

ANCESTRAL STATES AND SEX-DETERMINING GENES

Effects of Sterility Mutations

For separate sexes to evolve from a cosexual state, at least two mutations must occur: one to create males and one to create females (**Figure 2**). Two advantages might allow a male-sterility mutation to spread in a plant population (67). First, females cannot produce seeds by self-fertilization; avoidance of inbreeding depression increases progeny quality compared with that of cosexuals, unless these are completely outcrossing, which is rare among species related to dioecious plants (22). Females may also benefit by reallocating resources from male to female reproductive functions. Both benefits have been empirically demonstrated in natural plant populations (e.g., 30, 38, 61, 62, 91, 92).

A unisexual phenotype will not spread throughout the population, because it requires the other sex to fertilize its gametes. For example, females created by an advantageous male-sterility mutation will establish a polymorphism along with the ancestral hermaphrodite or monoecious individuals (a situation called gynodioecy). To establish dioecy, a second mutation must change the ancestral cosexual type into a male phenotype and must also remain segregating in the population.

The pathway via gynodioecy is more likely than one in which males evolve first, for the following reasons. Selfing reduces the availability of other hermaphrodites' ovules for outcrossing, so male mutants in a population of partially self-fertilizing hermaphrodites cannot greatly gain outcrossing opportunities. Considerably increased male fertility is therefore required to establish an androdioecious population (18, 68). Androdioecy is indeed rare and appears most often to evolve via mutations through which females in dioecious populations gain some male function, and full dioecy breaks down (21, 23, 78, 82, 90). In a gynodioecious population, however, hermaphrodites that reallocate resources from female to male functions increase their siring of ovules available in the females' flowers, which can allow the spread of female-sterility mutations provided that they sufficiently increase male fertility, leading to dioecy (18).

This model is clearly oversimplified, but the chief results apply more generally (18); for example, if full maleness arises not through a single mutation but by partially reallocating resources toward male functions, hermaphrodites could gradually become more male. Indeed, many plants are subdioecious, with "inconstant males" that have some female function, often expressed only in particularly favorable environments (32, 36, 45, 68, 69). Subdioecy is predicted to evolve when trade-offs between growth and reproduction are allowed in the model (95).

The genetic details are also important. Given the complexity of anther development and functioning, recessive loss-of-function male-sterility mutations can occur at many nuclear genes (e.g., 79, 107, 114, 117). However, because advantageous nuclear mutations rarely spread in populations if they are recessive, generating homozygous females requires some self-fertilization, reinforcing

the tendency for dioecy to evolve from populations without strong outcrossing mechanisms such as self-compatibility. The expectation that dioecy usually evolves by an initial male-sterility mutation followed by one or more mutations that reduce female functions and increase male functions (**Figure 2**) predicts that females should generally be homozygotes and males heterozygous, consistent with the observation that, in plants, XY sex chromosome systems are more common than female heterogamety (**Table 1**).

Haplotype: the haploid genotype of a genome region; the DNA sequences of different "alleles" (or haplotypes) of the region may differ at multiple sites

Evidence Supporting the Two-Mutation Model

The two mutations needed could occur in the same gene (**Figure 2*a***) or in separate genes (**Figure 2*b***). The involvement of two genes in the evolution of dioecy in several plant taxa was deduced from genetic evidence (reviewed in 115), particularly from crosses between cosexual and dioecious populations of *Ecballium* (Cucurbitaceae). When a species' sex-determining locus has three apparently allelic states (corresponding to the situation shown in **Figure 2*b***), this implies at least two closely linked genetic differences. In papaya, for example, two Y chromosome haplotypes determine males versus hermaphrodites (reviewed in 111), whereas females are homozygous for a haplotype showing X linkage; the situation is similar in grapevine (*Vitis vinifera*) (85).

Such allelic states could arise by mutations in a single gene, although it seems implausible that mutations in a single gene in a cosexual could often create both males and females. Moreover, direct evidence supports the involvement of two distinct genes in *Silene latifolia*, a species with male heterogamety: Partial deletions demonstrate that distinct Y chromosome regions carry female-suppressing and male-promoting factors (115). Now that Y-linked marker genes have been identified, this conclusion has been confirmed by the finding that different sets of Y-linked sequences are co-deleted in hermaphrodite and neuter plants that respectively lack these sex-determining factors (reviewed in 41). Consistent with this, a two-gene system in which the genes recombine has been discovered in a subdioecious strawberry species in which females are the heterozygous sex (103).

Evolution from Monoecious Ancestors

The model outlined above can apply to populations that are initially hermaphroditic or monoecious. However, phylogenetic analyses show that dioecy often evolved from monoecy (32, 89), so it is important to consider whether monoecious plants might also evolve dioecy some other way. In monoecious plants, rather than sterility mutations affecting the development of male or female parts of hermaphrodite flowers or simply abolishing flowers of one sex (**Figure 1*c***, bottom right), the changes would probably control inflorescence development; for example, mutations might cause one sex of flower to develop as the other sex (**Figure 1*c***, bottom left), which might not involve loss-of-function mutations, so femaleness might not be predicted to be generally recessive. However, gynoecy in muskmelon (*Cucumis melo*) is a recessive loss-of-function mutation (70).

Alternatively, dioecy might evolve by successive mutations in a single gene controlling the proportions of male and female flowers. The available genetic evidence from monoecious species, including *Antennaria dioica* (Asteraceae), suggests the involvement of two genes (115). Furthermore, the observation that males show gender inconstancy more often than females supports the pathway through gynodioecy, implying the involvement of major male-sterility mutations rather than gradual modification of male-female flower ratios (36). However, there is some evidence for different pathways in the Asteraceae (108). Detailed studies of more dioecious plants that evolved from monoecious ancestors are needed.

A central assumption of the model outlined above is that the mutations are advantageous in one sex, with trade-offs making them disadvantageous in the other. In monoecious plants, mutations increasing the proportion of male flowers must decrease the proportion of female ones, creating such a trade-off. In hermaphrodites, however, it remains to be tested whether mutations improving male functions but decreasing female functions (i.e., "sexually antagonistic" male-enhancer/female-sterility factors) are involved in the evolution of dioecy. Identification of the gene(s) involved will allow this assumption to be tested, and recently evolved dioecious plants are most suitable for such tests, rather than species with physically large non-recombining regions.

RNA sequencing (RNA-Seq): a high-throughput method for sequencing mRNAs expressed by the genes in the tissue sampled

EVOLUTION OF RECOMBINATION-SUPPRESSED REGIONS

Why Do Sex Chromosomes Evolve Recombination-Suppressed Regions?

The assumption that having both sex functions involves trade-offs is crucial for generating selection for suppressed recombination as unisexuality evolves. Close linkage of a male-enhancer/female-sterility factor to the male-sterility gene minimizes the frequency of recombinants carrying both sterility factors, and such a mutation will therefore be more likely to increase in frequency the more closely it is linked to the male-sterility gene (and mutations at an unlinked female-sterility gene will not increase in frequency). If two loci are involved and the sexually antagonistic second mutation does invade the population, a two-gene polymorphism is generated; owing to recombination, the population will then be subdioecious, with females, males, and the original cosexual phenotype as well as haplotypes that carry both sterility mutations, which will be selectively disfavored (**Figure 2*b***). Closer linkage may therefore evolve (13). This model thus offers a simple explanation for the evolution of suppressed recombination in plant sex-determining regions.

Detecting Fully Sex-Linked Regions

Over time, alleles in a non-recombining Y-linked region acquire distinctive variants, leading to sequence divergence of the sex-determining genes and the other genes in the linked region (**Figure 2*c***). Sex linkage can thus be detected from the existence of variants restricted to one sex, including anonymous markers, such as amplified fragment length polymorphisms (AFLPs) or microsatellites, which are often variants in nongenic sequences. For example, Y-linked polymorphisms heterozygous in all males, but not in females, were used to detect sex-linked regions in the date palm (*Phoenix dactylifera*) (25) and grapevine (85). High-throughput methods, such as restriction site–associated DNA sequencing (RAD-Seq) (33, 84), are also being used to search for variants restricted to one sex, to detect non-recombining regions in plants whose chromosomes are small and difficult to reliably identify cytologically. **Table 1** lists plants in which sex-linked sequences have now been identified, including two species—*S. latifolia* (8, 26, 76) and *Rumex hastatulus* (53)—in which RNA sequencing (RNA-Seq) has now ascertained many sex-linked protein-coding genes in the large sex-linked regions.

For many dioecious plants with genetic sex determination, however, it remains unclear whether a fully sex-linked region that includes multiple genes has evolved. Some of the many dioecious plants without sex chromosome heteromorphism (74, 89, 115) may have small sex-linked regions. Physically small regions can now be detected by sequencing approaches, especially when the Y chromosome is considerably diverged in sequence from the X chromosome, providing good chances that male-specific variants will be found (24). **Table 1** includes some examples. Plants with no fully sex-linked markers yet found, including the kiwifruit (*Actinidia chinensis*; Ericales) (40), could, however, have single-gene systems.

A Possible Single-Gene System

A possible single-gene system has recently been discovered in persimmon (*Diospyros lotus*; Ebenaceae), whose fully Y-linked (male-determining) region is inferred to be small, because few male-specific sequences were found in either RNA-Seq data or genome sequences (1). The candidate sex-determining gene (*OGI*) is a Y-linked duplication that is also present in several other dioecious *Diospyros* species and is expressed only in male flower buds. Its autosomal progenitor, called *MeGI*, is expressed in females, producing a male-suppressing regulatory RNA that is opposed by *OGI* in males. This system requires two mutational changes to evolve dioecy, but only *OGI* alleles now segregate. Females presumably evolved by a gain-of-function mutation in the *MeGI* gene of a cosexual ancestor, so, unlike in the model above, femaleness was probably not initially recessive. The *OGI* duplication could then have arisen in a hermaphrodite in the resulting gynodioecious population, creating a dominant maleness factor. However, because *OGI* is unlinked to *MeGI* and expressed male specifically, it should spread throughout the population, leaving *MeGI* in control of gender, rather than *OGI* establishing a polymorphic Y-linked region. Further study of this system is therefore needed.

CHANGES IN PLANT SEX CHROMOSOME SYSTEMS

Chromosome rearrangements (see **Figure 4** below) have caused formerly autosomal regions to become linked to a Y chromosome in at least four unrelated angiosperm species; in *Podocarpus*, a genus of gymnosperm plants; and in a bryophyte (reviewed in 74). X-autosome fusions have occurred in *Humulus japonicus*, several *Rumex* species, and *Viscum fischeri*. Such rearrangements leave the sex-determining system unchanged but add a new (Y2) chromosome that segregates from the X like the original Y (Y1). The Y designation implies only that Y2 segregates from the X, not that it is non-recombining. Like the additions to the PAR detected in *S. latifolia* (11), additions to a fully sex-linked region may add partially sex-linked regions, because these rearrangements should not stop recombination. Indeed, the newly sex-linked region formed by a reciprocal translocation that fused an autosome to a Y chromosome in *Silene diclinis* still recombines (55). Current information from the other species is scanty, although in *R. hastatulus*, genetic evidence suggests full sex linkage of Y2 genes, and signs of genetic degeneration (see below) support suppressed recombination. In *Rumex acetosa*, both Y chromosomes appear to be heterochromatic (however, as explained below, it is not certain that they arose by an X-autosome fusion).

Changes in heterogamety are also known in plants. For example, within the almost wholly dioecious family Salicaceae, *Salix viminalis* has female heterogamety (3, 52, 87, 96), and its sex-determining locus is on a linkage group that is not homologous with that in *Populus* species; in *Populus*, some species have XY systems, and female heterogamety may also exist (80, 109), although this is uncertain (44). In the genus *Silene*, *S. colpophylla*, with a homomorphic genetically XY system, is closely related to *S. otites*, with female heterogamety (99). These changes may represent either new genes replacing ancestral (yet to be uncovered) sex-determining loci and taking over control of gender, or independent evolution of sex determination. Independent evolution might fail to be recognized when it occurs in related species with the same heterozygous sex, and identifying such cases requires detailed genetic work, which has only recently become possible in plant systems. The best current study tested four genes that are sex linked in *S. latifolia* and found that they map to a single autosomal *S. colpophylla* linkage group (75); sex-linked genes in *S. colpophylla* are presumably autosomal in *S. latifolia*, but this has not yet been demonstrated.

To determine whether different species share an ancestral sex-determining system, the distribution of dioecy in phylogenies should therefore be supplemented by testing whether the

sex-determining genes (or genome regions) are homologous. Alternatively, widely different ages should be excluded by estimating the times when suppressed recombination evolved. Species with X-autosome balance systems, rather than actively male-determining Y-linked regions, in *R. acetosa* and two species in the family Cannabaceae, *Cannabis sativa* and *Humulus lupulus* (115), also probably changed from ancestral XY systems.

X-autosome balance: sex determination based on the ratio of X chromosomes and autosomes rather than on a dominant Y-linked actively male-determining factor

AGES OF PLANT SEX CHROMOSOME SYSTEMS AND SEX-LINKED REGIONS

Because dioecy has evolved repeatedly in the angiosperms, plants are excellent organisms for estimating when recombination was suppressed and testing questions such as whether X-autosome balance systems and chromosome heteromorphism are derived states and are more common in older systems. However, finding sex-linked regions in non-model plants and determining their ages and sizes remain difficult, even with genome sequencing.

In mammals and birds, the sex-determining regions have remained on the same chromosome since early in these groups' radiations, with few rearrangements apart from sex chromosome–autosome translocations that added genome regions to pre-existing sex chromosomes (16, 81, 113). Short-read sequencing data can then be assembled using genome sequence assemblies from related model species, to reveal sex-linked regions—the logic is that, when one sex chromosome has lost most of the genes present on the other, the homogametic sex will have all genes present in the diploid state, and consequently twice the depth of coverage of the other sex (5, 27, 120). This approach cannot be used for non-model plants with independently evolved sex-linked regions, especially as these regions often evolved recently and may have lost few genes (although if a closely related species exists with an assembled genome, the localized presence of abundant heterozygous variants might identify sex-linked regions; see below).

Without a good reference genome sequence, assembly is very difficult, as illustrated by the problems in genera such as *Populus* (44) and *Salix* (52, 87). Non-recombining regions pose particular problems, making it difficult to determine the extent of sex-linked regions. Moreover, the sex-linked regions of some plants may be in centromeric regions with low gene density (and high repetitive content; see below), as is the case in papaya (56) and possibly also in *Populus* and *Asparagus* (106). Young systems may also often have few fully sex-linked genes and little sequence divergence, making them difficult to find; in older systems, however, Y-linked sequences from a single male may be treated as paralogs by genome assembly software, rather than mapping as alleles of X-linked genes. This possibility can be tested by studying multiple males to check whether candidate Y-linked variants are truly male specific. The RAD-Seq and RNA-Seq approaches mentioned above can ascertain sex-linked genes in non-model dioecious species, including genes present in both sex chromosomes but regularly heterozygous for different sequences in one sex, which also indicate the heterogametic sex (43, 73).

Sequence Divergence Between Y- and X-Linked Genes

Sequences of even small numbers of Y- and X-linked genes are very valuable because they provide age estimates. After recombination suppression, Y-linked sequences diverge from their X-linked counterparts. Neutral sequence divergence between the sex chromosomes accumulates with the number of generations since recombination stopped (60). If this molecular clock can be calibrated (for example, using sequence divergence between species whose last common ancestor can be dated from the fossil record), the number of years can be estimated. Available estimates for plants in which sex-linked genes have been sequenced (**Tables 1** and **2**) support the conclusion that

Table 2 Genetic degeneration in the species listed in Table 1 where information is available

Species	Estimated time since recombination stopped (thousands of generations)	Homozygotes for Y (or Z) viable[a]	Lower function or expression of Y than X genes	Evidence of gene loss	Heterochromatin	Reference(s)
Silene latifolia	6,615	No	Yes	Yes	No constitutive heterochromatin	8, 10, 26, 28, 64, 75, 82a
Rumex hastatulus XY race	1,446	Probably not	Yes	Perhaps (based on RNA-Seq)	—	51
Rumex hastatulus Y1-X	1,592					
Rumex hastatulus Y2-X	438					
Carica papaya	1,846	No	Yes	Yes	Male-specific heterochromatin knobs	56, 112, 118
Marchantia polymorpha	64,615	NA	—	Yes	Yes	119

Abbreviations: NA, not applicable; RNA-Seq, RNA sequencing. Dashes indicate that no data are available.
[a]Data are from References 53, 73, and 114.

suppressed recombination between the sex chromosomes of the plants so far studied evolved much more recently than those of eutherian mammals [roughly 181 Mya (27)] or birds [102 Mya (120)]; even the oldest plant estimate, for *Marchantia polymorpha*, is much more recent than these dates. Divergence has been estimated for few sex-linked genes in haploids, but sex linkage appears to be much younger in *Ceratodon purpureus* than in *M. polymorpha* (**Table 1**). More data from bryophytes would be valuable, and finding sex-linked sequences could provide evidence about which species have genetic control of gametophytes' sex.

Plants with highly heteromorphic sex chromosomes, such as *R. acetosa*, may have long-established sex-linked regions, but X-Y divergence is currently unknown in this species. Based on nuclear internal transcribed spacer sequences, dioecy in *Rumex* probably first evolved between 15 and 16 Mya (77). However, X-Y divergence for most *R. hastatulus* genes is small, even in the older Y-linked region (53), so this species may have evolved suppressed recombination much more recently and perhaps independently. There are currently too few data to be certain that no ancient plant sex chromosomes exist, and more studies are needed in haploid plants. It is, however, unsurprising that many systems are much younger than those found in animals, because reliable pollination service is essential for dioecious plants (32), and they may revert to cosexuality if pollinator abundance, or the availability of conspecific individuals, decreases. Hermaphrodite revertant plants are often self-compatible and able to self-fertilize (as in the examples of papaya and grapevine hermaphrodites mentioned above) and can continue to breed in such situations, unlike many hermaphrodite animals. In addition to such reproductive assurance, seed production by self-fertilization is advantageous through increased transmission of genes to the progeny generation. These advantages can potentially outweigh the disadvantage of inbreeding depression suffered by progeny produced by self-fertilization. Young systems are particularly likely to break down. First, a single change, such as loss of a Y-linked female suppressor by mutation or recombination, can generate functional hermaphrodites (115); dioecious plant populations often include inconstant males with significant female functions, facilitating breakdown. Second, variant Y chromosomes determining hermaphroditism can spread through a diploid population if pollen carrying the Y-linked region is functional (as in the Y deletion mutants mentioned above), and if YY homozygotes are still viable (29), as in plants with homomorphic sex chromosomes, including *Thalictrum* species, *Actinidia chinensis*, and *Asparagus officinalis* (reviewed in 74 and 115), and perhaps in *Urtica dioica* (45). Alternatively, hermaphrodites could evolve by reversion of the X-linked male-sterility factor (see above), or by suppression of its effect, followed by loss of the Y chromosome. A possible case is *U. dioica*, which may have broken down to monoecy.

Evolutionary Strata

Sequence divergence is not uniform across fully sex-linked regions, but regions closest to the PARs of papaya (112) and *S. latifolia* (9) are the least diverged, indicating particularly recent recombination suppression (**Figure 3**), as is also inferred in animal sex-linked regions adjoining the PAR boundary (27, 63, 120). In plants with many X-linked genes that have retained Y-linked copies, many such pairs can be analyzed, giving valuable information about the time course of recombination suppression and its consequences (see next section).

However, strata create a problem for testing whether the sex-linked regions of different species are homologous. Genes from a stratum established before two species' common ancestor should show sex linkage and similar X-Y divergence in both species, and the sequences should form X- and Y-linked clusters rather than clustering by their species (44). However, genes in a young stratum in one plant used as a reference species might remain partially sex linked in a relative (**Figure 3**). Comparisons of divergence should therefore include genes from the old stratum of the reference

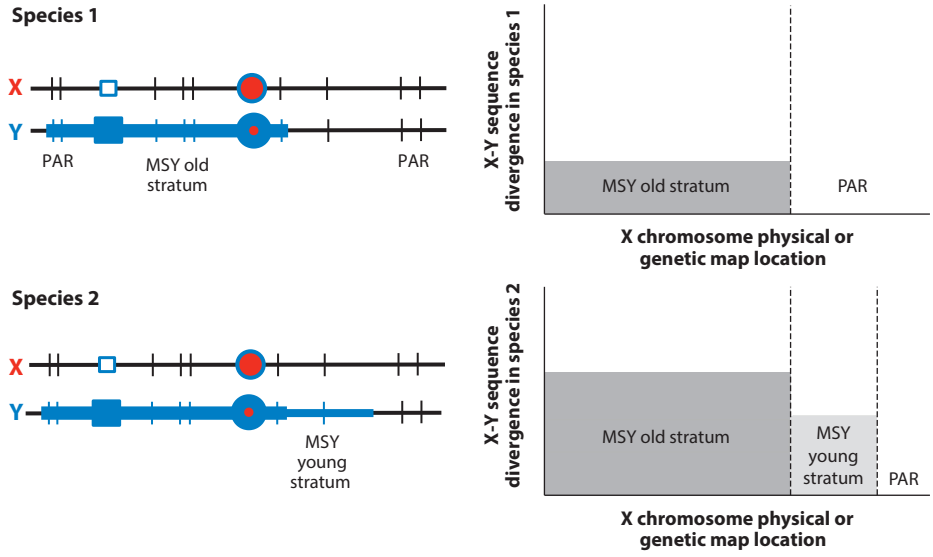

Figure 3

Sequence divergence between X- and Y-linked genes in two related species. The top section shows a fully sex-linked region after enough time has passed for the Y-linked genes of that species (species 1) to diverge from their X-linked alleles. The bottom section illustrates a species (species 2) in which a new stratum has evolved. Here, the Y-linked genes in the older stratum have diverged further from their X-linked alleles than in species 1, and genes in the new stratum have started to diverge. Note, however, that if the recombination suppression event that created the old stratum occurred in a common ancestor, then the divergence might be similar for these genes in both species. Moreover, if young-stratum genes from species 2 were sequenced in species 1, their sex linkage would probably not be detected, as it is only partial. Abbreviations: MSY, male-specific region of the Y chromosome; PAR, pseudoautosomal region.

species' X chromosome, and comparative maps should include genes spanning much of the X map, as was done when comparing *S. latifolia* and *S. colpophylla* (75).

The finding that some plants have undergone further recombination suppression events suggests that recombination suppression evolves not just to preserve correct combinations of alleles at the sex-determining loci. It will be interesting to obtain sequence divergence data to test whether new sex-linked strata have evolved in species with sex chromosome–autosome fusions. If so, this finding would lend support to the hypothesis that large sex-linked regions evolve after sexually antagonistic mutations arise in partially sex-linked regions, improving male functions at the expense of female fitness (reviewed in 94, 102). In turn, this would imply that alleles in the cosexual ancestors evolved under trade-offs between male and female functions (**Figure 3**).

THE MANY CONSEQUENCES OF SUPPRESSED RECOMBINATION

In addition to sequence divergence of Y- from X-linked alleles, sex-linked regions have several unusual characteristics, and their differing ages in plants offer valuable systems for studying the timescales over which these characteristics evolve. As mentioned above, homozygotes for the Y-linked regions are viable in some diploid dioecious plants, but, if recombination is suppressed for long evolutionary times, the Y may lose essential functions.

Genetic Degeneration of Y Chromosomes

The lack of recombination reduces the size of the Y chromosome population (in population genetic terms, the effective population size that controls the importance of genetic drift; see 17), making selection against mildly detrimental mutations less effective in Y-linked than in X-linked genes. In addition, the spread of a strongly advantageous Y-linked mutation through the Y population will cause mildly detrimental mutations carried by the same Y chromosome to spread. Both of these processes may cause Y-linked genes to accumulate more nonsynonymous substitutions and loss-of-function mutations than their X-linked alleles (loss of entire Y-linked genes can also occur, if the effects on survival and fertility are not too severe). These processes can explain why the long-established Y or W chromosomes of mammals and birds have lost most genes carried by the X or Z chromosomes and why most remaining genes are pseudogenes (93, 100).

Transposable element (TE): a dispersed repetitive sequence that can increase in copy number; TEs tend to accumulate differentially in non-recombining genome regions

Stronger genetic drift also causes faster fixation of variants in Y-linked genes than in other sequences, which predicts reduced sequence diversity, as is observed in *S. latifolia*, implying that degeneration is occurring (reviewed in 64). However, degeneration may often be minor in plants (13). First, as explained above (**Table 1**), sex linkage probably often evolved much more recently than the animal systems just mentioned.

Second, many plants have small sex chromosome–like regions, and genetic degeneration is predicted to be fastest when a non-recombining region includes many genes under selection (4, 19). The numbers of genes within sex-linked regions are currently not accurately known for most plants (**Table 1**) owing to the difficulties mentioned above. In papaya, the Y-linked region is approximately 8 Mb, but only approximately 1.6 Mb is nonrepetitive, and gene density is very low (111). Excluding transposable element (TE) sequences, this study annotated 66 intact protein-coding genes with start and stop codons, although another study estimated many more (110). Third, selection on genes expressed in the haploid stages should oppose their degeneration, even in diploid plants, and unisexual haploid plants might be predicted to undergo particularly little degeneration (13).

Young plant Y-linked regions are excellent for studying degeneration. Outgroups are often available to distinguish substitutions in Y- versus X-linked alleles, and many X-Y gene pairs can be analyzed in plants with minor gene losses from the Y-linked region. Higher accumulation of nonsynonymous substitutions compared with X-linked alleles is detectable in *S. latifolia* (8, 26, 76) and *R. hastatulus* (53), consistent with degeneration; the Y-linked sequences also have lower expression than their X-linked alleles. In papaya, the genome region has been completely sequenced, and complete sequences of many genes have been obtained in *S. latifolia* (82a), allowing pseudogenes to be identified. When only intact papaya genes were analyzed, Y degeneration was not detected, implying that—at least in this species—the signal is largely contributed by substitutions in pseudogenes, in which selection no longer opposes nonsynonymous changes (118). With only partial sequences, pseudogenes cannot always be recognized, so the excess nonsynonymous substitutions in Y-linked sequences may be due to their presence. Neither gene loss from the Y chromosome nor pseudogenization is major in papaya, but both may be greater in *S. latifolia* (**Table 2**); studies of older plant sex-linked regions should clarify the extent of gene losses. The very small size and gene content of both *M. polymorpha* sex chromosomes does suggest loss of genes (119), but an outgroup is needed to test the alternative that the ancestral chromosome was small.

Repetitive Sequence Accumulation

Plant Y chromosomes are generally larger than X chromosomes, dramatically so in *Coccinia indica* (101). This suggests that an early consequence of recombination suppression may be accumulation of repetitive elements, which is predicted to occur in non-recombining genome

regions (20). The *S. latifolia* Y, for instance, is 40% bigger than the X (71) and has abundant repetitive elements as well as sequences duplicated from the plastid and mitochondrial genomes (15). Two out of five BACs tested yielded more abundant FISH signals on the sex chromosomes than on other chromosomes, consistent with accumulation of repetitive elements (50); however, the signals were non-Y-specific (65).

Direct evidence that the X has less such element content is scanty, as sequencing genome regions with high repetitive sequence content is difficult. The sex chromosomes have rarely been compared with recombining genome regions on other chromosomes, which is important because X-linked regions recombine only in females (whereas autosomes recombine in both sexes); thus, the X may also accumulate repetitive sequences, making it hard to detect accumulation on the Y. Sequencing in papaya showed that repetitive elements make up at least 81% of the older Y-linked stratum, not much higher than that of the X region carrying the homologous genes (77%), perhaps because non-genic sequences can accumulate changes freely and quickly become unrecognizable (112). Moreover, this region is thought to be near the centromere of the chromosome in both papaya and its relative *V. parviflora* (56), and it may have been non-recombining since before the sex-determining locus evolved. Repetitive sequences have, however, clearly accumulated in the younger sex-linked stratum (which stopped recombining approximately 1.9 Mya); the Y region is twice the physical size of its X chromosome counterpart and has 80% repetitive sequences, compared with 61% in the X (112). This X-linked region has a higher density of repetitive sequences than the homologous region in an outgroup that does not have sex chromosomes, proving that accumulation has occurred on both sex chromosomes, but less on the X than on the Y (49).

Consistent with repetitive sequence accumulation, sequences of BACs from sex chromosomes suggest lower gene densities in the *S. latifolia* Y than the X, although the sequence overlaps are not sufficient to allow paired comparisons of numbers of TEs or genes across large regions (12). However, average Y gene densities are low (only 16 genes/Mb overall, compared with 34 genes/Mb in the X or 22 genes/Mb in the six X BACs for the same probe genes). More sequence data of this kind are needed, including analyses of regions in the younger stratum, to test for TE accumulation early in sex chromosome evolution. TE insertions may make Y chromosomes prone to breakage, perhaps promoting deletions (**Figure 4**) and gene losses. For example, the Y chromosome of *H. lupulus* is small, unlike the Y1 or Y2 in *H. japonicus* (34).

Heterochromatin

The relationships between heterochromatinization, repetitive sequence accumulation, and genetic degeneration of plant Y chromosomes are currently unclear, as are the relationships with the times when the recombining ceased, and estimates of these times for more plants are needed (**Table 2**). Moreover, heterochromatin in plant genomes has several different origins and compositional features (6). Nevertheless, as reviewed by Jamilena et al. (58), several plants, including *Coccinia indica* (101) and *Cannabis sativa* (35), have Y-specific heterochromatic regions, as predicted for non-recombining regions. However, others do not. For example, the large *S. latifolia* Y-linked region is almost entirely euchromatic (14, 46). Papaya, a somewhat younger system than *S. latifolia*, has four Y-specific heterochromatic knobs, plus one present on both the X and Y (perhaps reflecting its pericentromeric location and non-recombining history), and the related *V. parviflora* also has one Y-specific knob (56).

The *R. acetosa* sex chromosomes (which are perhaps older than those of *S. latifolia*) are largely heterochromatic, with large amounts of similar Y-specific repeats in both copies (98, 104). It has been suggested that the two Y chromosomes arose by a process involving breakage at the centromere, followed by duplication of the arms (88). However, an X-autosome fusion has not

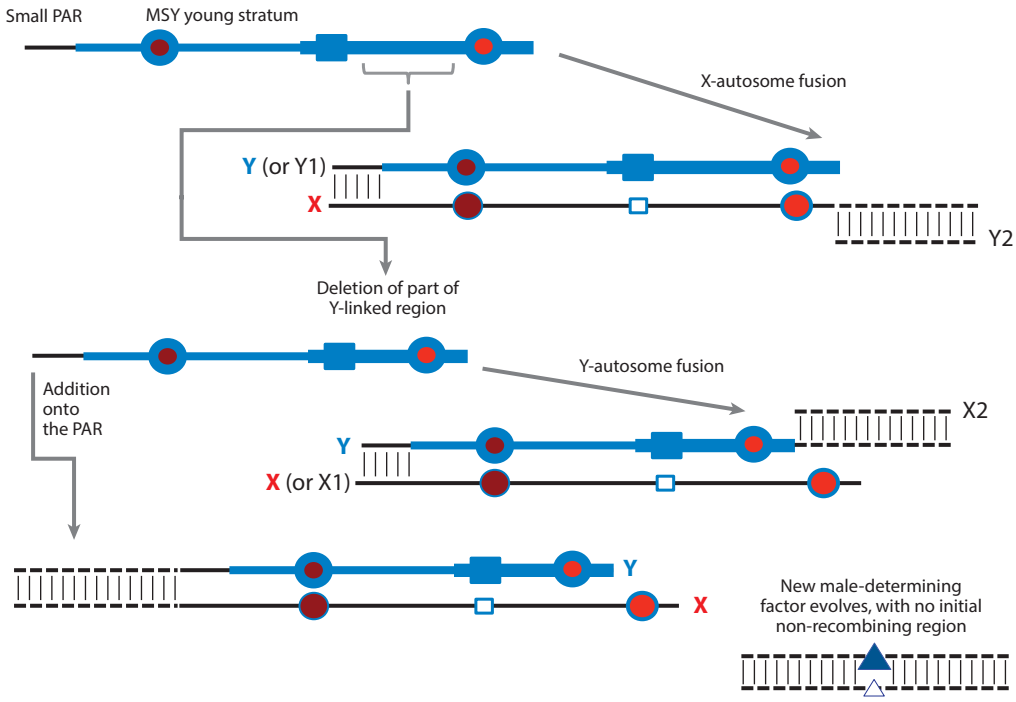

Figure 4

Changes in sex chromosome systems. At the top, an ancestral Y chromosome with a large non-recombining region is indicated by solid lines, with thicker and thinner lines indicating older and younger strata, respectively, and a thin black line indicating a small pseudoautosomal region (PAR) at one end. The older stratum includes the sex-determining genes explained in the main text; the younger stratum may have evolved in response to the appearance of another male-promoting/female-suppressing mutation in a former PAR gene, symbolized by a blue circle with a darker red center than the first such gene. This Y chromosome may become smaller through deletion(s) or larger by the addition of autosome regions (*dashed lines*, with *regions of vertical lines* indicating recombining regions). Additions may be onto the PAR (adding recombining genome regions to both the X and the Y) or to the non-PAR end (often by reciprocal translocations between the autosome and the sex chromosome), creating Y- or X-autosome fusions. X-autosome fusion is detectable because the X chromosome will be larger than the homologous chromosome in related species, and there will be two Y chromosomes, the original one and a new one, often called Y2; deletions may make fusions less readily detectable. Finally, a new male-determining gene may evolve on an autosome or in a new sex chromosome location and may change the system from an XY to a ZW one. Additional abbreviation: MSY, male-specific region of the Y chromosome.

been excluded; if the resulting Y2 chromosome (**Figure 4**) stopped recombining in males, it could have accumulated repetitive sequences like those on the original Y. Distinguishing these possibilities requires identifying genes to test whether the gene contents of the two arms of each Y chromosome are similar. Consistent with a much younger age (53), the *R. hastatulus* Y2 chromosome is euchromatic, but the older Y1 is described as highly heterochromatic (47), which is surprising given its slight divergence from the X (see **Table 1**). Similarly, the *H. lupulus* Y appears to be heterochromatic, and in *H. japonicus*, which has a chromosome fusion, both Y chromosomes show stronger signals of heterochromatinization (4′,6-diamidino-2-phenylindole, or DAPI, signals) than other chromosomes (48).

4′,6-Diamidino-2-phenylindole (DAPI): a fluorescent stain used in cytological research to detect AT-rich and other heterochromatic regions in genomes

Heterochromatinization is not the same as genetic degeneration, although it may indicate that gene losses have occurred, and TE accumulation could contribute to genetic degeneration. In the early stages, TE insertions may destroy or reduce the genes' functions by insertions in noncoding regions, reducing expression either directly (7) or through methylation to suppress TE functioning

(51). Thus, TEs may in part explain the lower expression of Y- than X-linked alleles that is already apparent in plant sex chromosomes such as those of *R. hastatulus* (53) and *S. latifolia* (8, 26, 76).

If a Y chromosome becomes largely degenerated, TE accumulation will be little opposed by purifying selection, because insertions into the coding sequences of genes whose expression is suppressed, or noncoding regions whose functions are already destroyed by TE insertions, will not reduce fitness. Ultimately, massive TE accumulation may occur, perhaps explaining the hugely expanded *C. indica* Y chromosome (more than three times larger than the X) in the past 3 million years (101). A similar expansion may have occurred in *Spinacia tetrandra*, whose Y chromosome is larger than those of close relatives, with no apparent rearrangement that added genome material (42). Expansion may be followed by ectopic recombination between TEs in different Y or W chromosome regions, leading to deletions of potentially large chromosome regions if they no longer carry essential genes. Although, as explained above, parts of the *S. latifolia* Y chromosome can be deleted, and Y chromosome polymorphism in *R. acetosa* (98, 116) suggests that deletions readily occur, it is not yet clear whether any large parts of plant Y chromosomes are devoid of genes and correspond with heterochromatic regions.

QUESTIONS FOR FUTURE RESEARCH

More work is now possible on many of the questions raised in this article, because approaches to discover sex-linked genes have now been developed for non-model organisms using DNA and RNA sequencing. The well-established foundations of cytological data and distribution information about dioecious plants, together with reliable phylogenetic relationships, should allow modern genetic and sequencing approaches to make new progress on several currently unanswered questions, including those listed in the Future Issues box below.

SUMMARY POINTS

1. In some plant species, called dioecious plants, individuals are unisexual and have either male or female functions only. Such unisexual plants are scattered across many families of flowering plants, gymnosperms, and haploid plants.
2. Cytologically visible heteromorphic sex chromosomes, implying genetic sex determination, have long been known in some dioecious plant species. However, heteromorphism is often absent, including in species related to plants with heteromorphic sex chromosomes, suggesting that many plants with genetic sex determination evolved sex-linked regions recently.
3. Molecular genetic studies are now starting to discover fully sex-linked markers and genes in dioecious plants, confirming genetic sex determination even in species with physically small, cytologically undetectable, fully sex-linked regions; such work should help test the genetic basis of plant sex determination, including testing whether dioecy that evolved from monoecious ancestry or in organisms with complex genetics (such as *Urtica dioica*; 45, 97) differs from that from hermaphrodite progenitors.
4. The plant Y chromosomes so far studied have lost only modest proportions of the genes present in the homologous X-linked region, in keeping with the prediction that selection in the haploid stages opposes loss of functions.
5. Sequences of the retained genes provide estimates of the times when they became sex linked, confirming the conclusion from distributional data that many plant sex-linked regions are young.

6. Sex-linked regions in plants are therefore interesting for studying recombination suppression and subsequent gene losses; for detecting subtle footprints of genetic degeneration, including altered gene expression; and for studying accumulation of repetitive sequences and the timescale of heterochromatin formation and evolution of chromosome heteromorphism.

FUTURE ISSUES

1. Which (if any) dioecious plants without chromosome heteromorphism nevertheless have a small sex-linked region, and how many genes are included?
2. Does any dioecious plant have a truly single-gene sex-determining system, and are such cases newly evolved, or were they derived by takeover events from ancestral sex chromosome systems still present in related plants?
3. When sex-linked regions have evolved in plants, when did recombination stop, how many recombination suppression events were involved, were chromosome inversions responsible, and to what extent have plants evolved genetic degeneration and/or sex-linked genes with specialized male and female functions or expression patterns?
4. What are the relative ages of XY, ZW, and X-autosome balance systems, and did the latter two generally evolve from ancestral XY systems?
5. How many haploid plants have genetic sex-determination and sex-linked regions, how old are the many haploid sex chromosome systems, and are these chromosomes less degenerated than those of diploids?
6. Are the sex-determining genes in different species within families or genera with multiple dioecious species the same (or in regions with the same gene content), or did they evolve independently? How many separate origins of sex-linked regions have occurred, and what were their times of origin?
7. Why do very large non-recombining regions and heteromorphic sex chromosomes sometimes evolve? Is their large DNA content largely due to repetitive sequence accumulation, and do the oldest sex chromosomes lose this content and become smaller?
8. Did systems with multiple Y chromosomes evolve by X-autosome fusions or by fission or duplication of preexisting Y chromosomes?

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