

*Chapter I*

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# Plant Sex-Chromosome Evolution

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*Pedro J. Sola-Campoy, Carmelo Ruiz Rejón,  
Roberto de la Herrán, and Rafael Navajas-Pérez\**

Departamento de Genética, Facultad de Ciencias,  
Universidad de Granada, Granada, Spain

## ABSTRACT

Sex chromosomes are thought to have evolved from an autosomal pair through the accumulation of sex-determining genes and the disruption of X-Y recombination that ultimately led to the formation of heteromorphic sex chromosomes. Early evolved plant-sex chromosomes have given rise to many studies in recent years, which have proved chromosomal rearrangements and repetitive-DNA accumulation crucial events that play an important role in sex-chromosome evolution. In this review, we combine evidence gathered by different approaches and report the most recent findings in three model species on plant sex-chromosome analyses, including different levels of sex-chromosome differentiation: *Carica papaya*, homomorphic sex chromosomes; *Silene latifolia*, heteromorphic and undifferentiated; and *Rumex acetosa*, heteromorphic and highly differentiated. The data are discussed in the light of plant sex-chromosome differentiation and evolution.

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\* Corresponding author: Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Campus de Fuentenueva s/n, 18071. Granada, SPAIN, email: rnavajas@ugr.es

## INTRODUCTION

Reproduction can be as simple as a cell dividing (asexual reproduction) or can involve the combination of genetic material from two taxonomically related organisms through sexual reproduction. In terms of energy cost, sexual reproduction can jeopardize proliferation, although it is preponderant all through the fungus, plant, and animal kingdoms. The importance of sexual reproduction lies in ensuring a new genetic combination by avoiding self fertilization, and decreasing deleterious mutation rates by recombination, reasons advantageous enough to develop strong mechanisms for sex-determination maintenance, such as sex chromosomes bearing sex-determining genes [1, 2].

**Table 1. Glossary of most common terms related to sex determination in plants.**

### Glossary

Hermaphrodite	plants with both male and female reproductive parts in the same flower
Dioecious	plants in which female and male reproductive organs are separated in different individuals
Androdioecious	plant species in which male and hermaphrodite flowers are borne on separate individuals
Gynodioecious	plants that bear female and hermaphrodite flowers on separate individuals
Monoecious	plants in which female and male reproductive organs are separated in different floral structures on the same plant
Andromonoecious	plant species in which male and hermaphrodite flowers are borne separately on the same individual
Gynomonoecious	plants that bear female and hermaphrodite flowers on the same individual
Trioecious	plants that bear unisexual (dioecious) and hermaphrodite flowers in different individuals
Polygamous	general term to describe plants that bear hermaphrodite and unisexual flowers on the same individual or different

Sex determination in plants is controlled by genetic factors and, in some species, can be influenced by growth hormones and environmental factors. Contrary to the situation in animals, such mechanisms seem to be more flexible, and related representatives with intermediate sexual conditions are found (see Table 1). Dioecy is a rare condition in plants (38% of all angiosperms and very few gymnosperms, bryophyta and algae), and only around 40 representatives of such species have sex chromosomes (Table 2 [3, 4].

**Table 2. List of most representative plant species with sex chromosomes**

Family	Species	Sex chromosome	Sex det. mechanism	References
Angiosperms				
<i>Actinidiaceae</i>	<i>Actinidia deliciosa</i>	Heterogametic male	Active-Y	[74]
	<i>Actinidia chinensis</i>	Heterogametic male	Active-Y	[75]
<i>Amaranthaceae</i>	<i>Acnida</i> sp.	Heterogametic male	Active-Y	[3]
<i>Asparagaceae</i>	<i>Asparagus officinalis</i>	Heterogametic male	Active-Y	[76]
<i>Asteraceae</i>	<i>Antennaria dioica</i>	Heterogametic male	-	[13]
<i>Cannabidaceae</i>	<i>Cannabis sativa</i>	♀XX/ ♂XY	X/A ratio	[77]
	<i>Humulus lupulus</i>	♀XX/ ♂XY	X/A ratio	[78]
	<i>Humulus japonicus</i>	♀XX/ ♂XY <sub>1</sub> Y <sub>2</sub>	X/A ratio	[79]
<i>Cariaceae</i>	<i>Carica papaya</i>	Heterogametic male	Active-Y	[80]
	<i>Vasconcellea</i> sp.	Heterogametic male	Active-Y	[80]
<i>Caryophyllaceae</i>	<i>Silene latifolia</i>	♀XX/ ♂XY	Active-Y	[81]
	<i>Silene dioica</i>	♀XX/ ♂XY	Active-Y	[15]
<i>Chenopodiaceae</i>	<i>Spinacia oleracea</i>	Heterogametic male	Active-Y	[82]
<i>Cucurbitaceae</i>	<i>Bryonia dioica</i>	Heterogametic male	Active-Y	[66]
	<i>Ecballium elaterium</i>	Heterogametic male	Active-Y	[3]
	<i>Coccinia indica</i>	♀XX/ ♂XY	Active-Y	[83]
<i>Dioscoreaceae</i>	<i>Dioscorea tokoro</i>	Heterogametic male	Active-Y	[84]
<i>Euphorbiaceae</i>	<i>Mercurialis annua</i>	Heterogametic male	Active-Y	[3]
<i>Polygonaceae</i>	<i>Rumex acetosa</i> group	♀XX/ ♂XY <sub>1</sub> Y <sub>2</sub>	X/A ratio	[85, 52]
	<i>Rumex acetosella</i>	♀XX/ ♂XY	Active-Y	[49]
	<i>Rumex hastatulus</i>	♀XX/ ♂XY or ♂XY <sub>1</sub> Y <sub>2</sub>	Active-Y- X/A ratio	[49]
	<i>Rumex suffruticosus</i>	♀XX/ ♂XY	Active-Y	[17]
<i>Ranunculaceae</i>	<i>Thalictrum</i> sp.	Heterogametic male	-	[3]
<i>Rosaceae</i>	<i>Fragaria vesca</i>	Heterogametic female	Active-W	[86]
	<i>Fragaria chiloensis</i>	Heterogametic male	Active-Y	[86]
	<i>Fragaria virginiana</i>	Heterogametic male	Active-Y	[86]
<i>Salicaceae</i>	<i>Populus nigra</i>	♂ZZ/♀ZW	Active-W	[67]
<i>Vitaceae</i>	<i>Vitis</i> sp.	♀XX/ ♂XY	Active-Y	[63]
Gymnosperms				
<i>Cycadaceae</i>	<i>Cycas revoluta</i>	♀XX/ ♂XY	Active-Y	[87]
	<i>Cycas pectinata</i>	♀XX/ ♂XY	Active-Y	[69]
<i>Ginkgoaceae</i>	<i>Ginkgo biloba</i>	♀XX/ ♂XY	Active-Y	[68]
<i>Podocarpaceae</i>	<i>Podocarpus macrophyllus</i>	♀XX/ ♂XXY	-	[88]
<i>Zamiaceae</i>	<i>Zamia</i> sp.	Heterogametic male	-	[89]
Bryophyta				
<i>Marchantiaceae</i>	<i>Marchantia polymorpha</i>	♀XX/ ♂XY	Active-Y	[71]
<i>Sphaerocarpaceae</i>	<i>Sphaerocarpos donnellii</i>	♀XX/ ♂XY	-	[90]
Algae				
<i>Phaeophyceae</i>	<i>Ectocarpus siliculosus</i>	-	-	[72]

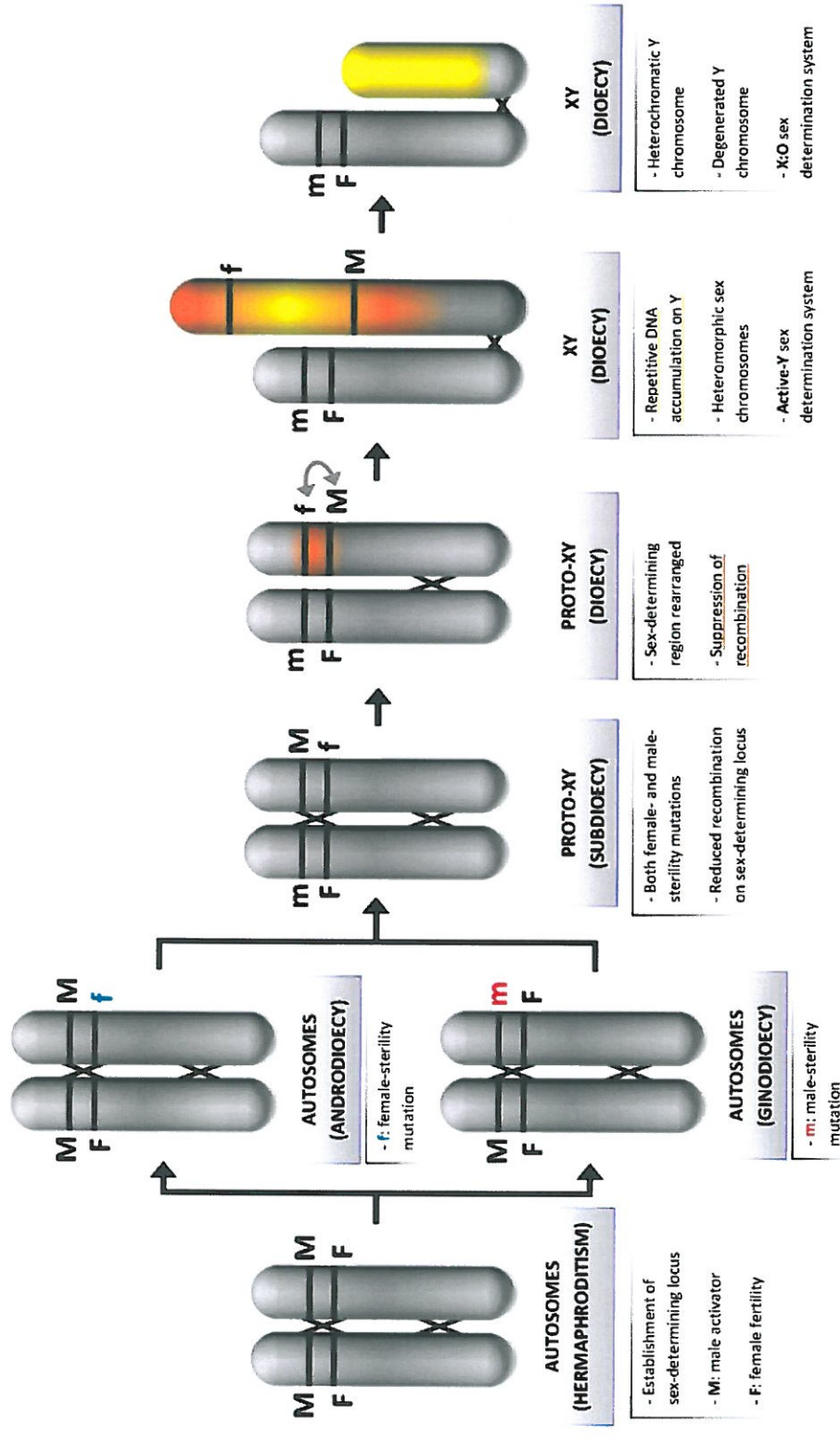


Figure 1. Evolutionary pathway to dioecy from an hermaphroditic ancestor through androdioecious or gynodioecious intermediates, and establishment of sex-determining region and sex chromosomes. Grey represents autosomal and pseudo-autosomal recombining regions, orange stands for suppression of recombination and transposable DNA element accumulation in Y chromosome.

In dioecious representatives, sex chromosomes are thought to have evolved from a standard autosomal chromosome pair as a consequence of a rarely recombining region containing genes involved in sex determination, followed by Y-chromosome degeneration [5]. Dioecy has evolved separately in the evolution by a stepwise process that implies three evolutionary stages [5]. In some cases this derives from monoecious ancestors through andromonoecious or gynomonoeocious intermediates, by the genetic fixation of sex ratios between individuals [6] , and more frequently, from hermaphroditic ancestors through gynodioecious or androdioecious intermediates, the latter being extremely rare in plants (**Figure 1** [7, 8]). The first stage involves the establishment of the sex-determining locus (or loci) on an autosomal pair. At least two mutations, male-sterility and female-sterility mutations, are required (**Figure 1**). In the second stage, the suppression of recombination on the sex-determining region, which is favoured mainly by some sort of chromosomal rearrangement, triggers the molecular degeneration of Y chromosomes (or W in WZ systems). In the final third stage, recombination occurs only in a small region because X and Y chromosomes are too diverged, the Y chromosome remains highly degenerated and heterochromatic (see **Figure 1**).

That progressive suppression of recombination promoted by chromosomal rearrangements (i.e. local duplications, inversions, and translocations; [9, 10]), is the ultimate consequence of the accumulation of diverse repetitive sequences, such as mobile elements and satellite DNAs. This may further inhibit recombination between X and Y chromosomes and ensure the maintenance of dimorphic sex chromosomes, while conferring them with exceptional evolutionary features. This suppression of recombination has been crucial for the rise and evolution of sex chromosomes and has occurred separately in different plant lineages [11].

The theoretic importance of the sex-chromosome study lies in the understanding of sex-determining mechanisms, this information also having practical application in crop improvement. Plant sex chromosomes are particularly informative because they have evolved much more recently than those of mammals or *Drosophila* (**Figure 2**), allowing access to early stages in the process of sex-chromosome emergence. Another fact particular to plants is that most species with separate sexes have evolved directly from ancestors with both sex functions. In this context, the enormous increase in studies on plant sex determination during the last decade is not surprising (see for example reviews by Ming and Moore [12] or Charlesworth [13]). The most intensively studied plants are *Carica papaya* [14], *Silene latifolia* [15], and *Rumex acetosa* [16, 17]. In this chapter, we review the most important findings regarding those models.

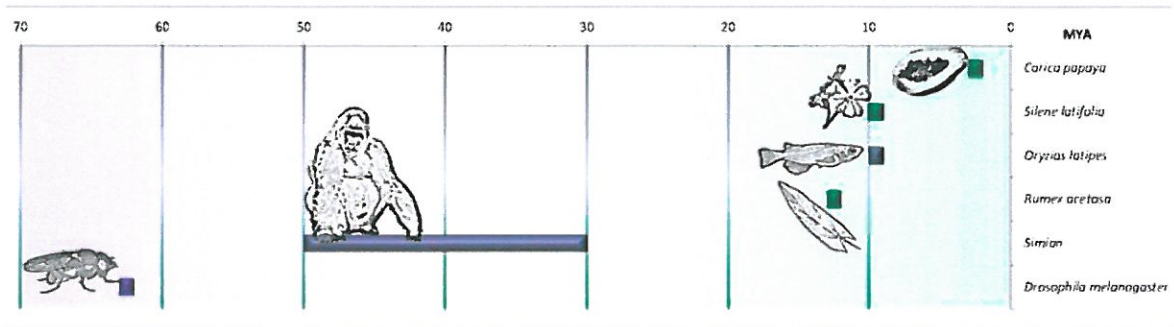


Figure 2. Scaled-temporal diagram representing the origin of sex-chromosomes system in model species based on molecular data: insects, *Drosophila melanogaster* [91]; fishes, *Oryzias latipes* [92]; mammalians, simians [45]; and flowering plants, *Rumex acetosa* (XX/XY1Y2 system) [16], *Silene latifolia*[15], and *Carica papaya* [22].

## DIFFERENT STAGES ON SEX-CHROMOSOMES EVOLUTION

### 1. Papaya (*Carica Papaya*), Brand-New Sex Chromosomes

The molecular mechanism of sex determination in papaya is not fully known, but the papaya male-specific Y region (MSY) may contain a female-suppressing gene and a male-fertility gene. Some authors have suggested that sex determination in this species is determined by a single gene corresponding to SEX1 locus, which has three alleles: two dominant alleles for males (SEX1-M) and hermaphrodites (SEX1-H) over the third one for females (*sex1-f*) [18, 19]. This locus is involved in stamen and carpel development/suppression and also it is hypothesised to bear genes responsible for cross-over suppression (C) and lethality (L), both present in the dominant alleles, giving rise to a 2:1 segregation for sex type [18]. Sex determination in papaya is mediated by the presence of a pair of sex chromosomes [14]. Thus, this species is polygamous with  $2n=16 + XX$  in females, and XY in males or  $XY^h$  in hermaphrodite individuals. Papaya sex chromosomes are morphologically indistinguishable and represent an early stage on sex-chromosome evolution.

The centromere of the papaya Y chromosome has mapped within the MSY region [20]. This centromeric domain has accumulated significantly more DNA than the corresponding X chromosomal locus, which has contributed significantly to a partial cease of recombination, leading to abnormal chromosome pairing. Fine genetic mapping analyses on the MSY region and its counterpart in the X

chromosome have shown a reduced number of expected genes and an accumulation of retroelements in the Y chromosome [21, 22]. Also, comparisons of BACs [23] and AFLP-segregation data have demonstrated that the rate of change in this region is significantly higher than in other papaya genomic regions (reviewed in [24]). It is noteworthy that homozygotes for the MSY region (YY individuals) are lethal, suggesting that some essential gene is lost, but, according to the current data, this might be due to lethal mutations in one gene or a few genes, or to silencing by methylation, rather than to a massive molecular degeneration. Methylation is thought to trigger the suppression of recombination in some sex chromosomes [25]. In fact, although some signs of heterochromatinization are visible in the MSY region in form of five knob-like heterochromatic blocks, the meiotic behaviour of sexual pairs is not affected except for the small region around the centromere [20]. The sex-determining region in papaya is still small, representing only 13% of Y chromosome, and no massive chromosomal rearrangements that could have resulted in a complete suppression of recombination are detected [20]. Other well-studied plants, such as asparagus (*Asparagus officinalis*, [26]), for which YY genotypes have been described, kiwi (*Actinidia deliciosa*, [27]) or wild strawberry (*Fragaria virginiana*, [28]) might show a similar situation; indistinguishable homomorphic sex-chromosomes, signs of suppression of recombination around a sex-determining region, and no clear evidence of molecular degeneration of Y chromosome.

A first draft of the whole genome sequence has been recently published for papaya [29]. This will benefit comparative analyses and will enable the characterization of sex-determining gene(s) when the Y chromosome is fully sequenced. To date, comparative analyses have been made using a BAC-by-BAC comparison bearing sequences from MSY region and its X counterpart. This has allowed estimates of the age of papaya sex chromosomes of about 0.5-2.5 million years ago (mya) (**Figure 2**), while the divergence between Y and Y<sup>h</sup> is estimated to be 73000 years [23]. Similar analyses using the sister genus *Vasconcellea* dated the origin of papaya sex chromosomes at approximately 2-3 mya [30].

## 2. White Campion (*Silene Latifolia*), on the Way to Y-Chromosome Degeneration

*Silene latifolia* has  $2n=22$  autosomes + XY in males and XX in females. Sex chromosomes in white campion are morphologically distinguishable

(heteromorphic) and constitute the largest pair of the karyotype; the Y chromosome being about 50% longer than the X chromosome and containing approximately the 9% of the diploid genome [31]. *S. latifolia* sex-determining system has been extensively studied and is possibly the best-known plant model in this area to date. At least three different groups of genes have been established in the Y chromosome: groups A and D, located in the short arm and related to gynoeceium suppression and stamen-promoting activity, respectively; and group BC, located in long arm and related to stamen development and male fertility [32]. About a dozen Y-chromosome-linked genes and their counterparts in the X chromosome have been isolated such SIY1, SIY3, SIY4, DD44Y, and SlssY (Revised in [33] and [34]). However, genes expressed preferentially or exclusively located in Y chromosome have not been characterized so far. Only SLAP3Y (a MADS box gene) has been demonstrated to be involved in male-specific flower-bud maturation, but this gene has counterparts present in the X chromosome and in one autosome [35].

There is no recombination along almost the entire length of X and Y chromosomes except for a short region, the pseudoautosomal region (PAR), located on the distal end of the X short arm and the Y long arm [36]. Although the non-recombining process might have started shortly before, a large pericentric inversion triggered the gradual process of recombination restriction and led to Y-chromosome erosion [15, 33]. Some evidence points in this direction; thus, Marais et al. (2008) [37] showed a significantly faster evolution in four Y-linked genes than did their counterparts in X-linked. Another sign of degeneration comes from the fact that some introns of Y-linked genes are longer than those copies in the X chromosome. A detailed examination of the larger intron shows sequences belonging to transposable elements such LRT retrotransposons present in DD44Y or inverted repeats in SIY3 intron [37]. Transposable elements, together with tandem repeats, microsatellites, and chloroplast DNA have in fact accumulated in the no-recombining region, playing an important role in sex-chromosome differentiation [38, 39]. The *S. latifolia* YY genotype is not viable, further indicating deterioration of genes not related to maleness, as is predicted for the sex-chromosome-evolution model [40].

However, recent data would support the contention that the degeneration of the Y chromosome is still in an early stage. Concretely, a hermaphrodite-inducing mutation has been proved to be located in the gynoeceium-suppression region of the Y chromosome of *S. latifolia*, suggesting a lack of strong degeneration of the Y chromosome [41]. Also, Qiu et al. (2011) [42], analysing the patterns of codon-usage bias in X- and Y-linked genes in *Silene latifolia*, found similar results.



Based on comparisons between X- and Y-linked genes, and considering a mutation rate of  $10^{-8}$  mutations per base pair per generation, sex-chromosomes of *S. latifolia* have been dated 5-10 mya [43], this resembling findings in other plant species (**Figure 2**).

### 3. Sorrel (*Rumex Acetosa*), a Y-Chromosome Dead End

The last stage in the process of sex-chromosome formation comprises not only the suppression of recombination but also the accumulation of a repertory of repetitive elements -mainly transposable elements and satellite DNAs- in the Y chromosome. This final step gives rise to a dead-end molecular differentiation between X and Y chromosomes accompanied by dramatic morphological differences, i.e. heteromorphism. As a result, the Y chromosome remains as a non-recombining desert that preserves exclusively genes that are crucial for the maleness [44]. Most known sex-chromosome systems, including those of mammals and insects, reached this third stage after tens of millions of years of evolution (**Figure 2**; [45-47]). However, this terminal differentiation is highly unusual in organisms with younger sex-chromosome systems such as plants and fishes (revised in [48]).

In plants, *Rumex acetosa* and closely related species (the so-called *Acetosa* group) are characterized by the presence of an atypical XX/XY<sub>1</sub>Y<sub>2</sub> sex-chromosome system that has been widely studied [49-54, 17]. This chromosomal complex, formed by unequal translocation or misdivision, is unique in the plant kingdom because both Y chromosomes are almost entirely heterochromatic, representing the most advanced stage in plant sex-chromosome evolution [11, 17]. In fact, the two Y chromosomes recombine only with their X-chromosome counterpart by a small PAR and do not recombine between them, constituting a sexual trivalent during meiosis. They are also almost fully heterochromatic and stain strongly with the fluorochrome DAPI [55]. A more detailed molecular survey reveals that Y-chromosome heterochromatin is constituted mainly by several satellite-DNA families, RAYSI, RAYSII, RAYSIII, and RAE180 [56-58] and several types of transposable elements [59]. Navajas-Pérez et al. (2009) [60] mapped comparatively RAYSI and RAE180 sequences by chromosome painting and characterized the structure of Y chromosomes in two related species, *R. acetosa* and *R. papillaris*. Thus, it was possible to infer that massive chromosomal rearrangements are taking place within Y-chromosome heterochromatin. This may have suppressed the recombination completely, leading to massive molecular

degeneration. Although the accumulation of similar sorts of repetitive sequences has been described in sex chromosomes of other plant species such as in *Silene* or papaya [61, 21], the *R. acetosa* complex seems to have reached a deeper coverage and higher degeneration. In this context, considering the pattern of accumulation of such repetitive sequences, it is possible to infer different degrees of sex-chromosome differentiation in *Rumex*; species with undifferentiated homomorphic sex chromosomes, species with incipient XX/XY systems, and highly degenerated XX/XY<sub>1</sub>Y<sub>2</sub> species (reviewed in [54]).

The *R. acetosa* sex-determination mechanism is *Drosophila*-like, depending on the ratio between X chromosomes and autosome sets [62]. By contrast, in papaya and white campion, sex-determination is human-like, controlled by the presence of a male-determining Y chromosome. The presence of *Rumex* relatives with both systems has offered proof that X/A ones evolved secondarily from male-determining Y ones [16], as suggested before by Westergaard (1958) [63]. This would represent a new piece of evidence that points to *R. acetosa* as the most evolved plant sex-chromosome system. Considering the mean rate of change in nuclear DNA of 0.6% per site per million years for plants [64], we used our data on internal spacer sequences of rDNA (ITS) to infer that dioecy would have appeared in *Rumex* between 15-16 mya, while the divergence time between the *R. acetosella*/*R. suffruticosus* (related group of species with XX/XY system) leading to the *Acetosa* XX/XY<sub>1</sub>Y<sub>2</sub> clade should be 12-13 mya (Figure 2; [16]). Unfortunately, no candidate genes for sex-determining have been characterized so far and further analysis will be needed to keep clarifying the interesting sex-determining system of this group of species.

Finally, a last step in sex-chromosome evolution observed in animals is the dosage compensation of X-linked genes for maintaining correct gene-expression levels in females. That may have evolved as an adaptive response to the loss of gene activity on the Y chromosome. This stage has not been fully demonstrated, either in *Rumex* or in other plant species, although some level of differential methylation in one of the two X chromosomes in homogametic female cells has been reported in white campion [65].

## CONCLUSIONS

Here, we have highlighted the most outstanding findings with regard to sex-chromosome systems in three model plant species: *Carica papaya*, papaya; *Silene*

*latifolia*, white campion; and *Rumex acetosa*, sorrel. In all cases analysed, sex dimorphism and sex chromosomes dated between 2.5-13 mya (Figure 2). Although these estimates should be taken with caution due to the incompleteness of the fossil record and that different approaches and DNA sequences have been used to gather the data (reviewed in [73]), the results indicate that sex-chromosome systems in plants are very young. This, together with the fact that dioecious plants have evolved directly from ancestors with both sex forms, make plants excellent models to study sex determination and the evolution of the sex-chromosome systems.

Model plant species with sex-chromosomes parallel the three-step theory proposed by Charlesworth (1996) [5] for sex-chromosome evolution (Figure 1). An initial stage comprises species with undifferentiated homomorphic sex chromosomes. This stage, represented by papaya, asparagus or wild strawberry, is characterized by a small region involved in sex determination, incipient mechanisms for suppression of recombination such as methylation, and faint evidence of molecular erosion of Y chromosome chromatin, such as a low density of genes or accumulation of transposable elements in the MSY region. In a second stage, the extension of the region with suppressed recombination, normally promoted by drastic chromosomal rearrangements, becomes evident, leading to heteromorphic sex-chromosomes. However, the degree of Y molecular degeneration is still moderate. The maximum exponent to exemplify this situation is white campion. The third stage, represented by sorrel, includes highly differentiated sex chromosomes. In this stage, chromosomal rearrangements and massive accumulation of repetitive DNA are responsible of Y degeneration and the establishment of heteromorphic sex chromosomes.

As predicted theoretically, we have showed that in plants several mechanisms such as methylation, chromosomal rearrangements or the accumulation of repetitive elements may be actively contributing to sex-chromosome formation, triggering the suppression of recombination and favouring the subsequent molecular degeneration of Y chromosomes. It is noteworthy that, contrary to the situation in mammals, in all cases, there is a tendency for the Y chromatin to expand. This may further confirm that sex chromosomes in plants recently evolved and are still differentiating by heterochromatin expansion.

In short, sex chromosomes are found in different angiosperms, such as: *Carica papaya*, papaya; *Silene* spp. white campion; *Rumex* spp., sorrels; *Cannabis sativa*, hemp; *Actinidia deliciosa*, kiwi; or *Bryonia dioica*, red bryony [66]. In these species, the XX/XY system is preponderant but not exclusive; meanwhile, in *Populus nigra*, black poplar females are heterogametic ZW while

males are homogametic ZZ [67]. Sex chromosomes have also been described in other groups such as: gymnosperms, *Ginkgo biloba*, ginkgo [68, 93] and *Cycas pectinata*, cycad [69]; mosses, *Ceratodon purpureus* [70]; hepatics, *Marchantia polymorpha* liverwort [71]; and algae *Ectocarpus siliculosus* [72] (Table 2). As the volume of genomic data from different plant species increases, the number of species with sex chromosomes is likely to increase as well. This opens a promising scenario for sex-determination studies, and will allow comparative analyses. The comparison of X- and Y-specific sequences will reveal chromosomal rearrangements and mutations of the sex-determining region after the suppression of recombination and will ultimately shed light on several aspects of plant sex chromosomes: i) to unravel chromatin rearrangements involved in X-Y differentiation, ii) to clarify the comparative evolution of sex-determining regions in diverse plant genomes, and ultimately iii) to characterize the genes responsible for sex determination in plants.

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