

## Letter

# Sex-chrom, a database on plant sex chromosomes

## Introduction

Sex determination and sex chromosomes have been studied extensively owing to their importance in evolutionary biology. Species of dioecious plants, which may have sex chromosomes, offer us unique insights into the history of this phenomenon across the Tree of Life. Here, we present Sex-chrom: a database on plant sex chromosomes ([www.sexchrom.csic.es](http://www.sexchrom.csic.es)), which aims to provide an easily accessible and organized information source for scientists and a general audience interested in this field. Basic data such as complete taxonomic classification of the species, chromosome number, genome size (2C), ploidy level, sex determination mechanism, presence of homomorphic or heteromorphic sex chromosomes and their corresponding sources are readily available for 178 species of 84 genera and 65 families. Besides, the database contains specific sections for 10 selected model systems in plant sex chromosome research such as *Silene latifolia* and *Rumex acetosa*. In these sections, more detailed information also is available, including data on sex-linked genes, transposable elements or tandemly repeated DNA present in sex chromosomes. Data have been extracted from 431 sources published between 1919 and 2020.

Although most plant species are hermaphroditic, carrying both female and male reproductive organs, some are monoecious in which some flowers are either female or male in the same individual, and only *c.* 6% of angiosperms are dioecious, characterized by the appearance of individuals having separate sexes (Renner, 2014). The sex determination pathways differ significantly among plant species with various breeding systems (Fig. 1), and in dioecious species, they are presumed to be under control of nuclear gene(s), usually located in the sex chromosomes. Among species with sex chromosomes, only a few have heteromorphic sex chromosomes, in a similar way to most animals. Although in plants dioecy is uncommon, it has usually emerged from hermaphroditism: it is likely that the 15 600 known dioecious species would have appeared in 900–5000 independent transitions from hermaphrodite ancestors (Renner, 2014), usually through an intermediate (Barrett, 2002). Also, their sex chromosomes have emerged from a pair of autosomes (Charlesworth, 2015) in which there is initially partial lack of recombination. It can be assumed that sex-determining genes often are located in the sex chromosomes, albeit sex determination has been so far studied in a minority of dioecious plant species and in many cases, the nonrecombining region of the sex chromosomes is too small to be easily revealed (Hobza *et al.*, 2018). The relatively rare plant species with known sex chromosomes have become the subject of wide research. The

main topics deal with the process of the Y/W chromosome degeneration, searching for sex-linked genes underlying the sex determination, or with mechanisms of epigenetic control of the dosage compensation (reviewed by Charlesworth, 1996).

## Types of plant sex chromosomes, sex determination systems and their diversity

Most dioecious plants possess morphologically indistinguishable (homomorphic) sex chromosomes, whereas only a minority have conspicuously morphologically different (heteromorphic) sex chromosomes (Fig. 2). There are different opinions on this classification, because some chromosomes may seem homomorphic when observed by light microscopy but their heteromorphism is further revealed via other cytogenetic methods such as fluorescent *in situ* hybridization (FISH), or it is only apparent in meiotic chromosomes. In this report, we consider heteromorphism of the sex chromosomes if there is any karyological or molecular cytogenetics evidence.

We can distinguish between several types of sex determination, which could be very diverse across the Tree of Life. The best-characterized and most common in the Plant Kingdom is the one in which males are the heterogametic sex (XY) and females are the homogametic sex (XX) (Westergaard, 1958), which is the same system as mammals and some insects. Some models are derived from the XY, as the XX/X<sub>1</sub>Y<sub>2</sub> system – reported in some species, in which males possess two Y chromosomes (e.g. *Humulus japonicus* or *R. acetosa*) – and the X<sub>1</sub>X<sub>1</sub>X<sub>2</sub>X<sub>2</sub>/X<sub>1</sub>X<sub>2</sub>Y – where there are four and two X chromosomes in females and males, respectively, as in some *Podocarpus* species (Zhou & Gu, 2001). Another mechanism observed in plants, although much less frequently, is the ZZ/ZW system: in this case, females are the heterogametic sex (ZW), whereas males are homogametic (ZZ) (Ming *et al.*, 2011). This system has been found in genera *Fragaria*, *Salix*, *Silene* and a few other genera (reviewed by Slancarova *et al.*, 2013, and Balounova *et al.*, 2019); it also is the sex determination of birds, and of some reptiles and insects. Besides, when sex is determined genetically in organisms with haploid-phase sex determination systems (such as green algae, bryophytes and brown algae), the chromosomes containing the sex-determining region (SDR) are referred to as U (female) and V (male) sex chromosomes (Bachtrog *et al.*, 2011; Coelho *et al.*, 2018). The sex determination systems vary largely amongst plant species, and we can observe this variability even within one genus or species. The last systems known in plants are the cytoplasmic male sterility, which is encoded by mitochondria, and the nuclear-encoded male fertility restorer genes (Saumitou-Laprade *et al.*, 1994; Caruso *et al.*, 2012). In some cases, sex expression is labile and is controlled by environmental conditions (Korpelainen, 1998). Often an interplay between genetic, epigenetic and physiological factors (e.g. the role

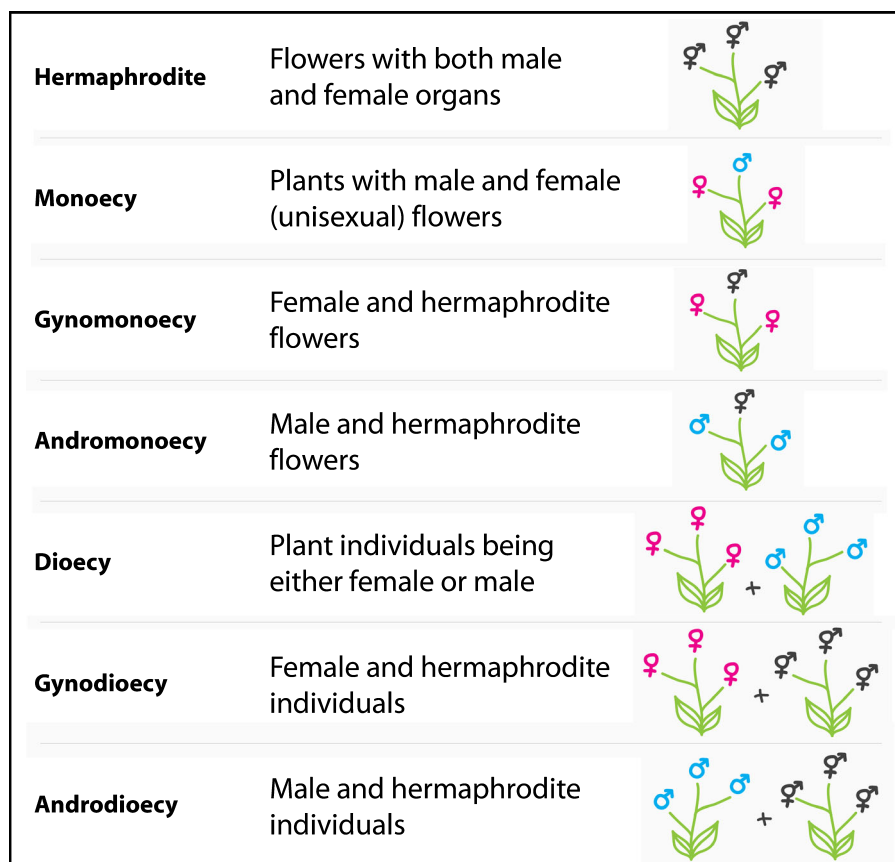


Fig. 1 Overview of possible plant breeding systems.

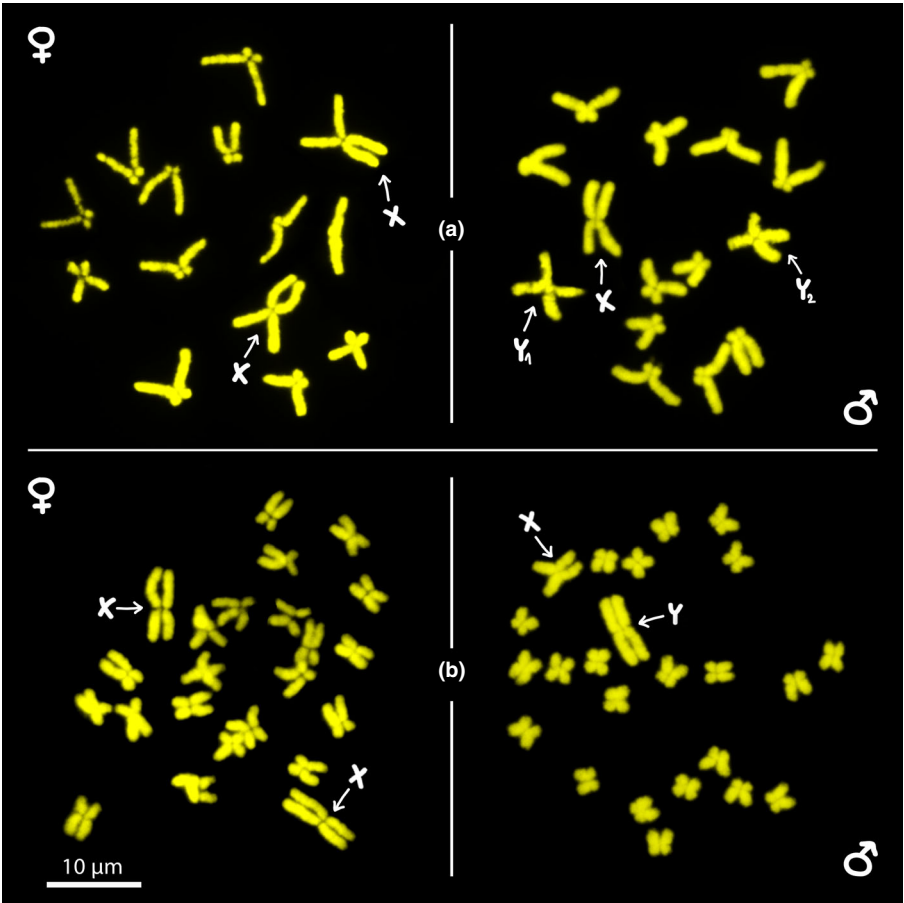
of phytohormones) also can take part in sex determination (Aryal & Ming, 2014).

### Model systems in sex chromosome research

The database compiles detailed information for certain species that have frequently attracted the interest of many researchers on plant sex chromosomes (Table 1). This selection is based in different searches performed with the databases Scopus and Web of Science (WOS) (see the Materials and Methods section for further details). In particular, an outstanding model of variability in sex-determination systems is genus *Silene* (family Caryophyllaceae), whose species *S. latifolia* is one of the most investigated and reviewed in plant sex chromosomes research. There is a huge diversity in breeding systems across the genus in which hermaphrodite, gynodioecious, androdioecious and dioecious species are found (Casimiro-Soriguer *et al.*, 2015). Actually, dioecy has appeared at least twice independently in the genus. In one section of *Silene*, there are only species in which males are heterogametic with one Y chromosome (XY), whereas females are homogametic (XX). The only exception is *S. diclinis* in which a reciprocal translocation may have caused the simultaneous presence of two distinct X chromosomes (X and neo-X) and two distinct Y chromosomes (Howell *et al.*, 2009). In another section, however, the original sex-determining system was probably based on female heterogamety (ZW), as found in *S. otites*, *S. borysthénica* and *S. wolgensis* among

others; interestingly, male heterogamety (XY) appeared again in two species, *S. colpophylla* and *S. pseudotites*, in this mostly ZW section (Balounova *et al.*, 2019; Martin *et al.*, 2019). Besides, we find examples of both heteromorphic and homomorphic sex chromosomes in the genus. Unlike in animals, there are very few examples of plant species heterogametic in females (Slancarova *et al.*, 2013) and there is not any other known case of heterogamety switch with such recent origin of dioecy in such closely related species.

Another interesting model species is *Carica papaya*, a trioecious tropical fruit tree, from the small family Caricaceae, carrying three sexes – female, male and hermaphrodite. Papaya has very young sex chromosomes with an estimated time of divergence *c.* 7 Myr ago (Ma) (Wu & Moore, 2015). Its sex chromosomes are homomorphic, facing an early degeneration stage of its Y chromosome. Due to its trioecious character, papaya enables us to observe the differences between the Y chromosome of hermaphrodite individuals ( $Y^h$ ) and males (Y). It has been presumed that sex determination is controlled by three alleles of one gene, but other possibilities such as epigenetic factors are being considered (Aryal & Ming, 2014). Small SDRs have been found on the Y and  $Y^h$  chromosomes, called MSY (from the male-specific region of the Y) and  $MSY^h$ , respectively. These regions show signs of chromosomal rearrangements, inversions and translocations typical for the nonrecombining region of the Y chromosome (Ming *et al.*, 2007). Papaya constitutes an important example of the origin of the SDR



**Fig. 2** Metaphase chromosomes from female and male individuals of *Rumex acetosa* (a) and *Silene latifolia* (b) with marked sex chromosomes (both species possess heteromorphic sex chromosomes). Staining of chromosomes was carried with DAPI (4',6-diamidino-2-phenylindole) fluorescent stain and artificially colored in yellow with PHOTOSHOP CS5 for better contrast. Pictures by Boris Vyskot and Magda Soukupová.

in the chromosomal part already showing reduced recombination (Charlesworth, 2019).

Sex chromosomes of *Cannabis sativa* have been characterized recently by Prentout *et al.* (2020), and it is apparently one of the oldest sex chromosome systems known in the Plant Kingdom so far. Although it has an estimated time of divergence between 12 and 29 Ma, its sex chromosomes are not conspicuously heteromorphic in simple staining and light microscopy (but they can be

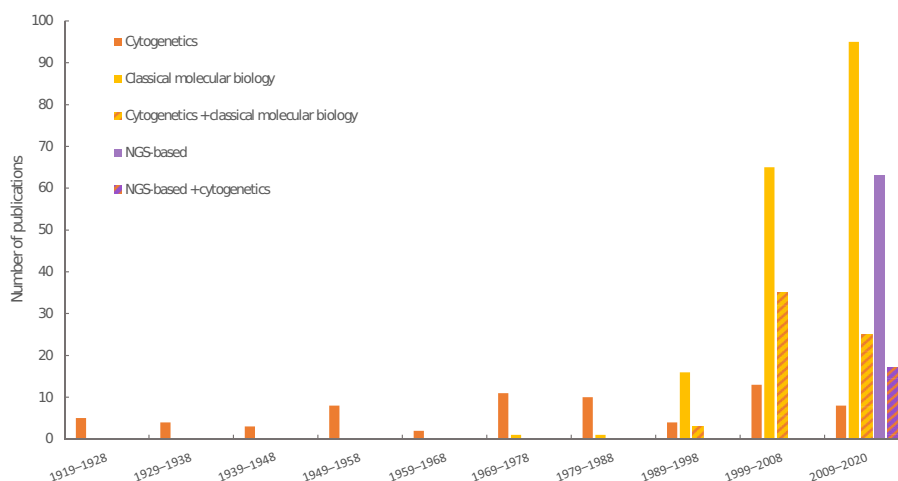
**Table 1** Model systems for which detailed information (i.e. description of sex-linked and sex-biased genes, transposable elements, tandem repeats or other specific loci or sequences, and other relevant data) is available online in separate sections of the Sex-chrom database.

Model system	Publications (WOS)	Publications (Scopus)	Data entries
<i>Asparagus officinalis</i>	50	17	26
<i>Cannabis sativa</i>	37	17	31
<i>Carica papaya</i>	72	55	43
<i>Rumex acetosa</i>	117	69	42
<i>Silene latifolia</i>	341	189	95
<i>Marchantia polymorpha</i>	35	15	27
<i>Fragaria</i>	23	14	22
<i>Humulus</i>	41	22	24
<i>Populus</i>	36	22	41
<i>Mercurialis annua</i>	14	6	19

Data entries indicate the number of individual data records extracted.

distinguished via C-banding and FISH; Divashuk *et al.*, 2014). As shown by Prentout *et al.* (2020) the *Cannabis* Y chromosome presents advanced signs of degeneration, suggesting that the nonrecombining region is taking part in *c.* 70% of the Y chromosome length. The Cannabaceae has only two genera – *Cannabis* and *Humulus* – which are both dioecious. Two of their species (*C. sativa* and *Humulus lupulus*) are of agronomical importance and also appear to be promising model systems for the research of sex chromosome evolution.

*Asparagus officinalis*, another agronomically important dioecious plant, also stands out as a recent model in plant chromosome research. In this case, it has homomorphic but young sex chromosomes in which a nonrecombining region has been recently recognized via genome comparison between double-haploid YY supermale and XX female (Deng *et al.*, 2012; Harkess *et al.*, 2017). Several mutation experiments were performed, shedding light on genes responsible for the suppression of female function and promotion of the male, supporting the Westergaard's two-loci theory for the evolution of sex chromosomes (reviewed in Charlesworth, 2018). These findings are of double importance: they make *Asparagus* an even more convenient model for the evolution of sex determination and also provide us with valuable information for commercial breeding approaches favouring production of all-male seeds – preferred over female seeds because male plants do not produce fruits which would deviate the plant's energy from stalk growth.



**Fig. 3** Number of publications reporting sex chromosomes in plants over 10 successive 10-yr periods, between 1919 and 2020. The right axis indicates cumulative data in number of publications.

*Rumex acetosa* is a dioecious plant (weedy but also cultivated for food purposes) whose sex-determination is controlled by the ratio of X chromosomes to autosomes (X:A) (Ainsworth *et al.*, 1995). This species has been studied intensively mainly regarding the repetitive elements and their relationship to the evolution of sex chromosomes (Lengerova & Vyskot, 2001; Puterova *et al.*, 2018). Interestingly, the males of *R. acetosa* possess two Y chromosomes, which are morphologically easily distinguishable and thus favorable for cytological experiments. Other *Rumex* species such as *R. hastatulus* and *R. acetosella* appear to have Y dominance rather than the X:A balance system (Singh & Smith, 1971). The interest in dioecious species in this genus (including *R. hastatulus* and *R. rothschildianus* among others) has increased with the advent of Next Generation Sequencing (NGS) approaches (Hough *et al.*, 2014; Sandler *et al.*, 2018) although, for example, cytological work showing chromosome fusion in *R. hastatulus* was already well known from the early 1960s (Smith, 1963).

More recently, other model systems have been studied intensively, such as the octoploid *Fragaria*, ZW-based (Tennessen *et al.*, 2018); species from genus *Populus*, showing male heterogamety and used as model for the study of the turnover of the SDR (Gerald *et al.*, 2015); *Mercurialis annua*, also presenting male heterogamety and a model of metapopulation studies influenced by polyploidization and dioecy (Russell & Pannell, 2015); *Humulus*, another model of the X:A balance system (Divashuk *et al.*, 2011); and the bryophyte *Marchantia polymorpha*, which is UV-based and a model for the study of adaptation to the land environment and evolution of nonvascular plants (Bowman *et al.*, 2017).

Given that information on plant sex chromosomes is scattered across myriad sources and that there is a certain complexity regarding the understanding of the multiple sex determination systems and sex chromosomes typologies in plants, we have constructed a new publicly available resource, the Sex-chrom database, freely accessible at [www.sexchrom.csic.es](http://www.sexchrom.csic.es). Currently there is another database, [treeofsex.org](http://treeofsex.org) (The Tree of Sex Consortium, 2014) whose main goal is the analysis of sex determination in eukaryotes with a strong evolutionary/phylogenetic focus, whereas

the objective of Sex-chrom is to provide detailed, summarized and easily available information on plant sex chromosomes for the scientific community bridging the gap between cytological, genetic and genomic data, given the growing interest that the field is receiving in the recent years (see Fig. 3).

## Materials and Methods

### Information sources

A search procedure was designed to retrieve scientific documents that contained data on plant sex chromosomes. The databases used were Scopus (Elsevier) and WOS (Web of Science, Clarivate Analytics). The reason for selecting them was their wide coverage (Mongeon & Paul-Hus, 2016). In an initial step, we used the keywords 'sex chromosome', and later added 'plant', but the search results were too unspecific, and in most cases, publications with sex chromosomes in animals were retrieved. Therefore, we investigated each plant genus with species having sex chromosomes (reviewed in Ming *et al.*, 2011, and Charlesworth, 2016, 2019) and the search strategy consisted of the name of each of the genera and several keywords such as 'sex chromosome', 'sex linked', 'female linked', 'male linked', 'karyotype', 'cytotaxonomy' or 'cytogenetic\*'. In a further step, the name of the family where species with sex chromosomes had been found was included in a new search strategy in order to find possible overlooked results in these genera. As searches were performed, results were downloaded in CSV format. During this process, duplicate and not relevant documents were discarded, and as a result 431 articles, books and meeting proceedings were sourced to provide significant information of plant sexual chromosomes.

In order to select the most appropriate model species for which to provide more detailed information, we listed the number of papers resulting from the search of 'sex chromosome\*' and the name of each plant genera with species having sex chromosomes (extracted from Sex-chrom), in both WOS and Scopus databases (search options: 'Topic' in WOS and 'Title/Abstract/Keywords' in



Scopus). As a result, the model systems mentioned in the Introduction section were selected, because these were either those for which more papers were available (see Supporting Information Table S1) or represented a variety of sex determination systems and plant diversity. Although in some cases we selected a single species as a model (e.g. the well-known cases of *S. latifolia* and *R. acetosa* for which there is extensive specific research), in some others the genus was the model 'unit' as several species from a single genus were the focus, rather than a given unique species (e.g. *Fragaria* or *Populus*).

### Data mining


The information was extracted manually from each source publication and when available (in most cases), the presence and type of sex chromosomes were visually checked in the paper figures. Data were introduced in a Google Sheet (Google Inc.) and the

following fields were filled for each entry: (1) clade (upper, large plant group without a clear taxonomic category); (2) clade (lower, smaller plant group without clear taxonomic category); (3) order; (4) family; (5) genus; (6) specific epithet; (7) species name; (8) infraspecific category; (9) genome size; (10) taxonomic status; (11) accepted name; (12) accepted authorship (fields 10, 11 and 12 correspond to the taxonomic verification of species names and authorships by the Taxonomic Name Resolution Service v.4.0, a tool from iPlant Collaborative (<http://tnrs.iplantcollaborative.org/TNRSapp.html>) for correcting and standardizing plant names); (13) sexual system; (14) chromosome number ( $2n$ ); (15) ploidy level; (16) sex determination mechanism; (17) sex chromosomes male; (18) sex chromosomes female; (19) heteromorphic or homomorphic sex chromosomes; (20) source; (21) method of the primary characterisation of sex-determining system; (22) miscellaneous genomic information (regarding specific chromosomes or

Search

## Welcome to Sex-chrom! v 1.1

**Sex-chrom: a database on plant sex chromosomes**



Most angiosperms are hermaphrodite, and only few have separate sexes, either in different flowers in the same individual (monoecy) or different flowers in distinct individuals (dioecy). Among dioecious plants some have environmental control of sex determination. Some others have genetic control of sex determination, but without cytologically differentiated sex chromosomes and a minority do have sex chromosomes. Conversely, in animals, separate sexes and heteromorphic chromosomes, are common. The low frequency and scattered taxonomic distribution suggest that sex chromosomes have evolved repeatedly and quite recently in plants.

Sex-chrom database 2019



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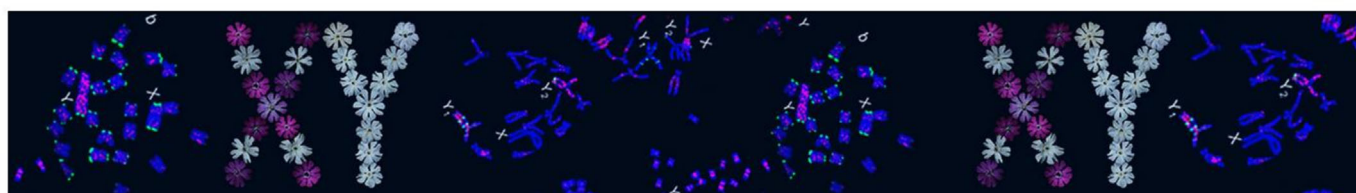


Fig. 4 Screenshot of the Home page of the Sex-chrom database ([www.sexchrom.csic.es](http://www.sexchrom.csic.es)).

the whole genome); (23) source of miscellaneous genomic information; (24) other relevant information; (25) source of other relevant information. An additional Google Sheet was constructed to include the additional data provided for the chosen model systems (*A. officinalis*, *C. sativa*, *C. papaya*, *Fragaria*, *Humulus*, *M. polymorpha*, *M. annua*, *Populus*, *R. acetosa* and *S. latifolia*) with fields including (1) the options (information on specific loci/sequence, presence of sex-biased genes and of sex-linked genes, tandemly repeated sequences, transposable elements or other relevant data), (2) the specific name of these options, if applicable, (3) a brief description, (4) data on protein homology (only for the option 'sex-linked genes'), (5) additional information and (6) data source (linked to the publication). Each entry and each publication had unique identification numbers. Data (spreadsheets) are stored in the institutional and open-access repository Digital CSIC (digital.csic.es).

### Database web environment and construction

The first release of Sex-chrom (Fig. 4) was launched in February 2019. The database can be easily queried through a search box at the

front of the web page where genus or species name of interest can be inserted. Besides, information for the model systems can be browsed by clicking at the individual applications for each species. Data regarding sex-linked genes, sex-biased genes, loci/sequence, tandemly repeated sequences, transposable elements or other relevant information can be browsed by selecting the specific options. Search results are downloadable by a CSV file and also from the institutional repository Digital CSIC. The tab 'Publications' offers a complete reference list of the publication sources, grouped by genus or by its review character, used for data construction, hyperlinked to their DOIs, whereas the tab 'Links' offers directions of other resources or databases interesting to research in plant sex chromosomes.

The structure of the database was performed in the MySQL server and hosted in [www.sexchrom.csic.es](http://www.sexchrom.csic.es); it is implemented in a responsive web design that can be easily accessible from a variety of devices (e.g. laptops, tablets, mobile phones) and screen sizes. The Google sheets including the information were imported to a CSV file. The website uses LARAVEL v.5.3 (<https://laravel.com/>; for PHP 5 developments) and BOOTSTRAP v.3.3.7 (with HTML, CSS and JS; <http://getbootstrap.com/>) open source frameworks.

**Table 2** The 20 journals (including publisher information) providing most articles on plant sex chromosomes (journals still publishing in 2019).

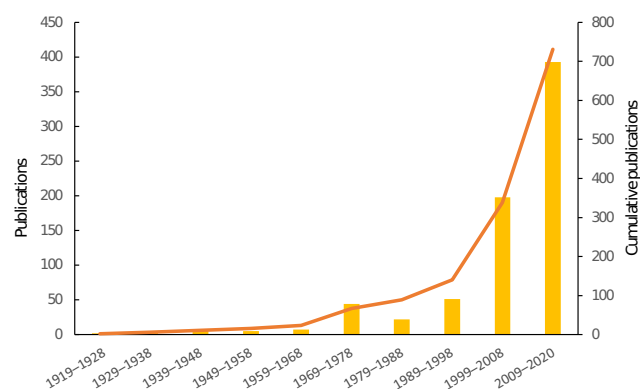
	Journal	Publisher	IF (2018)	Articles
1	<i>Evolution</i>	Society for the Study of Evolution	3.573	92
2	<i>Genetics</i>	Genetics Society of America	3.564	89
3	<i>Genetica</i>	Springer Nature	1.229	87
4	<i>Chromosome Research</i>	Springer Nature	2.183	73
5	<i>Heredity</i>	Nature Publishing Group	3.179	65
6	<i>Journal of Heredity</i>	Oxford University Press	2.618	64
7	<i>Chromosoma</i>	Springer Nature	3.530	60
8	<i>Genome</i>	Canadian Science Publishing	2.152	50
9	<i>Nature</i>	Nature Publishing Group	43.070	49
10	<i>PLoS One</i>	Public Library of Science	2.776	49
11	<i>Molecular Genetics and Genomics</i>	Springer Nature	2.879	48
12	<i>New Phytologist</i>	Wiley-Blackwell	7.299	41
13	<i>G3: Genes, Genomes &amp; Genetics</i>	Genetics Society of America	2.630	38
14	<i>Molecular Ecology</i>	Wiley-Blackwell	5.855	37
15	<i>Current Biology</i>	Cell Press	9.193	37
16	<i>PLoS Genetics</i>	Public Library of Science	5.224	35
17	<i>Molecular Biology and Evolution</i>	Oxford University Press	14.797	34
18	<i>Hereditas</i>	BioMed Central	0.896	34
19	<i>PNAS*</i>	National Academy of Sciences	9.580	30
20	<i>Cytologia</i>	Japan Mendel Society	0.922	28

IF, Impact Factor.

\*Proceedings of the National Academy of Sciences, USA.

### Results and Discussion

Data were extracted from 431 sources published between 1919 and 2020. Fig. 3 shows the publication frequency in this period, data extracted from a search strategy in Web of Science (WOS) with the keywords 'plant\*' and 'sex chromosome\*'. According to this, the present decade is the most productive in terms of papers published on sex chromosomes, highlighting the growing interest in the topic. Indeed, although global scientific production is under exponential growth, with estimations of the number of publications doubling every 24 yr (Bornmann & Mutz, 2015), the growth experienced by papers on plant sex chromosomes largely exceeds this estimation, being *c.* 155% more productive this decade than the last. Other databases collecting cytogenetics-related research (Garcia *et al.*, 2014; D'Ambrosio *et al.*, 2017; Sochorová *et al.*, 2018; Vitales *et al.*, 2019) do not show such a growth, apart from the Chromosome Counts Database (CCDB, <http://ccdb.tau.ac.il/>)



**Fig. 5** Publications consulted in the Sex-chrom database over successive 10-yr periods, between 1919 and 2020, and classified according to the predominant methodological approach. NGS, Next Generation Sequencing.

home/, Rice *et al.*, 2015) whose actual data increase is difficult to calculate, but is probably also very high in the recent years. The CCDB also contains information on sex chromosome presence for certain taxa, although it is mainly focused on chromosome number. Table 2 presents a list of the 20 most frequent journals publishing research on plant sex chromosomes; although most of them are focused on chromosome research, some more general journals such as *Evolution* or *Nature* also account for many papers on plant sex chromosomes.

An inspection of the methodology used when addressing plant sex chromosome research (Fig. 5) shows a clear shift from cytological approaches (involving classical karyology and molecular cytogenetics), which were abundant until the 1990s, to those based on genomics tools, most commonly used from 2015 onwards. Although the close relationship between the DNA sequence and the chromosome structure are better understood if genomics and cytogenetic data are integrated, few research works use both approaches when studying plant sex chromosomes – *c.* 15 papers out of the > 400 here analyzed. As Deakin *et al.* (2019) noted, the field of genomics has largely distanced itself from cytogenetics, and this change is particularly relevant in the study of sex chromosomes; the causes of such a decline on the use of cytogenetics should be found in the descending numbers of researchers trained in cytogenetics worldwide.

In total, Sex-chrom contains data for 65 families, 84 genera and 178 species of plants, and for those whose ploidy level is known, 83.89% are diploid, 12.79% polyploid and 3.32% haploid. The 258 entries available include: 87.60% (226 entries) of angiosperms (eudicots 92.92%, monocots 6.63%, 0.44% ANA grade), 5.04% gymnosperms (13 entries), 5.04% Bryophytes (13 entries) and the remaining 2.32% belong to green, red and brown algae. The most represented families are the Cucurbitaceae (12.02%), Actinidiaceae (8.14%), Polygonaceae (7.75%), Salicaceae (7.75%) and Caryophyllaceae (5.81%), all of them with a high ecological or economic importance, which may explain, at least partly, the research intensity in these groups (i.e. a positive research bias). As for algae, data are available only for six species, probably reflecting a negative research bias or technical difficulties related to small or numerous chromosomes in these taxa. However, most of the represented species belong to Chlorophyta and Rhodophyta, groups that comprise most algae; hence, the information on sex chromosomes available for algae may be minimally representative of its diversity.

As for the sex determination mechanism, entries belong mainly to plants with male heterogamety (XY, 68.99%), followed by female heterogamety (ZW, 16.30%), and the UV sex chromosomes system (5.43%), present in some Bryophytes and algae. It appears, therefore, that the ZW sex-determining systems are not so rare in plants as thought before the advent of molecular markers and NGS. With respect to sex chromosome morphology, close to half of the dataset (48.45%) presents homomorphic sex chromosomes, whereas 31.01% have heteromorphic sex chromosomes; the remaining (20.54%) are not clearly determined in one or the other category (e.g. incipient heteromorphic sex chromosomes are found in *Arceuthobium americanum* but they have not been included in any of both groups; Dwarka *et al.*, 2011). As expected, most entries

(92.64%) correspond to the dioecious sexual system, but we also have included other types not truly dioecious, showing a dioecy-derived sex-determination systems, such as androdioecy and gynodioecy, from families Datisceae and Ebenaceae, respectively. In these taxa, the evolution of the sex-linked region seems to proceed in a similar way to dioecious species.

The database also covers information on the method for the primary characterization of the sex-determining system. Certainly, classical and molecular cytogenetics are the most used approaches to identify sex chromosomes, although in some cases the visual distinction of chromosome size and morphology is still difficult. However, genetic mapping based on AFLP, RAPD, SSR or EST markers also have been used quite often to detect them. Albeit these techniques are able to answer some important questions (e.g. if sex is determined genetically, or which sex is heterogametic) more advanced techniques need to be used in the future for further characterization of sex determination and sex chromosomes in many species. More recently, approaches based on genome-wide association studies and even whole-genome sequencing of individuals of both sexes have been used for example in poplar, strawberry and some green algae (Geraldes *et al.*, 2015; Tennesen *et al.*, 2018; Zhang *et al.*, 2019). Another efficient approach, particularly useful in nonmodel organisms, is SEX-DETECTOR (Muyle *et al.*, 2016), a probabilistic method that using RNAseq high-throughput sequencing of parental species and their progeny can infer autosomal and sex-linked genes. More recently, a double digest RADseq (ddRADseq) has been used to address sex determination across the radiation of dioecious *Nepenthes* (Scharmann *et al.*, 2019).

The individual databases for model species include additional information for plants whose sex chromosomes have been more intensively studied from different points of view. With this, we aim to bridge the gap between cytological and genetic/genomic data by facilitating the access to very specific information. The selected species or genera are spread across the angiosperm phylogeny, including Rosids (*Fragaria*, *Cannabis*, *Carica*, *Humulus*, *Mercurialis* and *Populus*) and Asterids (*Silene*, *Rumex*) among the eudicots, one monocot (*Asparagus*) and one bryophyte (*Marchantia*). In several cases they are important crops (*A. officinalis*, *C. sativa*, *C. papaya*, *Fragaria*), which has probably driven the greater abundance of information for these taxa. For example, information on repetitive DNA is valuable as it plays an important role in plant sex chromosome evolution, yet it is only starting to be understood. Repetitive sequences often are ubiquitous and can constitute more than one half of genome size (in *S. latifolia*, they represent > 60% of all genome content; Macas *et al.*, 2011). They include satellites, microsatellites, transposons (both DNA and retroelements) among others, and they may have regulatory functions in some cases, although at present most research works are limited to detect their presence and abundance. Transposable elements accumulation can account for low gene densities in sex-linked regions (Wang *et al.*, 2012). Because of their transposition mechanism, they can cause genetic degeneration such as mutations and chromosome rearrangements (Charlesworth, 2019). In *S. latifolia* and *R. acetosa*, fluorescent *in situ* hybridization (FISH) analyses clearly showed that satellites, microsatellites, some transposons or transferred plastid sequences are more abundant in the Y



than in the X chromosomes and autosomes (Kubat *et al.*, 2008; Steffova *et al.*, 2014). Conversely, it also has been shown that certain retrotransposons are less abundant in the Y chromosomes: Kubat *et al.* (2014) found that one family of Ogre retrotransposons was present only on autosomes and in the X chromosome of *S. latifolia*. They realized that this Y chromosome devoid of Ogre was connected with its elimination in the male (pollen) line. This process is now being studied by editing candidate genes using CRISPR-Cas9 technology (Hudzieczek *et al.*, 2019). It seems likely that in the coming years, research on repetitive DNA in plant sex chromosomes will be giving more clues on their probably overlooked functionality.

Sex-chrom also contributes information on sex-linked and sex-biased genes in model plants. As already presumed (Bachtrog *et al.*, 2014), genetic content plays a key role in the establishment of sex determination. On the one hand, sex-linked genes – genes physically located on either one of the sex chromosomes – are being mapped in several species, and an intensive effort is being put in characterizing those, which are responsible for the separation of sexes (reviewed in Ming *et al.*, 2011). The function of numerous sex-linked genes, located within the nonrecombining region of sex chromosomes has been elucidated, with most (but not all) having a housekeeping character (Matsunaga, 2006) such as those directly responsible for sex expression. As an example of such data collected in Sex-chrom, two Y-linked genes in *Asparagus* were characterized by Harkess *et al.* (2017), one of which is responsible for the female-suppression function (*SOFF*) and the other considered to be the male-promoting gene (*AspTDF1*). On the other hand, sex-biased genes – genes transcriptionally enriched in one of the sexes – do not necessarily originate from the pair of sex chromosomes; they can be located at any autosome, contributing to sexual dimorphism and are often expressed in sex-specific tissues. Examples of information on sex-biased genes gathered in the database are ovule development genes in *A. officinalis*, exhibiting female-biased expression (Harkess *et al.*, 2015) or the *CCLS4* gene in *S. latifolia* males which contributes to anther development (Hinnisdaels *et al.*, 1997).

### Future directions

There are several questions open for further work in the field of plant sex chromosome evolution. One of them is which mechanism keeps the nonrecombining region small, in spite of a long history of dioecy in many cases. Small nonrecombining regions are probably advantageous, because they are less influenced by the negative impacts of lack of recombination than large ones (Rodríguez Lorenzo *et al.*, 2018). In *Salix* and *Populus*, with shared dioecious ancestry (45 Ma), their homomorphic sex chromosomes are indeed much younger than dioecy (Boucher *et al.*, 2003; Manchester *et al.*, 2006), mostly having small nonrecombining regions in the species studied so far (Geraldes *et al.*, 2015; Hou *et al.*, 2015; Pucholt *et al.*, 2017; Zhou *et al.*, 2018). It can be deduced that the sex-determining pathways in both genera further diverged and evolved independently via repeated recruitment of new master genes controlling sex determination (Pucholt *et al.*, 2017), inducing several rounds of *de novo* sex chromosome differentiation in

different genomic locations. We can expect such switches in other families, even in groups in which dioecy originated relatively recently (Balounova *et al.*, 2019). For example, in the octoploid *Fragaria* (which show recent dioecy evolved from a group of mostly diploid and hermaphroditic taxa) the sex-determining region locates in a small ‘cassette’ which moved during the evolution from one homeologue to another (Tennessen *et al.*, 2018), again causing a *de novo* start of sex chromosome differentiation. It would be interesting to see if this or a similar mechanism evolved in other species. There are unexpectedly high proportions of nonrecombining loci, recently found by high throughput sequencing, in the sex chromosomes of *Mercurialis* (c. 30%) (Veltsos *et al.*, 2018) and *Cannabis* (c. 70%) (Prentout *et al.*, 2020). These examples show the importance of multidisciplinary approaches (including more genetic maps), as these species were supposed to have recent homomorphic sex chromosomes that are now revealed as more ancient and differentiated (but see Divashuk *et al.*, 2014 on *Cannabis*).

Another area of future research would be the sex-determination systems of families where sex-specific differences in recombination frequencies (heterochiasmy) are found (Mehra & Kashyap, 1984; Lenormand & Dutheil, 2005), to test if the model of homomorphic X-Y recombination caused by sex-reversal in, for example, some amphibians (Perrin, 2009) could apply to some plants (although probably playing a minor role). There are several dioecious species among Orchidaceae (Milet-Pinheiro *et al.*, 2015) that may be studied in this respect; despite being one of the largest plant families, the presence of sex chromosomes is still unknown in this family. In Liliaceae there are several examples of heterochiasmy (Burt *et al.*, 1991) and the only known plant example of achiasmy in male meiosis (in the *Fritillaria japonica* group) (Noda, 1975). Although no dioecious species have been revealed in this family so far, cryptic dioecy is sometimes found among species originally evaluated as gynodioecious or androgynodioecious (Mayer & Charlesworth, 1991). Heterochiasmy also can be region-specific, as observed in the large pseudoautosomal region of *Fragaria chiloensis* (Tennessen *et al.*, 2016). Such a local heterochiasmy can be provoked by external factors as an increased growing temperature, as observed in barley (Phillips *et al.*, 2015). Further research can, therefore, bring surprising results even in dioecious species from families with no heterochiasmy yet revealed. It also would be interesting to extend our knowledge of sex determination in more species of gymnosperms and algae, whose coverage remains limited. More data on these groups will provide further comparison opportunities.

We also aim to include more dioecious species, requiring further research and also cosexual relatives of species with sex chromosomes, as part of the database pool in the future, so that comparative analyses are facilitated. Sex-chrom is conceived as a long-term project and future updates will include the new findings that will be uncovered in the coming years. Our research group already has successfully implemented online databases with a plant cytogenetic-genomic focus since 2011 and this new resource joins a catalogue of web-based databases on Asteraceae genome sizes (Garnatje *et al.*, 2011; Garcia *et al.*, 2014; Vitales *et al.*, 2019), plant and animal ribosomal DNA loci (Garcia *et al.*, 2012; Vitales *et al.*,



2017, Sochorová *et al.*, 2018) and B-chromosomes (D'Ambrosio *et al.*, 2017), that are updated and maintained regularly like Sex-chrom. Sex-chrom is an already comprehensive resource on plant sex chromosome data uniquely relating cytogenetic, genetic and genomic information. What is more, it will inevitably expand in scope as current developments in bioinformatics and molecular biology, including novel advances in biotechnology, allow the discovery of even more new facets of sex chromosome biology in the future.

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## Author contributions

SG, SB, JPP-D and NS initiated the project; SG designed the online database; MPA-L and KB designed and performed the bibliographical search strategies; SB, JPP-D, NS, MB, UDA, HM, VP and IPL retrieved the information from the literature searches; BJ, SG, JPP-D and MB reviewed and corrected the retrieved information; SB, JPP-D, BV and SG designed the manuscript figures; SG, BJ, AK and SB wrote the initial draft of the manuscript and all authors participated in the manuscript revision. SB, JPPD and NS contributed equally to this work.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Table S1** Number of articles retrieved by WOS (Web of Science, Clarivate Analytics) and Scopus (Elsevier) databases by the search 'sex chromosome\*' and the name of each genus known to contain species with plant sex chromosomes.

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**Key words:** dioecy, heteromorphic sex chromosomes, homomorphic sex chromosomes, sex-biased genes, sex-determining region, sex-linked genes, tandem repeats, transposable elements.

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