



Labile sex expression in angiosperm species with sex chromosomes

Jos Käfer, Marcos Méndez, Sylvain Mousset

► To cite this version:

Jos Käfer, Marcos Méndez, Sylvain Mousset. Labile sex expression in angiosperm species with sex chromosomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2022, 377 (1850), 10.1098/rstb.2021.0216 . hal-03624906

HAL Id: hal-03624906

<https://hal.science/hal-03624906>

Submitted on 30 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Labile sex expression in angiosperm species with sex chromosomes

Jos Käfer^{1,2,*}, Marcos Méndez³ and Sylvain Mousset¹

¹ Université de Lyon; Université Lyon 1; CNRS; UMR 5558; Laboratoire de Biométrie et Biologie Evolutive; F-69622, Villeurbanne, France; ² CESAB – FRB; 34000 Montpellier, France; ³ Área de Biodiversidad y Conservación; Universidad Rey Juan Carlos; Tulipán s/n; E-28933 Móstoles (Madrid), Spain

*To whom correspondance should be sent: jos.kafer@univ-lyon1.fr

Abstract Here we review the literature on sexual lability in dioecious angiosperm species with well-studied sex chromosomes. We distinguish three types of departures from strict dioecy, concerning either a minority of flowers in some individuals (leakiness) or the entire individual, which can constantly be bisexual or change sex. We found that for only four of the 22 species studied, reports of lability are lacking. The occurrence of lability is only weakly related to sex chromosome characteristics (number of sex-linked genes, age of the non-recombining region). These results contradict the naive idea that lability is an indication of the absence or the recent evolution of sex chromosomes, and thereby contribute to a growing consensus that sex chromosomes do not necessarily fix sex determination once and for all. We discuss some implications of these findings for the evolution of sex chromosomes, and suggest that more species with well-characterized lability should be studied with genomic data and tools.

1 Introduction

About 5 to 6 % (more than 15000 species) of flowering plants are dioecious, meaning they have separate female and male individuals [1]. Currently, sex-determining systems are known for less than 100 species, mostly through the discovery of sex chromosomes [cf 2–5]. Dioecy, and therefore sex chromosomes, evolved many times independently in the angiosperms.

The knowledge about sex chromosomes in plants is still scarce: fewer than 30 species have been studied in detail with genomic data [see reviews 4–6]. Most described chromosome systems are of the XY type (male heterogamety), but some species have ZW type chromosomes (with female heterogamety). It is thought that the evolution of sex chromosomes starts with a small sex-determining region on a chromosome pair, from which recombination suppression spreads to a larger part of the chromosomes. Initially, the YY or WW genotypes are viable (although they might be infertile). The non-recombining region of the Y or W chromosome is expected to degenerate through the reduced efficacy of selection, which leads to an accumulation of deleterious mutations and transposable elements. Transposable elements might initially cause these chromosomes to grow in size (which seems to be specific to plants), but at later stages, non-functional genes can be lost, leading to a decrease in size and maybe even to the loss of the entire Y or W chromosome.

Recent genomic studies have shown that not all plant sex chromosomes follow similar evolutionary trajectories [cf 5, 7]. Among the most striking observations is that some relatively recently evolved sex chromosomes have large non-recombining regions and strong chromosome heteromorphy (e.g. *Silene latifolia*, *Coccinia grandis*) whereas some older systems have homomorphic sex chromosomes with small non-recombining regions (e.g. *Diospyros lotus*, *Vitis vinifera*). The process that causes recombination suppression to spread (e.g. interference of the sex-determining genes creating infertile individuals, sexually antagonistic selection) is not yet clear [7, 8]. Reports of genetic sex determination remain rare compared to the total number of dioecious species, but this is mostly attributed to the difficulty of detecting sex chromosomes, especially if they are of recent origin [3, 6]. Thus, it is suspected that many more species with genetic sex determination will be described as sequencing techniques continue to improve.

Environmental sex determination is less well known in plants. Note that this term is used differently in plants and in animals. In vertebrates, by far the best-studied group, sex is often fixed during their lifetime, thus the influence of the environment occurs at a specific period early in development (although sex changes within an individual's lifetime are documented in fish [9]). In plants, the organs for sexual reproduction are produced anew in each flower (or similar structure), thus allowing more flexibility [10]. Some striking examples of sex change in plants, also called “sex choice” or “gender diphasy”, have been documented [11, 12], for example in *Arisaema* and *Gurania*, which change sex according to their size. As their size depends on the growing conditions, this can be termed environmental sex determination [10]. It is considered evidence for the absence of genetic sex determination; indeed, in species in which all individuals change sex, no genetically based dimorphism is

supposed. Lloyd and Bawa [11] considered the possibility that sex choice might occur in sexually dimorphic species, but concluded that the evidence is weak.

As can be inferred from previous reviews [11–14], variation in sex expression most often concerns a few individuals in a population or a few flowers on a plant. Such variation might be fixed (i.e. some individuals have different patterns of sex expression than the majority) or variable (different patterns of sex expression occur every now and then). In both cases, the plants are able to produce flowers of the sex they normally don’t produce. This lability has sometimes been considered a sign for weak genetic differentiation between the sexes [e.g. 5]. Indeed, the main hypothesis for the expansion of sex-linked non-recombining regions has been the accumulation of genes with sex-antagonistic effects [15] [but see 16]. Such genes are thought to increase specialization in one of the sexual functions (female or male) and to confer less fitness, or even sterility, to intermediate phenotypes. Thus, well-differentiated sex chromosomes have sometimes been regarded as an example of irreversible evolution [17] or “evolutionary traps” [9]. The existence of deviations from the purely female and purely male individuals is sometimes classed as “subdioecy”, a transitory state between hermaphroditism (monocliny) and “full dioecy” [e.g. 18–20].

While reviews on sex changes or other forms of labile sex expression exist and have noted that some species with sex chromosomes display lability [13, 14], more sex chromosomes have been discovered and have been studied with genomic data since their publication. Here we compile data on lability in species with sex chromosomes for which there are estimates of the number of sex-linked genes and the age of these chromosomes. We show that lability occurs in almost all species investigated, and test whether the occurrence of lability is related to the characteristics of their sex chromosomes.

2 Methods

2.1 Terminology and classification of sexual lability

In the strict sense, sexual lability refers to the variation of sex expression in time. It has been known at least since the early 20th century, under many names: sex change [21], sex reversal [22], sex choice [11], sequential hermaphroditism [14], among others. The term “leaky dioecy” was coined by Baker and Cox [23] to indicate “the condition that prevails where hermaphroditism or bisexuality occurs at low levels in populations of otherwise dioecious species”. According to this definition, leaky dioecy includes trioecious species [24], in which male, female and cosexual (usually monoclinal) individuals coexist in the same population. This is however not the sense that most authors nowadays tend to give to the term leakiness [e.g. 25], as it is considered similar to “inconstancy” [26]; Cronk [10], in this issue, prefers to use the almost forgotten term “pleogamy”.

While sexual lability (in the strict sense), leaky dioecy, and trioecy refer to different aspects of sexual expression in plants, they are difficult to separate in practice for two reasons. First, in many cases only a static picture of the sex ratio is available for a given population. Second, sex change often involves bisexual stages, and not simply switches from male to female or vice versa [27]. This means that at a given time, more than two sexual forms may be present in the population of sex-changing species. Therefore, some claims of leaky dioecy, subdioecy or trioecy based on a snapshot of sex expression in a population could be cases of sex change. In addition, as we will show below, plant species can exhibit both sex change and leaky dioecy. In this review, we have adopted a broad definition of sexual lability that includes leaky dioecy and trioecy as special cases, for which sex expression does not necessarily vary in time.

Table 1 is an attempt to classify the different forms of lability that are observed in dioecious plants. Note that this classification is purely based on the observed phenotypic variation, and does not use any knowledge about the possible mechanisms of lability, which are unknown in the vast majority of cases. We chose to separate leakiness and trioecy by reserving the latter term to cases in which individuals are fully monoclinal or monoecious. Leakiness indicates the presence of a minority of aberrant flowers in an individual, which can thus still be considered mostly female or male. To subdivide the major types of lability, we use the classical terms of sexual systems to indicate the phenotype of the aberrant individuals [cf 1], although some prefer to reserve these terms for use at the population-level only [10].

2.2 Sex chromosome data

We gathered information about sexual lability for species in which the sex chromosomes have been studied with genomic data. Information about many of these species was recently compiled by Renner and Müller [5], and we added a few corrections and species, notably those for which no chromosome-level assembly was available. We included species studied with transcriptomic data, but results from low-density genetic maps were not used, nor approaches using RADseq or similar data, because in the absence of a well-assembled genome, they do not permit an estimation of the total number of sex-linked genes.

The original studies of sex chromosomes use different methods to calculate the number of genes in the non-recombining region. All methods have performance limits, but almost no study seeks to quantify the false

Table 1: Classification of types of lability in dioecious species. The terms “aberrant flowers” and “aberrant individuals” refer to the deviations from strict dioecy.

Lability type	Sex of aberrant individuals	Sex of aberrant flowers	Frequency of aberrant flowers in aberrant individuals	Temporal variation within an individual’s lifetime
Leakiness	Monoecious	Female or male	Low ($\ll 50\%$, usually $< 10\%$)	Possibly
	Gynomonoecious	Hermaphrodite		
	Andromonoecious	Hermaphrodite		
	Trimonoecious	Female or male plus hermaphrodite		
Trioecy	Monoclinous	Hermaphroditic	(almost) 100%	No (or very limited)
	Monoecious	Female or male	Similar proportions	
Sex change	Female or male (sequentially)	Female or male (sequentially)	(almost) 100%	Yes (by definition)

discovery rate and the miss rate. Furthermore, some rely on well-assembled genomes, others on *de novo* assemblies with more uncertainties; coverage-based methods more easily recover Y- or W-specific genes, while methods using segregating alleles perform better on XY or ZW gametologs. We chose to use point estimates of the numbers of sex-linked genes and the ages of the sex chromosomes, as the variation among species (approximately two orders of magnitude) is much larger than the confidence intervals per species.

2.3 Data on lability

Evidence of departure from strict dioecy was obtained from a review of the literature on reproductive biology of the species selected according to the criteria mentioned above. In some cases, lability has been explicitly reported. In other cases, this information was retrieved from sources documenting taxonomy, sexual system, sex determination mechanisms, teratology in flowers, population sex ratio, or reproductive success within and among reproductive events. We documented all the types of lability reported for each species but not all the references of lability per species. We also documented whether cases of lability were present in other species of the same genus. We have mainly restricted our review to cases of naturally occurring lability, or observations in cultivated plants that were exposed to nearly natural conditions (notably excluding the application of hormones).

Whenever available, the sex of the aberrant flowers was scored, as well as the direction of sex change, the resulting sex of the individuals (monoclinous, monoecious, gynomonoecious or andromonoecious), the frequency of the aberrant individuals, and the viability of the aberrant flowers (whether pollen was viable or viable seeds were produced).

2.4 Statistical analyses

Prior to the analysis, we merged closely related species to avoid phylogenetic biases: *Silene otites* and *S. pseudotites*, the *Populus* species, and the *Salix* species. These species did not differ in observed lability; for the sex chromosome characteristics, the average value was taken into account in the analysis. We used two statistical tests to assess the effect of sex chromosome characteristics on the presence or absence of the different types of lability: Wilcoxon’s rank sum test and binomial regression using a Generalized Linear Model (GLM). For the binomial regression, we used the presence or absence of the three types of lability, and the presence of absence of any kind of lability. There is only a relatively small number of species with well-described sex chromosomes, so we performed a power analysis to test the robustness of our results. Full descriptions of the GLM and the power analysis are provided in Supplementary File 1.

3 Results

Information about the presence or absence of the types of lability is shown in Table 2. No lability has been reported for *Actinidia chinensis*, *Diospyros lotus*, *Salix viminalis* and *S. purpurea*. but there are reports of lability for other species occurring in the same genera: *Actinidia deliciosa* [28], *Diospyros kaki* [29] and *D. egrettarum* [30], and many *Salix* species ([31]; see Supplementary Table 1). Fourteen species show more than one type of lability, and four show all kinds of lability.

In the species studied, the non-recombining regions of the sex chromosomes have between seven (*Fragaria chiloensis*) and 1600 genes (*Cannabis sativa*, *Rumex hastatulus*; Supplementary Table 2), but it should be noted that the highest estimates come from studies using transcriptome data, which likely represent only a proportion

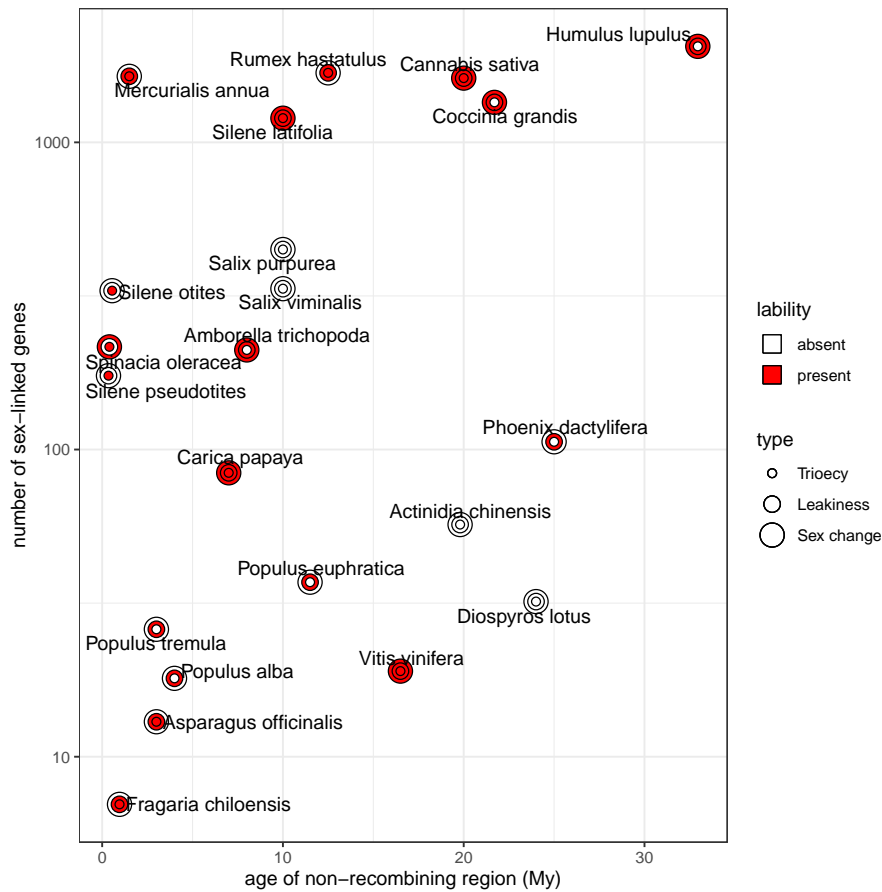


Figure 1: Presence or absence of the different types of sexual lability in species with well-characterized sex chromosomes. For each species, the circles show whether the type of lability has been observed (red) or not (white): small (inner circles) for trioecy, the intermediate circles for leakiness, and the large (outer) circles for sex change.

Table 2: Sexual lability in 22 species with well-described sex chromosomes. The lability types correspond to those summarized in Table 1. Am = andromonoecious, Gm = gynomonoecious, M = monoecious, Tm = trimonoecious. For species with reported leakiness, the leaky sex is indicated in parenthesis: f = female, m = male, b = both sexes. For clarity, species without reported lability are indicated with a dash in all columns. For details, see Supplementary Table 1. This table also includes references to the literature on sex chromosomes.

Species	Sex change	Type of lability		References
		Leakiness	Trioecy	
<i>Actinidia chinensis</i>	-	-	-	[28, 33, 34]
<i>Amborella trichopoda</i>	Yes	Am * (m)		[35, 36]
<i>Asparagus officinalis</i>		Am * (m)	H	[5, 37–39]
<i>Cannabis sativa</i>	Yes	M, Gm (f)	M	[21, 22, 32, 40–42]
<i>Carica papaya</i>	Yes	Am, Tm (m)	H	[5, 43–46]
<i>Coccinia grandis</i>	Yes	Gm (f)		[47, 48]
<i>Diospyros lotus</i>	-	-	-	[5, 30]
<i>Fragaria chiloensis</i>		Am (m)	H	[49–51]
<i>Humulus lupulus</i>	Yes	M (b)		[52–55]
<i>Mercurialis annua</i>		M, Tm (b)	M	[5, 56–58]
<i>Phoenix dactylifera</i>		Am, Gm * (b)		[5, 59]
<i>Populus alba</i>		M (m)		[5, 60]
<i>Populus euphratica</i>		M (f)		[5, 61]
<i>Populus tremula</i>		Am, M (m)		[5, 62, 63]
<i>Rumex hastatulus</i>		Am, Gm (b)	H	[64–66]
<i>Salix purpurea</i>	-	-	-	[31, 67, 68]
<i>Salix viminalis</i>	-	-	-	[31, 67, 69]
<i>Silene latifolia</i>	Yes	Am, M (m)	H	[70–75]
<i>Silene otites</i>			H	[76–78]
<i>Silene pseudotites</i>			H?	[77–79]
<i>Spinacia oleracea</i>	Yes		M	[5, 80–83]
<i>Vitis vinifera</i>	Yes	Am * (m)	H	[84–86]

*: evidence that leaky individuals actually change sexual expression between flowering events, *i.e.* are inconstant in sex expression.

of the genes. The ages of recombination suppression range from 0.25 My (*Silene pseudotites*) to 33 My (*Humulus lupulus*).

As shown in Figure 1, there is no clear correlation between the occurrence of lability and characteristics of the sex chromosomes. Table 3 shows the tests for sex chromosome differences for all types of lability. Only one of these tests was significant at the 5% level: species with younger sex chromosomes more often produce stable bisexual (monoclinous or monoecious) individuals. These results were confirmed by the binomial regression analysis (Supplementary File 1). The additional power analysis shows that we can only expect to find significant results when the strongest change in the probability of occurrence of lability (the so-called “switch”) lies within the range of values (*i.e.*, ages or number of sex-linked genes) that are effectively observed in the dataset. Thus, if this switch occurs before or immediately at the onset of sex chromosome evolution, or after several tens of millions of years of existence of the sex chromosomes, it cannot be discovered using these data. This also indicates that the significant negative correlation between the occurrence of trioecy and sex chromosome age is a robust result, even with the low sample size.

It is difficult to draw conclusions about the frequency of sex change, leakiness, and the occurrence of monoclinous or monoecious individuals within each of the species, as many studies report observations in a qualitative manner, and the quantitative data presented are often not directly comparable. From the few measurements we gathered, one can infer that the frequencies of aberrant individuals and flowers are often low, except in some cases where the plants were grown in extreme conditions. Species with younger sex chromosomes or fewer genes do not consistently have higher frequencies of sex change or leakiness; some of the highest frequencies of lability have been reported for *Cannabis sativa* [22, 32], which has rather large and old sex chromosomes.

Sex change was documented from male to female and vice versa in four species. Leakiness was more frequently reported for males (eight species) than for females (three species) or for both sexes (four species; Table 2).

We illustrate these results by describing two plant species for which detailed studies are available. *Silene latifolia* stands out among angiosperms as it has strikingly dimorphic sex chromosomes. The Y chromosome is larger than the X, and it is considerably degenerated [73, 87], although these chromosomes evolved only approximately 10 million years ago [75]. Sex determination is considered quite stable in *S. latifolia* [88]. Completely monoclinous individuals (trioecy) have received some attention as they arise through deletions of parts of the

Table 3: Summary and tests of the effect of sex chromosome characteristics and sexual lability. Two-sided p -values were calculated using Wilcoxon’s rank sum test.

	Median number of sex-linked genes			Median age of non-recombining region (My)		
	without lability	with lability	p -value	without lability	with lability	p -value
Sex change	81.5	707.5	0.27	8.08	13.3	0.48
Leakiness	216	211	0.78	10.0	10.0	0.58
Trioecy	159	234	1	20.8	5.00	0.021
Overall	57	216	0.58	19.8	10.0	0.26

Y chromosome and allow one to investigate the genetic architecture of dioecy in this species [2, 72, 89, 90]. Leakiness does not seem to be caused by a mutation, at least in one report where it was found not to be heritable [91]. An extensive study of the occurrence of sexual lability has been performed by Frick and Cavers [70], who found both leakiness and sex changes by monitoring plants in common garden experiments and the greenhouse for several flowering seasons.

In *Amborella trichopoda*, sex chromosomes of the ZW type have a small non-recombining region that evolved quite recently (less than 16.5 My ago, most probably around 8 My ago) [36]. A detailed study using cultivated individuals during two flowering seasons showed variable proportions of leakiness in males (thus, inconstancy in the literal sense) and sex change in about 1% of individuals [35]. The individual grown in the Santa Cruz arboretum (California), from which the reference genome is derived [92], has been shown to be genetically female (*i.e.* has both Z and W chromosomes [36]). It is striking that this individual has changed sex, having flowered as mostly male in the past but recently as female. The botanical garden in Bonn also observed sex changes in an *A. trichopoda* plant [93], and although its history is not completely clear, based on the relatedness ϕ statistic [94] of 0.361 calculated on resequencing data [92], this individual should be considered a clone of the Santa Cruz individual. Furthermore, sex changes have been reported in cuttings of the Santa Cruz plant [95].

4 Discussion

We have shown, using a set of species whose sex chromosomes have been studied with genomic data, that sexual lability is only weakly influenced by the age of the sex chromosomes and the number of sex-linked genes. More precisely, sex change and leakiness did not seem to be influenced by the characteristics of the sex chromosomes, while the occurrence of trioecy diminished with the age of the sex chromosomes but not with the number of sex-linked genes. Importantly, in the vast majority of the species included in this analysis, at least some form of lability has been reported.

These results should be considered preliminary as this review is limited by the availability of data. First, we were able to include only a limited number of species, *i.e.* those for which the sex chromosomes were sufficiently well-characterized. We cannot rule out that the size of the non-recombining region or the sex chromosome age affect the occurrence of lability. However, it seems plausible that strong correlations, if they existed, would have been visible in this small sample as well, as shown by the power analysis (Supplementary File 1). We do not know if the species included in this review are a representative sample of all species with sex chromosomes, because such chromosomes have simply not been characterized in the vast majority of dioecious species. In addition, differences in the probability of lability between dioecious species with and without sex chromosomes could not be explored because sex-determining mechanisms are only understood in a small subset of dioecious plants.

Second, the estimates of the number of sex-linked genes or the age of the sex chromosomes depend on the methods used in the primary studies. The estimates that are used in this review will most likely become more precise when more genomic resources will be available. Note however that there is large variation in the current sample, and the rank sum tests presented in Table 3 are, to a certain degree, robust to inaccuracies.

Third, reports on sexual lability are scarce and difficult to compare to one another. As lability typically concerns only a few individuals in a population, observations of many plants, preferably including several flowering seasons, are needed to discover spontaneously occurring sex changes, leakiness, or bisexual individuals, as we have described for *S. latifolia* and *A. trichopoda* [35, 70]. Such studies seem to have been more frequent before 1990 than today, with some notable exceptions. Not all species have received the same amount of attention from researchers, such that the absence of sexual lability in some species is likely to be the effect of the absence of studies. For the species completely lacking reports of sexual lability, we found evidence of lability in closely related species; it seems thus plausible that all of the studied species are capable of some degree of lability. Finally, we could only compare the presence or absence of lability as quantitative information (e.g. fraction of labile individuals) was available for only a subset of species (Supplementary Table 1).

Despite these limitations, our results show that lability can occur in dioecious plants regardless of the characteristics of their sex chromosomes. These findings are contrary to the naive idea that sex chromosomes

represent a stable and irreversible way to fix dioecy [cf 17]. This idea has been largely questioned in recent years, and data in animals show that exceptions to this rule are frequent [e.g. 9]. Whether more subtle effects exist, and whether lability changes in dioecious species before sex chromosomes evolve, remains to be explored but currently the data to test these hypotheses are not available.

We have only limited understanding of the causes of lability. Some aberrant sexual phenotypes have been linked to mutations on the sex chromosomes and can be transmitted to descendants, as is often the case with trioecy (e.g. mutations on the Y chromosome in *Carica papaya* [96], *Silene latifolia* [72, 89] and *Vitis vinifera* [97], or modified X chromosomes in *Cannabis sativa* [98] and *Mercurialis annua* [99]). One could hypothesize that leakiness, which involves only some flowers of a plant, is more often the result of “developmental noise” [10] or of some local action of hormones than the other types of lability that involve whole plants and that might have genetic causes. However, the picture that emerges from this review is that different types of lability often co-occur, and the distinction might thus be partially artificial.

The classical cases of sex change seem to involve plant size or resource status, or maybe external environmental conditions [cf 12, 13]. But the example of *A. trichopoda*, in which cuttings and clones grown in different locations change sex, seems to fit neither of the proposed mechanisms. Furthermore, there could be a role of somatic mutations, leading to an occasional branch of a different sex (leakiness), or even sex change in plants that regenerate frequently, but this hypothesis has only rarely been considered. The study of the causes of lability in species with sex chromosomes will only advance when sex-determining genes start to be described, but this currently is only the case for a handful of species [cf 5]. Sexually labile individuals might help in this enterprise by allowing to identify mutations, differences in gene expression, or epigenetic mechanisms [100]. In the case of trioecy with a genetic basis, monoclinal or monoecious individuals can easily be selected and maintained artificially, as is the case for some crop species (*Cannabis sativa*, *Vitis vinifera*, *Carica papaya*). Leakiness in *Mercurialis annua* has been the subject of intensive studies, that show that it can be selected for when mates (pollen) are limited [101], but is also influenced by the environment [57, 102]. Such experiments combining the effects of population density, mate availability, environmental conditions and inheritance could be performed for other species with sex chromosomes, and will probably show that sex determination is not entirely genetic nor environmental, as several authors have already suggested [e.g. 5, 103].

The wide occurrence of lability in dioecious species suggests that this sexual system is maintained through selection and not because of intrinsic (developmental or genetic) constraints. Indeed, if dioecy was not advantageous, it would be counter-selected and lost, together with the sex chromosomes [cf 101, 104]. For example, if a species went through a short period of monoclinal or monoecy, the sex chromosomes could be lost, and a new pair might evolve when the species transitions back to dioecy. If and when such transitions happen is not known, but such events might be an additional mechanism to consider in the study of sex chromosome turnover [cf 6, 105, 106].

Sex chromosome research is currently benefiting from the “genomic revolution”: technologies for whole-genome sequencing are improving, their costs are diminishing and many tools are available to infer sex-linkage [6, 106, 107]. On the contrary, studies including large-scale screening and long-term monitoring of sexual lability seem to have lost popularity (with some exceptions, notably economically important species), as illustrated by the age of the references we used to extract data from. We suppose this is due to the current research context in which long-term studies with uncertain outcomes are disfavoured. So we invite researchers to consider the reports of sexual lability in a species as an incentive to study it; the most time-consuming part of the work is already done. Of course, it is unlikely that sex chromosomes will be found in species in which sex change is common (e.g. *Arisaema*, Cataglyphidae, *Gurania*, *Panax* [11]) as no genetic dimorphism is expected, but other species seem clearly dimorphic with a substantial, albeit minor, proportion of sex change, such as some *Acer*, *Ilex*, *Solanum* and *Atriplex* species [13].

5 Acknowledgements

We thank Aline Muyle, Helene Badouin and Djivan Prentout for bringing several interesting studies to our attention. Cornelia Löhne (Botanische Gärten der Universität Bonn) and Holly Forbes (University of California Botanical Garden at Berkeley) provided information about cultivated *Amborella* plants. We also thank the participants of the workshop on plant sex chromosome evolution (June 2021) for stimulating discussions, and in particular Susanne Renner and Niels MÄCeller for the organisation and comments on the manuscript. This work benefited from discussions in the “DiveRS” group funded by the synthesis centre CESAB of the French Foundation for Research on Biodiversity (FRB; www.fondationbiodiversite.fr), and we thank Andrew Helmstetter (CESAB) for critically reading the manuscript.

6 Authors' contributions

JK conceived the study, gathered data on sex chromosomes, carried out data analysis, and drafted the manuscript. MM gathered and compiled data on sexual lability, assisted in interpreting the results, and critically revised the manuscript. SM conceived and performed additional statistical tests. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

7 Competing Interests

We have no competing interests.

References

- [1] S. S. Renner. The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. *Am. J. Bot.*, 101(10):1588–1596, October 2014. doi: 10.3732/ajb.1400196.
- [2] M. Westergaard. The mechanism of sex determination in dioecious flowering plants. *Adv. Genet.*, 9: 217–281, 1958.
- [3] R. Ming, A. Bendahmane, and S. S. Renner. Sex chromosomes in land plants. *Annu. Rev. Plant Biol.*, 62:485–514, June 2011. doi: 10.1146/annurev-arplant-042110-103914.
- [4] D. Charlesworth. Plant sex chromosomes. *Annu. Rev. Plant Biol.*, 67:397–420, April 2016. doi: 10.1146/annurev-arplant-043015-111911.
- [5] S. S. Renner and N. A. Müller. Plant sex chromosomes defy evolutionary models of expanding recombination suppression and genetic degeneration. *Nat. Plants.*, 7(4):392–402, April 2021. doi: 10.1038/s41477-021-00884-3.
- [6] A. Muyle, R. Shearn, and G. A. Marais. The evolution of sex chromosomes and dosage compensation in plants. *Genome Biol. Evol.*, 9(3):627–645, March 2017. doi: 10.1093/gbe/evw282.
- [7] D. Charlesworth. When and how do sex-linked regions become sex chromosomes? *Evolution.*, 75(3): 569–581, March 2021. doi: 10.1111/evo.14196.
- [8] S. Ponnikas, H. Sigeman, J. K. Abbott, and B. Hansson. Why do sex chromosomes stop recombining? *Trends Genet.*, 34(7):492–503, July 2018. doi: 10.1016/j.tig.2018.04.001.
- [9] D. Bachtrog, J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto, T. L. Ashman, M. W. Hahn, J. Kitano, I. Mayrose, R. Ming, N. Perrin, L. Ross, N. Valenzuela, and J. C. Vamasi. Sex determination: why so many ways of doing it? *PLoS Biol.*, 12(7):e1001899, July 2014. doi: 10.1371/journal.pbio.1001899.
- [10] Q. Cronk. The distribution of sexual function in the flowering plant: from monoecy to dioecy. *Phil. Trans. R. Soc. B*, in press, 2022.
- [11] D. G. Lloyd and K. S. Bawa. Modification of the gender of seed plants in varying conditions. *Evol. Biol.*, 17:255–338, 1984.
- [12] M. A. Schlessman. Gender diphasy (“sex choice”). In J Lovett Doust and L Lovett Doust, editors, *Plant reproductive ecology: patterns and strategies*, pages 136–153. Oxford University Press, Oxford, 1988.
- [13] D. C. Freeman, K. T. Harper, and E. L. Charnov. Sex change in plants: Old and new observations and new hypotheses. *Oecologia.*, 47(2):222–232, January 1980. doi: 10.1007/BF00346825.
- [14] H. Korpelainen. Labile sex expression in plants. *Biol. Rev.*, 73(2):157–180, May 1998. doi: 10.1017/S0006323197005148.
- [15] D. Charlesworth, B. Charlesworth, and G. Marais. Steps in the evolution of heteromorphic sex chromosomes. *Heredity*, 95(2):118–128, August 2005. doi: 10.1038/sj.hdy.6800697.
- [16] D. L. Jeffries, J. F. Gerchen, M. Scharmann, and J. R. Pannell. A neutral model for the loss of recombination on sex chromosomes. *Phil. Trans. R. Soc. Lond. B Biol. Sci.*, 376(1832):20200096, August 2021. doi: 10.1098/rstb.2020.0096.

- [17] J. J. Bull and E. L. Charnov. On irreversible evolution. *Evolution*, 39(5):1149–1155, 1985 1985. doi: 10.2307/2408742.
- [18] B. Charlesworth and D. Charlesworth. Model for evolution of dioecy and gynodioecy. *Am. Nat.*, 112(988): 975–997, 1978.
- [19] B. K. Ehlers and T. Bataillon. 'Inconstant males' and the maintenance of labile sex expression in subdioecious plants. *New Phyt.*, 174(1):194–211, 2007 2007. doi: 10.1111/j.1469-8137.2007.01975.x.
- [20] R. B. Spigler and T. L. Ashman. Gynodioecy to dioecy: are we there yet? *Ann Bot.*, 0(O):O, August 2011. doi: 10.1093/aob/mcr170.
- [21] F. J. Pritchard. Change of sex in hemp - Mutilation makes female plants of *Cannabis sativa* produce male flowers - Change in nutrition probably responsible for the result. *J. Heredity*, 7:325–329, 1916. doi: 10.1093/oxfordjournals.jhered.a110739.
- [22] J. H. Schaffner. Complete reversal of sex in hemp. *Science*, 50:311–312, JUL-DEC 1919. doi: 10.1126/science.50.1291.311.
- [23] H. G. Baker and P. A. Cox. Further thoughts on dioecism and islands. *Ann. Missouri Bot. Gard.*, 71(1): 244–253, 1984. doi: 10.2307/2399068.
- [24] C. Darwin. *The different forms of flowers on plants of the same species*. John Murray, London, 2 edition, 1884.
- [25] G. G. Cossard and J. R. Pannell. Enhanced leaky sex expression in response to pollen limitation in the dioecious plant *Mercurialis annua*. *J. Evol. Biol.*, 34(2):416–422, February 2021. doi: 10.1111/jeb.13720.
- [26] Mark A. Schlessman, Laura B. Vary, Jérôme Munzinger, and Porter P. Lowry II. Incidence, correlates, and origins of dioecy in the island flora of New Caledonia. *Intl. J. Plant Sci.*, 175(3):pp. 271–286, 2014. ISSN 10585893.
- [27] J. Lovett Doust and P. B. Cavers. Sex and gender dynamics in jack-in-the-pulpit, *Arisaema triphyllum* (Araceae). *Ecology*, 63(3):797–808, 1982. doi: 10.2307/1936800.
- [28] R. Testolin, G. Cipriani, and G. Costa. Sex segregation ratio and gender expression in the genus *Actinidia*. *Sex. Plant Repr.*, 8(3):129–132, May 1995.
- [29] T. Akagi, I. M. Henry, R. Tao, and L. Comai. Plant genetics. A Y-chromosome-encoded small RNA acts as a sex determinant in persimmons. *Science*, 346(6209):646–650, October 2014. doi: 10.1126/science.1257225.
- [30] S. Venkatasamy, G. Khittoo, and S. Keeley. Leaky dioecy in *Diospyros* (Ebenaceae) endemic to the Island of Mauritius. *Plant Ecol.*, 189(1):139–146, March 2007. doi: 10.1007/s11258-006-9171-y.
- [31] P. Mirski. Exceptions from dioecy and sex lability in genus *Salix*. *Dendrobiology*, 71:167, 2014. doi: 10.12657/denbio.071.017.
- [32] J. H. Schaffner. Sex determination and sex differentiation - Sex determination and sex differentiation in the higher plants. *Am. Nat.*, 59:115–127, JAN-FEB 1925. doi: 10.1086/280022.
- [33] T. Akagi, I. M. Henry, H. Ohtani, T. Morimoto, K. Beppu, I. Kataoka, and R. Tao. A Y-Encoded Suppressor of Feminization Arose via Lineage-Specific Duplication of a Cytokinin Response Regulator in Kiwifruit. *Plant Cell*, 30(4):780–795, April 2018. doi: 10.1105/tpc.17.00787.
- [34] T. Akagi, S. M. Pilkington, E. Varkonyi-Gasic, I. M. Henry, S. S. Sugano, M. Sonoda, A. Firl, M. A. McNeillage, M. J. Douglas, T. Wang, R. Rebstock, C. Voogd, P. Datson, A. C. Allan, K. Beppu, I. Kataoka, and R. Tao. Two Y-chromosome-encoded genes determine sex in kiwifruit. *Nat. Plants.*, 5(8):801–809, August 2019. doi: 10.1038/s41477-019-0489-6.
- [35] N. Anger, B. Fogliani, C. P. Scutt, and G. Gateble. Dioecy in *Amborella trichopoda*: evidence for genetically based sex determination and its consequences for inferences of the breeding system in early angiosperms. *Ann. Bot.*, 119(4):591–597, March 2017. doi: 10.1093/aob/mcw278.
- [36] J. Käfer, A. Bewick, A. Andres-Robin, G. Lapetoule, A. Harkess, J. Caius, B. Fogliani, G. Gâteblé, P. Ralph, C. W. DePamphilis, F. Picard, C. Scutt, G. A. B. Marais, and J. Leebens-Mack. A derived ZW chromosome system in *Amborella trichopoda*, representing the sister lineage to all other extant flowering plants. *New Phytol.*, in press, August 2021. doi: 10.1111/nph.17662.

- [37] C. M. Rick and G. C. Hanna. Determination of sex in *Asparagus officinalis* L. *Am. J. Bot.*, 30(9):711–714, November 1943. doi: 10.2307/2437718.
- [38] L. C. Peirce and T. M. Currence. The inheritance of hermaphroditism in *Asparagus officinalis* L. *Proc. Am. Soc. Hortic. Sci.*, 80:368–376, 1962.
- [39] A. A. Franken. Sex characteristics and inheritance of sex in asparagus (*Asparagus officinalis* L.). *Euphytica*, 19(3):277, 1970. doi: 10.1007/BF01904204.
- [40] A. M. Faux, X. Draye, R. Lambert, R. D’Andrimont, P. Raulier, and P. Bertin. The relationship of stem and seed yields to flowering phenology and sex expression in monoecious hemp (*Cannabis sativa* L.). *Eur. J. Agronomy*, 47:11–22, May 2013. doi: 10.1016/j.eja.2013.01.006.
- [41] Z. K. Punja and J. E. Holmes. Hermaphroditism in marijuana (*Cannabis sativa* L.) inflorescences - impact on floral morphology, seed formation, progeny sex ratios, and genetic variation. *Front. Plant Sci.*, 11:718, 2020. doi: 10.3389/fpls.2020.00718.
- [42] D. Prentout, O. Razumova, B. Rhoné, H. Badouin, H. Henri, C. Feng, J. Käfer, G. Karlov, and G. A. B. Marais. An efficient RNA-seq-based segregation analysis identifies the sex chromosomes of *Cannabis sativa*. *Genome Res.*, 30(2):164–172, February 2020. doi: 10.1101/gr.251207.119.
- [43] J. E. Higgins. Growing melons on trees - The papaya an important tropical fruit which offers great opportunities to breeders - Remarkable irregularities in sex - Changing male trees into female. *J. Heredity*, 7:208–220, 1916. doi: 10.1093/oxfordjournals.jhered.a110693.
- [44] P. Allan, J. McChlery, and D. Biggs. Environmental-effects on clonal female and male *Carica papaya* (L) plants. *Scientia Hort.*, 32(3-4):221–232, July 1987. doi: 10.1016/0304-4238(87)90089-6.
- [45] A. Kumar. Feminization of androecious papaya leading to fruit set by ethrel and chlorfloreinol. *Acta Hort.*, 463:251–260, 1998.
- [46] K. J. Niklas and T. E. Marler. *Carica papaya* (Caricaceae): A case study into the effects of domestication on plant vegetative growth and reproduction. *Am. J. Bot.*, 94(6):999–1002, June 2007. doi: 10.3732/ajb.94.6.999.
- [47] A. G. Ghadge, K. Karmakar, R. S. Devani, J. Banerjee, B. Mohanasundaram, R. K. Sinha, S. Sinha, and A. K. Banerjee. Flower development, pollen fertility and sex expression analyses of three sexual phenotypes of *Coccinia grandis*. *BMC Plant Biol.*, 14:1322–1334, November 2014. doi: 10.1186/s12870-014-0325-0.
- [48] C. Fruchard, H. Badouin, D. Latrasse, R. S. Devani, A. Muyle, B. Rhoné, S. S. Renner, A. K. Banerjee, A. Bendahmane, and G. A. B. Marais. Evidence for dosage compensation in *Coccinia grandis*, a plant with a highly heteromorphic XY system. *Genes (Basel)*, 11(7), July 2020. doi: 10.3390/genes11070787.
- [49] J. F. Hancock and R. S. Bringham. Hermaphroditism in predominately dioecious populations of *Fragaria chiloensis* (L) Duchn. *Bull. Torrey Bot. Club*, 106(3):229–231, 1979. doi: 10.2307/2484560.
- [50] M. S. Dillenberger, N. Wei, J. A. Tennessen, T. L. Ashman, and A. Liston. Plastid genomes reveal recurrent formation of allopolyploid *Fragaria*. *Am. J. Bot.*, 105(5):862–874, May 2018. doi: 10.1002/ajb2.1085.
- [51] J. A. Tennessen, N. Wei, S. C. K. Straub, R. Govindarajulu, A. Liston, and T. L. Ashman. Repeated translocation of a gene cassette drives sex-chromosome turnover in strawberries. *PLoS Biol.*, 16(8):e2006062, August 2018. doi: 10.1371/journal.pbio.2006062.
- [52] E. W. Weston. Changes in sex in the hop caused by plant growth substances. *Nature*, 188(4744):81–82, 1960. doi: 10.1038/188081b0.
- [53] H. L. Shephard, J. S. Parker, P. Darby, and C. C. Ainsworth. Sexual development and sex chromosomes in hop. *New Phytol.*, 148(3):397–411, December 2000. doi: 10.1046/j.1469-8137.2000.00771.x.
- [54] S. Skof, A. Cerenak, J. Jakse, B. Bohanec, and B. Javornik. Ploidy and sex expression in monoecious hop (*Humulus lupulus*). *Botany-Botanique*, 90(7):617–626, July 2012. doi: 10.1139/B2012-037.
- [55] D. Prentout, N. Stajner, A. Cerenak, T. Tricou, C. Brochier-Armanet, J. Jakse, J. Käfer, and G. A. B. Marais. Plant genera *Cannabis* and *Humulus* share the same pair of well-differentiated sex chromosomes. *New Phytol.*, 231(4):1599–1611, August 2021. doi: 10.1111/nph.17456.
- [56] C. Yampolsky. Further observations on sex in *Mercurialis annua*. *Am. Nat.*, 54:280–284, 1920. doi: 10.1086/279760.

- [57] G. G. Cossard and J. R. Pannell. A functional decomposition of sex inconstancy in the dioecious, colonizing plant *Mercurialis annua*. *Am. J. Bot.*, 106(5):722–732, May 2019. doi: 10.1002/ajb2.1277.
- [58] D. J. Obbard, S. A. Harris, R. J. Buggs, and J. R. Pannell. Hybridization, polyploidy, and the evolution of sexual systems in *Mercurialis* (Euphorbiaceae). *Evolution*, 60(9):1801–1815, September 2006.
- [59] A. Othmani, M. Collin, A. Sellemi, S. M. Jain, N. Drira, and F. Aberlenc. First reported case of spontaneous hermaphroditism in female date palm (*Phoenix dactylifera* L.), cv ‘alligue’. *J. Hort. Sci. Biotechnol.*, 92:376–388, 2017.
- [60] T. Meehan. Comment on “sexual variation in *Castanea americana*, michx.”. *Proc. Acad. Sci. Nat. Philadelphia*, 32:353–354, 1880.
- [61] A. Petzold, T. Pfeiffer, F. Jansen, P. Eusemann, and M. Schnittler. Sex ratios and clonal growth in dioecious *Populus euphratica* Oliv., Xinjiang Prov., Western China. *Trees Structure and Function*, 27(3): 729–744, June 2013. doi: 10.1007/s00468-012-0828-y.
- [62] H. Hjelmqvist. Studies on the floral morphology and phylogeny of the amentiferae. *Botaniska Not. Supp.*, 2:5–171, 1948.
- [63] M. Fladung, M. Schilbach, H. Hoenicka, B. Kersten, and N. A. Muller. Selfing of a single monoecious *Populus tremula* tree produces viable males, females and “supermales”. *Trees Structure and Function*, 33(3):803–816, June 2019. doi: 10.1007/s00468-019-01817-6.
- [64] B. W. Smith. The mechanism of sex determination in *Rumex hastatulus*. *Genetics*, 48:1265–1288, 1963.
- [65] R. Navajas-Perez, R. De la Herran, G. Lopez Gonzalez, M. Jamilena, R. Lozano, C. Ruiz Rejon, M. Ruiz Rejon, and M. A. Garrido-Ramos. The evolution of reproductive systems and sex-determining mechanisms within rumex (polygonaceae) inferred from nuclear and chloroplastidial sequence data. *Mol. Biol. Evol.*, 22(9):1929–1939, September 2005. doi: 10.1093/molbev/msi186.
- [66] D. Crowson, S. C. H. Barrett, and S. I. Wright. Purifying and positive selection influence patterns of gene loss and gene expression in the evolution of a plant sex chromosome system. *Mol. Biol. Evol.*, 34(5): 1140–1154, May 2017. doi: 10.1093/molbev/msx064.
- [67] Y. J. Zhao, X. Y. Liu, R. Guo, K. R. Hu, Y. Cao, and F. Dai. Comparative genomics and transcriptomics analysis reveals evolution patterns of selection in the Salix phylogeny. *BMC. Genomics.*, 20(1):253, March 2019. doi: 10.1186/s12864-019-5627-z.
- [68] R. Zhou, D. Macaya-Sanz, C. H. Carlson, J. Schmutz, J. W. Jenkins, D. Kudrna, A. Sharma, L. Sandor, S. Shu, K. Barry, G. A. Tuskan, T. Ma, J. Liu, M. Olson, L. B. Smart, and S. P. DiFazio. A willow sex chromosome reveals convergent evolution of complex palindromic repeats. *Genome Biol.*, 21(1):38, February 2020. doi: 10.1186/s13059-020-1952-4.
- [69] P. Almeida, E. Proux-Wera, A. Churcher, L. Soler, J. Dainat, P. Pucholt, J. Nordlund, T. Martin, A. C. Rönnberg-Wästljung, B. Nystedt, S. Berlin, and J. E. Mank. Genome assembly of the basket willow, *Salix viminalis*, reveals earliest stages of sex chromosome expansion. *BMC. Biol.*, 18(1):78, June 2020. doi: 10.1186/s12915-020-00808-1.
- [70] B. Frick and P. B. Cavers. Aberrations in sex expression in *Silene latifolia*. *Am. Nat.*, 134(4):562–573, October 1989. doi: 10.1086/284997.
- [71] B. Janousek, S. R. Grant, and B. Vyskot. Non-transmissibility of the Y chromosome through the female line in androhermaphrodite plants of *Melandrium album*. *Heredity*, 80:576–583, May 1998.
- [72] P. M. Miller and R. V. Kesseli. A sex-chromosome mutation in *Silene latifolia*. *Sex. Plant Reprod.*, 24(3): 211–217, September 2011. doi: 10.1007/s00497-011-0163-2.
- [73] A. S. Papadopoulos, M. Chester, K. Ridout, and D. A. Filatov. Rapid Y degeneration and dosage compensation in plant sex chromosomes. *Proc. Natl. Acad. Sci. U.S.A.*, 112(42):13021–13026, October 2015. doi: 10.1073/pnas.1508454112.
- [74] A. Muyle, J. Käfer, N. Zemp, S. Mousset, F. Picard, and G. A. Marais. SEX-DETECTOR: a probabilistic approach to study sex chromosomes in non-model organisms. *Genome Biol. Evol.*, 8(8):2530–2543, August 2016. doi: 10.1093/gbe/evw172.
- [75] M. Krasovec, M. Chester, K. Ridout, and D. A. Filatov. The mutation rate and the age of the sex chromosomes in *Silene latifolia*. *Curr. Biol.*, 28(11):1832–1838, June 2018. doi: 10.1016/j.cub.2018.04.069.

- [76] L. L. Soldaat, B. Vetter, and S. Klotz. Sex ratio in populations of *Silene otites* in relation to vegetation cover, population size and fungal infection. *J. Veg. Sci.*, 8(5):697–702, November 1997. doi: 10.2307/3237374.
- [77] H. Martin, F. Carpentier, S. Gallina, C. Gode, E. Schmitt, A. Muyle, G. A. B. Marais, and P. Touzet. Evolution of young sex chromosomes in two dioecious sister plant species with distinct sex determination systems. *Genome Biol. Evol.*, 11(2):350–361, February 2019. doi: 10.1093/gbe/evz001.
- [78] V. Balounova, R. Gogela, R. Cegan, P. Cangren, J. Zluvova, J. Safar, V. Kovacova, R. Bergero, R. Hobza, B. Vyskot, B. Oxelman, D. Charlesworth, and B. Janousek. Evolution of sex determination and heterogamety changes in section Otites of the genus *Silene*. *Sci. Rep.*, 9(1):1045, January 2019. doi: 10.1038/s41598-018-37412-x.
- [79] C. Desfeux, S. Maurice, J. P. Henry, B. Lejeune, and P. H. Gouyon. Evolution of reproductive systems in the genus *Silene*. *Proc. Biol. Sci.*, 263(1369):409–414, April 1996.
- [80] M. K. Chailakhyan. Genetic and hormonal-regulation of growth, flowering, and sex expression in plants. *Am. J. Bot.*, 66(6):717–736, 1979. doi: 10.2307/2442417.
- [81] D. C. Freeman and J. J. Vitale. The influence of environment on the sex-ratio and fitness of spinach. *Bot. Gaz.*, 146(1):137–142, 1985. doi: 10.1086/337508.
- [82] J. J. Vitale and D. C. Freeman. Secondary sex characteristics in *Spinacia oleracea* L - quantitative evidence for the existence of at least 3 sexual morphs. *Am. J. Bot.*, 72(7):1061–1066, 1985. doi: 10.2307/2443449.
- [83] N. W. West and E. M. Golenberg. Gender-specific expression of GIBBERELIC ACID INSENSITIVE is critical for unisexual organ initiation in dioecious *Spinacia oleracea*. *New Phytol.*, 217(3):1322–1334, February 2018. doi: 10.1111/nph.14919.
- [84] D. Lewis. The evolution of sex in flowering plants. *Biological Reviews*, 17(1):46–67, January 1942. doi: 10.1111/j.1469-185X.1942.tb00431.x.
- [85] S. S. Negi and H. P. Olmo. Sex conversion in a male *Vitis vinifera* L. by a kinin. *Science*, 152(3729):1624, 1966. doi: 10.1126/science.152.3729.1624.
- [86] M. Massonnet, N. Cochetel, A. Minio, A. M. Vondras, J. Lin, A. Muyle, J. F. Garcia, Y. Zhou, M. Delle-donne, S. Riaz, R. Figueroa-Balderas, B. S. Gaut, and D. Cantu. The genetic basis of sex determination in grapes. *Nat. Commun.*, 11(1):2902, June 2020. doi: 10.1038/s41467-020-16700-z.
- [87] A. Muyle, N. Zemp, C. Deschamps, S. Mousset, A. Widmer, and G. A. Marais. Rapid de novo evolution of X chromosome dosage compensation in *Silene latifolia*, a plant with young sex chromosomes. *PLoS Biol.*, 10(4):e1001308, April 2012. doi: 10.1371/journal.pbio.1001308.
- [88] C Ainsworth. Boys and girls come out to play: The molecular biology of dioecious plants. *Ann. Bot.*, 86(2):211–221, AUG 2000. ISSN 0305-7364. doi: 10.1006/anbo.2000.1201.
- [89] A. Lardon, S. Georgiev, A. Aghmir, G. Le Merrer, and I. Negrutiu. Sexual dimorphism in white campion: complex control of carpel number is revealed by y chromosome deletions. *Genetics*, 151(3):1173–1185, March 1999.
- [90] N. Fujita, C. Torii, K. Ishii, W. Aonuma, Y. Shimizu, Y. Kazama, T. Abe, and S. Kawano. Narrowing down the mapping of plant sex-determination regions using new Y-chromosome-specific markers and heavy-ion beam irradiation-induced Y-deletion mutants in *Silene latifolia*. *G3 (Bethesda)*, 2(2):271–278, February 2012. doi: 10.1534/g3.111.001420.
- [91] P. Veltsos and L. F. Delph. The X chromosome is necessary for ovule production in *Silene latifolia*. *PLoS One*, 14(5):e0217558, 2019. doi: 10.1371/journal.pone.0217558.
- [92] Amborella Genome Project. The *Amborella* genome and the evolution of flowering plants. *Science*, 342(6165):1241089, December 2013. doi: 10.1126/science.1241089.
- [93] B. Grosse-Veldmann, N. Korotkova, B. Reinken, W. Lobin, and W. Barthlott. *Amborella trichopoda* – cultivation of the most ancestral angiosperm in botanic gardens. *Sibbaldia*, 9:143–155, 2011.
- [94] A. Manichaikul, J. C. Mychaleckyj, S. S. Rich, K. Daly, M. Sale, and W. M. Chen. Robust relationship inference in genome-wide association studies. *Bioinformatics*, 26(22):2867–2873, November 2010. doi: 10.1093/bioinformatics/btq559.

- [95] M. Buzgo, P. S. Soltis, and D. E. Soltis. Floral developmental morphology of *Amborella trichopoda* (Amborellaceae). *Int. J. Plant Sci.*, 165(6):925–947, November 2004. doi: 10.1086/424024.
- [96] J. Wang, J. K. Na, Q. Yu, A. R. Gschwend, J. Han, F. Zeng, R. Aryal, R. VanBuren, J. E. Murray, W. Zhang, R. Navajas-Perez, F. A. Feltus, C. Lemke, E. J. Tong, C. Chen, C. M. Wai, R. Singh, M. L. Wang, X. J. Min, M. Alam, D. Charlesworth, P. H. Moore, J. Jiang, A. H. Paterson, and R. Ming. Sequencing papaya X and Yh chromosomes reveals molecular basis of incipient sex chromosome evolution. *Proc. Natl. Acad. Sci. U.S.A.*, 109(34):13710–13715, August 2012. doi: 10.1073/pnas.1207833109.
- [97] H. Badouin, A. Velt, F. Gindraud, T. Flutre, V. Dumas, S. Vautrin, W. Marande, J. Corbi, E. Sallet, J. Ganofsky, S. Santoni, D. Guyot, E. Ricciardelli, K. Jepsen, J. Käfer, H. Berges, E. Duchêne, F. Picard, P. Hugueney, R. Tavares, R. Bacilieri, C. Rustenholz, and G. A. B. Marais. The wild grape genome sequence provides insights into the transition from dioecy to hermaphroditism during grape domestication. *Genome Biol.*, 21(1):223, September 2020. doi: 10.1186/s13059-020-02131-y.
- [98] O. V. Razumova, O. S. Alexandrov, M. G. Divashuk, T. I. Sukhorada, and G. I. Karlov. Molecular cytogenetic analysis of monoecious hemp (*Cannabis sativa* L.) cultivars reveals its karyotype variations and sex chromosomes constitution. *Protoplasma*, 253(3):895–901, May 2016. doi: 10.1007/s00709-015-0851-0.
- [99] J. R. Russell and J. R. Pannell. Sex determination in dioecious *Mercurialis annua* and its close diploid and polyploid relatives. *Heredity (Edinb.)*, 114(3):262–271, March 2015. doi: 10.1038/hdy.2014.95.
- [100] A. Muyle, D. Bachtrog, G. A. B. Marais, and J. M. A. Turner. Epigenetics drive the evolution of sex chromosomes in animals and plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 376(1826):20200124, June 2021. doi: 10.1098/rstb.2020.0124.
- [101] G. G. Cossard, J. F. Gerchen, X. Li, Y. Cuenot, and J. R. Pannell. The rapid dissolution of dioecy by experimental evolution. *Curr. Biol.*, 31(6):1277–1283, March 2021. doi: 10.1016/j.cub.2020.12.028.
- [102] J. S. Sánchez Vilas and J. R. Pannell. Plasticity in sex allocation in the plant *Mercurialis annua* is greater for hermaphrodites sampled from dimorphic than from monomorphic populations. *J. Evol. Biol.*, 27(9):1939–1947, September 2014. doi: 10.1111/jeb.12447.
- [103] E. M. Golenberg and N. W. West. Hormonal interactions and gene regulation can link monoecy and environmental plasticity to the evolution of dioecy in plants. *Am. J. Bot.*, 100(6):1022–1037, June 2013. doi: 10.3732/ajb.1200544.
- [104] J. Käfer, G. A. Marais, and J. R. Pannell. On the rarity of dioecy in flowering plants. *Mol. Ecol.*, 26(5):1225–1241, March 2017. doi: 10.1111/mec.14020.
- [105] G. S. Van Doorn. Evolutionary transitions between sex-determining mechanisms: a review of theory. *Sex. Dev.*, 8(1-3):7–19, 2014. doi: 10.1159/000357023.
- [106] D. H. Palmer, T. F. Rogers, R. Dean, and A. E. Wright. How to identify sex chromosomes and their turnover. *Mol. Ecol.*, 28(21):4709–4724, November 2019. doi: 10.1111/mec.15245.
- [107] M. Tomaszewicz, P. Medvedev, and K. D. Makova. Y and W chromosome assemblies: Approaches and discoveries. *Trends Genet.*, 33(4):266–282, April 2017. doi: 10.1016/j.tig.2017.01.008.