



HAL
open science

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

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

53 As can be inferred from previous reviews [11–14], variation in sex expression most often concerns a few
54 individuals in a population or a few flowers on a plant. Such variation might be fixed (i.e. some individuals
55 have different patterns of sex expression than the majority) or variable (different patterns of sex expression occur
56 every now and then). In both cases, the plants are able to produce flowers of the sex they normally don't produce.
57 This lability has sometimes been considered a sign for weak genetic differentiation between the sexes [e.g. 5].
58 Indeed, the main hypothesis for the expansion of sex-linked non-recombining regions has been the accumulation
59 of genes with sex-antagonistic effects [15] [but see 16]. Such genes are thought to increase specialization in one
60 of the sexual functions (female or male) and to confer less fitness, or even sterility, to intermediate phenotypes.
61 Thus, well-differentiated sex chromosomes have sometimes been regarded as an example of irreversible evolution
62 [17] or “evolutionary traps” [9]. The existence of deviations from the purely female and purely male individuals
63 is sometimes classed as “subdioecy”, a transitory state between hermaphroditism (monocliny) and “full dioecy”
64 [e.g. 18–20].

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

71 2 Methods

72 2.1 Terminology and classification of sexual lability

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

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

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

98 2.2 Sex chromosome data

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

105 The original studies of sex chromosomes use different methods to calculate the number of genes in the
106 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)

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

112 2.3 Data on lability

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

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

125 2.4 Statistical analyses

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

135 3 Results

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

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

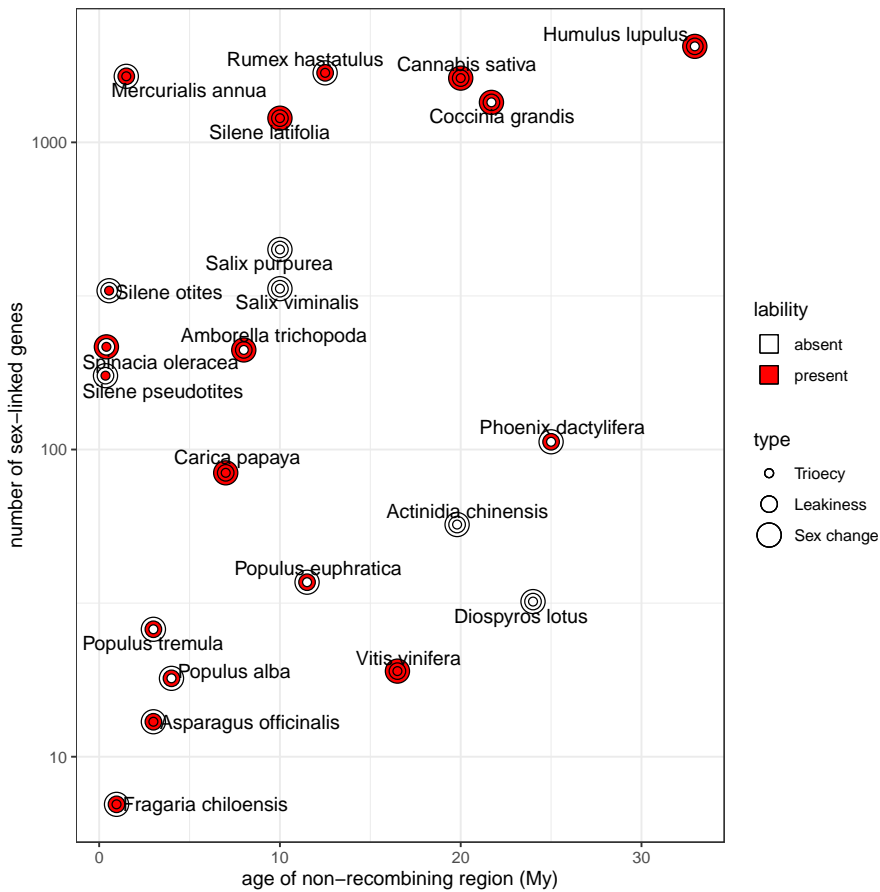


Figure 1: Presence or absence of the different types of sexual lability in species with well-characterized sex chromosomes. For each species, the circles show whether the type of lability has been observed (red) or not (white): small (inner circles) for trioecy, the intermediate circles for leakiness,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

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

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

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

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

166 We illustrate these results by describing two plant species for which detailed studies are available. *Silene*
167 *latifolia* stands out among angiosperms as it has strikingly dimorphic sex chromosomes. The Y chromosome is
168 larger than the X, and it is considerably degenerated [73, 87], although these chromosomes evolved only approx-
169 imately 10 million years ago [75]. Sex determination is considered quite stable in *S. latifolia* [88]. Completely
170 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

171 Y chromosome and allow one to investigate the genetic architecture of dioecy in this species [2, 72, 89, 90].
 172 Leakiness does not seem to be caused by a mutation, at least in one report where it was found not to be
 173 heritable [91]. An extensive study of the occurrence of sexual lability has been performed by Frick and Cavers
 174 [70], who found both leakiness and sex changes by monitoring plants in common garden experiments and the
 175 greenhouse for several flowering seasons.

176 In *Amborella trichopoda*, sex chromosomes of the ZW type have a small non-recombining region that evolved
 177 quite recently (less than 16.5 My ago, most probably around 8 My ago) [36]. A detailed study using cultivated
 178 individuals during two flowering seasons showed variable proportions of leakiness in males (thus, inconstancy
 179 in the literal sense) and sex change in about 1% of individuals [35]. The individual grown in the Santa Cruz
 180 arboretum (California), from which the reference genome is derived [92], has been shown to be genetically female
 181 (*i.e.* has both Z and W chromosomes [36]). It is striking that this individual has changed sex, having flowered
 182 as mostly male in the past but recently as female. The botanical garden in Bonn also observed sex changes in
 183 an *A. trichopoda* plant [93], and although its history is not completely clear, based on the relatedness ϕ statistic
 184 [94] of 0.361 calculated on resequencing data [92], this individual should be considered a clone of the Santa Cruz
 185 individual. Furthermore, sex changes have been reported in cuttings of the Santa Cruz plant [95].

186 4 Discussion

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

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

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

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

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

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

223 We have only limited understanding of the causes of lability. Some aberrant sexual phenotypes have been
224 linked to mutations on the sex chromosomes and can be transmitted to descendants, as is often the case with
225 trioecy (e.g. mutations on the Y chromosome in *Carica papaya* [96], *Silene latifolia* [72, 89] and *Vitis vinifera*
226 [97], or modified X chromosomes in *Cannabis sativa* [98] and *Mercurialis annua* [99]). One could hypothesize
227 that leakiness, which involves only some flowers of a plant, is more often the result of “developmental noise” [10]
228 or of some local action of hormones than the other types of lability that involve whole plants and that might
229 have genetic causes. However, the picture that emerges from this review is that different types of lability often
230 co-occur, and the distinction might thus be partially artificial.

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

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

253 Sex chromosome research is currently benefiting from the “genomic revolution”: technologies for whole-
254 genome sequencing are improving, their costs are diminishing and many tools are available to infer sex-linkage
255 [6, 106, 107]. On the contrary, studies including large-scale screening and long-term monitoring of sexual lability
256 seem to have lost popularity (with some exceptions, notably economically important species), as illustrated by
257 the age of the references we used to extract data from. We suppose this is due to the current research context
258 in which long-term studies with uncertain outcomes are disfavoured. So we invite researchers to consider the
259 reports of sexual lability in a species as an incentive to study it; the most time-consuming part of the work is
260 already done. Of course, it is unlikely that sex chromosomes will be found in species in which sex change is
261 common (e.g. *Arisaema*, *Catasetinae*, *Gurania*, *Panax* [11]) as no genetic dimorphism is expected, but other
262 species seem clearly dimorphic with a substantial, albeit minor, proportion of sex change, such as some *Acer*,
263 *Ilex*, *Solanum* and *Atriplex* species [13].

264 5 Acknowledgements

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

273 6 Authors' contributions

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

278 7 Competing Interests

279 We have no competing interests.

280 References

- 281 [1] S. S. Renner. The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy,
282 gynodioecy, and an updated online database. *Am. J. Bot.*, 101(10):1588–1596, October 2014. doi:
283 10.3732/ajb.1400196.
- 284 [2] M. Westergaard. The mechanism of sex determination in dioecious flowering plants. *Adv. Genet.*, 9:
285 217–281, 1958.
- 286 [3] R. Ming, A. Bendahmane, and S. S. Renner. Sex chromosomes in land plants. *Annu. Rev. Plant Biol.*,
287 62:485–514, June 2011. doi: 10.1146/annurev-arplant-042110-103914.
- 288 [4] D. Charlesworth. Plant sex chromosomes. *Annu. Rev. Plant Biol.*, 67:397–420, April 2016. doi:
289 10.1146/annurev-arplant-043015-111911.
- 290 [5] S. S. Renner and N. A. Müller. Plant sex chromosomes defy evolutionary models of expanding recombina-
291 tion suppression and genetic degeneration. *Nat. Plants.*, 7(4):392–402, April 2021. doi: 10.1038/s41477-
292 021-00884-3.
- 293 [6] A. Muyle, R. Shearn, and G. A. Marais. The evolution of sex chromosomes and dosage compensation in
294 plants. *Genome Biol. Evol.*, 9(3):627–645, March 2017. doi: 10.1093/gbe/evw282.
- 295 [7] D. Charlesworth. When and how do sex-linked regions become sex chromosomes? *Evolution.*, 75(3):
296 569–581, March 2021. doi: 10.1111/evo.14196.
- 297 [8] S. Ponnikas, H. Sigeman, J. K. Abbott, and B. Hansson. Why do sex chromosomes stop recombining?
298 *Trends Genet.*, 34(7):492–503, July 2018. doi: 10.1016/j.tig.2018.04.001.
- 299 [9] D. Bachtrog, J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto, T. L. Ashman, M. W. Hahn, J. Kitano,
300 I. Mayrose, R. Ming, N. Perrin, L. Ross, N. Valenzuela, and J. C. Vamosi. Sex determination: why so
301 many ways of doing it? *PLoS Biol.*, 12(7):e1001899, July 2014. doi: 10.1371/journal.pbio.1001899.
- 302 [10] Q. Cronk. The distribution of sexual function in the flowering plant: from monoecy to dioecy. *Phil. Trans.*
303 *R. Soc. B*, in press, 2022.
- 304 [11] D. G. Lloyd and K. S. Bawa. Modification of the gender of seed plants in varying conditions. *Evol. Biol.*,
305 17:255–338, 1984.
- 306 [12] M. A. Schlessman. Gender diphasy (“sex choice”). In J Lovett Doust and L Lovett Doust, editors, *Plant*
307 *reproductive ecology: patterns and strategies*, pages 136–153. Oxford University Press, Oxford, 1988.
- 308 [13] D. C. Freeman, K. T. Harper, and E. L. Charnov. Sex change in plants: Old and new observations and
309 new hypotheses. *Oecologia.*, 47(2):222–232, January 1980. doi: 10.1007/BF00346825.
- 310 [14] H. Korpelainen. Labile sex expression in plants. *Biol. Rev.*, 73(2):157–180, May 1998. doi:
311 10.1017/S0006323197005148.
- 312 [15] D. Charlesworth, B. Charlesworth, and G. Marais. Steps in the evolution of heteromorphic sex chromo-
313 somes. *Heredity*, 95(2):118–128, August 2005. doi: 10.1038/sj.hdy.6800697.
- 314 [16] D. L. Jeffries, J. F. Gerchen, M. Scharmann, and J. R. Pannell. A neutral model for the loss of recom-
315 bination on sex chromosomes. *Phil. Trans. R. Soc. Lond. B Biol. Sci.*, 376(1832):20200096, August 2021.
316 doi: 10.1098/rstb.2020.0096.

- 317 [17] J. J. Bull and E. L. Charnov. On irreversible evolution. *Evolution*, 39(5):1149–1155, 1985 1985. doi:
318 10.2307/2408742.
- 319 [18] B. Charlesworth and D. Charlesworth. Model for evolution of dioecy and gynodioecy. *Am. Nat.*, 112(988):
320 975–997, 1978.
- 321 [19] B. K. Ehlers and T. Bataillon. 'Inconstant males' and the maintenance of labile sex expression in subdi-
322 oecious plants. *New Phyt.*, 174(1):194–211, 2007 2007. doi: 10.1111/j.1469-8137.2007.01975.x.
- 323 [20] R. B. Spigler and T. L. Ashman. Gynodioecy to dioecy: are we there yet? *Ann Bot.*, 0(O):O, August
324 2011. doi: 10.1093/aob/mcr170.
- 325 [21] F. J. Pritchard. Change of sex in hemp - Mutilation makes female plants of *Cannabis sativa* produce
326 male flowers - Change in nutrition probably responsible for the result. *J. Heredity*, 7:325–329, 1916. doi:
327 10.1093/oxfordjournals.jhered.a110739.
- 328 [22] J. H. Schaffner. Complete reversal of sex in hemp. *Science*, 50:311–312, JUL-DEC 1919. doi: 10.1126/sci-
329 ence.50.1291.311.
- 330 [23] H. G. Baker and P. A. Cox. Further thoughts on dioecism and islands. *Ann. Missouri Bot. Gard.*, 71(1):
331 244–253, 1984. doi: 10.2307/2399068.
- 332 [24] C. Darwin. *The different forms of flowers on plants of the same species*. John Murray, London, 2 edition,
333 1884.
- 334 [25] G. G. Cossard and J. R. Pannell. Enhanced leaky sex expression in response to pollen limitation in the
335 dioecious plant *Mercurialis annua*. *J. Evol. Biol.*, 34(2):416–422, February 2021. doi: 10.1111/jeb.13720.
- 336 [26] Mark A. Schlessman, Laura B. Vary, Jérôme Munzinger, and Porter P. Lowry II. Incidence, correlates,
337 and origins of dioecy in the island flora of New Caledonia. *Intl. J. Plant Sci.*, 175(3):pp. 271–286, 2014.
338 ISSN 10585893.
- 339 [27] J. Lovett Doust and P. B. Cavers. Sex and gender dynamics in jack-in-the-pulpit, *Arisaema triphyllum*
340 (Araceae). *Ecology*, 63(3):797–808, 1982. doi: 10.2307/1936800.
- 341 [28] R. Testolin, G. Cipriani, and G. Costa. Sex segregation ratio and gender expression in the genus *Actinidia*.
342 *Sex. Plant Repr.*, 8(3):129–132, May 1995.
- 343 [29] T. Akagi, I. M. Henry, R. Tao, and L. Comai. Plant genetics. A Y-chromosome-encoded small RNA
344 acts as a sex determinant in persimmons. *Science*, 346(6209):646–650, October 2014. doi: 10.1126/sci-
345 ence.1257225.
- 346 [30] S. Venkatasamy, G. Khittoo, and S. Keeley. Leaky dioecy in *Diospyros* (Ebenaceae) endemic to the Island
347 of Mauritius. *Plant Ecol.*, 189(1):139–146, March 2007. doi: 10.1007/s11258-006-9171-y.
- 348 [31] P. Mirski. Exceptions from dioecy and sex lability in genus *Salix*. *Dendrobiology*, 71:167, 2014. doi:
349 10.12657/denbio.071.017.
- 350 [32] J. H. Schaffner. Sex determination and sex differentiation - Sex determination and sex differentiation in
351 the higher plants. *Am. Nat.*, 59:115–127, JAN-FEB 1925. doi: 10.1086/280022.
- 352 [33] T. Akagi, I. M. Henry, H. Ohtani, T. Morimoto, K. Beppu, I. Kataoka, and R. Tao. A Y-Encoded
353 Suppressor of Feminization Arose via Lineage-Specific Duplication of a Cytokinin Response Regulator in
354 Kiwifruit. *Plant Cell*, 30(4):780–795, April 2018. doi: 10.1105/tpc.17.00787.
- 355 [34] T. Akagi, S. M. Pilkington, E. Varkonyi-Gasic, I. M. Henry, S. S. Sugano, M. Sonoda, A. Firl, M. A.
356 McNeilage, M. J. Douglas, T. Wang, R. Rebstock, C. Voogd, P. Datson, A. C. Allan, K. Beppu, I. Kataoka,
357 and R. Tao. Two Y-chromosome-encoded genes determine sex in kiwifruit. *Nat. Plants.*, 5(8):801–809,
358 August 2019. doi: 10.1038/s41477-019-0489-6.
- 359 [35] N. Anger, B. Fogliani, C. P. Scutt, and G. Gateble. Dioecy in *Amborella trichopoda*: evidence for ge-
360 netically based sex determination and its consequences for inferences of the breeding system in early
361 angiosperms. *Ann. Bot.*, 119(4):591–597, March 2017. doi: 10.1093/aob/mcw278.
- 362 [36] J. Käfer, A. Bewick, A. Andres-Robin, G. Lapetoule, A. Harkess, J. Caius, B. Fogliani, G. Gâteblé,
363 P. Ralph, C. W. DePamphilis, F. Picard, C. Scutt, G. A. B. Marais, and J. Leebens-Mack. A derived ZW
364 chromosome system in *Amborella trichopoda*, representing the sister lineage to all other extant flowering
365 plants. *New Phytol.*, in press, August 2021. doi: 10.1111/nph.17662.

- 366 [37] C. M. Rick and G. C. Hanna. Determination of sex in *Asparagus officinalis* L. *Am. J. Bot.*, 30(9):711–714,
367 November 1943. doi: 10.2307/2437718.
- 368 [38] L. C. Peirce and T. M. Currence. The inheritance of hermaphroditism in *Asparagus officinalis* L. *Proc.*
369 *Am. Soc. Hortic. Sci.*, 80:368–376, 1962.
- 370 [39] A. A. Franken. Sex characteristics and inheritance of sex in asparagus (*Asparagus officinalis* L.). *Euphytica*,
371 19(3):277, 1970. doi: 10.1007/BF01904204.
- 372 [40] A. M. Faux, X. Draye, R. Lambert, R. D’Andrimont, P. Raulier, and P. Bertin. The relationship of stem
373 and seed yields to flowering phenology and sex expression in monoecious hemp (*Cannabis sativa* L.). *Eur.*
374 *J. Agronomy*, 47:11–22, May 2013. doi: 10.1016/j.eja.2013.01.006.
- 375 [41] Z. K. Punja and J. E. Holmes. Hermaphroditism in marijuana (*Cannabis sativa* L.) inflorescences - impact
376 on floral morphology, seed formation, progeny sex ratios, and genetic variation. *Front. Plant Sci.*, 11:718,
377 2020. doi: 10.3389/fpls.2020.00718.
- 378 [42] D. Prentout, O. Razumova, B. Rhoné, H. Badouin, H. Henri, C. Feng, J. Käfer, G. Karlov, and G. A. B.
379 Marais. An efficient RNA-seq-based segregation analysis identifies the sex chromosomes of *Cannabis*
380 *sativa*. *Genome Res.*, 30(2):164–172, February 2020. doi: 10.1101/gr.251207.119.
- 381 [43] J. E. Higgins. Growing melons on trees - The papaya an important tropical fruit which offers great
382 opportunities to breeders - Remarkable irregularities in sex - Changing male trees into female. *J. Heredity*,
383 7:208–220, 1916. doi: 10.1093/oxfordjournals.jhered.a110693.
- 384 [44] P. Allan, J. McChlery, and D. Biggs. Environmental-effects on clonal female and male *Carica papaya* (L)
385 plants. *Scientia Hort.*, 32(3-4):221–232, July 1987. doi: 10.1016/0304-4238(87)90089-6.
- 386 [45] A. Kumar. Feminization of androecious papaya leading to fruit set by ethrel and chlorflurenol. *Acta Hort.*,
387 463:251–260, 1998.
- 388 [46] K. J. Niklas and T. E. Marler. *Carica papaya* (Caricaceae): A case study into the effects of domest-
389 ication on plant vegetative growth and reproduction. *Am. J. Bot.*, 94(6):999–1002, June 2007. doi:
390 10.3732/ajb.94.6.999.
- 391 [47] A. G. Ghadge, K. Karmakar, R. S. Devani, J. Banerjee, B. Mohanasundaram, R. K. Sinha, S. Sinha, and
392 A. K. Banerjee. Flower development, pollen fertility and sex expression analyses of three sexual phenotypes
393 of *Coccinia grandis*. *BMC Plant Biol.*, 14:1322–1334, November 2014. doi: 10.1186/s12870-014-0325-0.
- 394 [48] C. Fruchard, H. Badouin, D. Latrasse, R. S. Devani, A. Muyle, B. Rhoné, S. S. Renner, A. K. Banerjee,
395 A. Bendahmane, and G. A. B. Marais. Evidence for dosage compensation in *Coccinia grandis*, a plant
396 with a highly heteromorphic XY system. *Genes (Basel)*, 11(7), July 2020. doi: 10.3390/genes11070787.
- 397 [49] J. F. Hancock and R. S. Bringham. Hermaphroditism in predominately dioecious populations of *Fragaria*
398 *chiloensis* (L) Duchn. *Bull. Torrey Bot. Club*, 106(3):229–231, 1979. doi: 10.2307/2484560.
- 399 [50] M. S. Dillenberger, N. Wei, J. A. Tennesen, T. L. Ashman, and A. Liston. Plastid genomes reveal recurrent
400 formation of allopolyploid *Fragaria*. *Am. J. Bot.*, 105(5):862–874, May 2018. doi: 10.1002/ajb2.1085.
- 401 [51] J. A. Tennesen, N. Wei, S. C. K. Straub, R. Govindarajulu, A. Liston, and T. L. Ashman. Repeated
402 translocation of a gene cassette drives sex-chromosome turnover in strawberries. *PLoS. Biol.*, 16(8):
403 e2006062, August 2018. doi: 10.1371/journal.pbio.2006062.
- 404 [52] E. W. Weston. Changes in sex in the hop caused by plant growth substances. *Nature*, 188(4744):81–82,
405 1960. doi: 10.1038/188081b0.
- 406 [53] H. L. Shephard, J. S. Parker, P. Darby, and C. C. Ainsworth. Sexual development and sex chromosomes
407 in hop. *New Phytol.*, 148(3):397–411, December 2000. doi: 10.1046/j.1469-8137.2000.00771.x.
- 408 [54] S. Skof, A. Cerenak, J. Jakse, B. Bohanec, and B. Javornik. Ploidy and sex expression in monoecious hop
409 (*Humulus lupulus*). *Botany-Botanique*, 90(7):617–626, July 2012. doi: 10.1139/B2012-037.
- 410 [55] D. Prentout, N. Stajner, A. Cerenak, T. Tricou, C. Brochier-Armanet, J. Jakse, J. Käfer, and G. A. B.
411 Marais. Plant genera *Cannabis* and *Humulus* share the same pair of well-differentiated sex chromosomes.
412 *New Phytol.*, 231(4):1599–1611, August 2021. doi: 10.1111/nph.17456.
- 413 [56] C. Yampolsky. Further observations on sex in *Mercurialis annua*. *Am. Nat.*, 54:280–284, 1920. doi:
414 10.1086/279760.

- 415 [57] G. G. Cossard and J. R. Pannell. A functional decomposition of sex inconstancy in the dioecious, colonizing
416 plant *Mercurialis annua*. *Am. J. Bot.*, 106(5):722–732, May 2019. doi: 10.1002/ajb2.1277.
- 417 [58] D. J. Obbard, S. A. Harris, R. J. Buggs, and J. R. Pannell. Hybridization, polyploidy, and the evolution
418 of sexual systems in *Mercurialis* (Euphorbiaceae). *Evolution*, 60(9):1801–1815, September 2006.
- 419 [59] A. Othmani, M. Collin, A. Sellemi, S. M. Jain, N. Drira, and F. Aberlenc. First reported case of spontan-
420 eous hermaphroditism in female date palm (*Phoenix dactylifera* L.), cv 'alligue'. *J. Hort. Sci. Biotechnol.*,
421 92:376–388, 2017.
- 422 [60] T Meehan. Comment on “sexual variation in *Castanea americana*, michx.”. *Proc. Acad. Sci. Nat. Phil-*
423 *adelphia*, 32:353–354, 1880.
- 424 [61] A. Petzold, T. Pfeiffer, F. Jansen, P. Eusemann, and M. Schnittler. Sex ratios and clonal growth in
425 dioecious *Populus euphratica* Oliv., Xinjiang Prov., Western China. *Trees Structure and Function*, 27(3):
426 729–744, June 2013. doi: 10.1007/s00468-012-0828-y.
- 427 [62] H. Hjelmqvist. Studies on the floral morphology and phylogeny of the amentiferae. *Botaniska Not. Supp.*,
428 2:5–171, 1948.
- 429 [63] M. Fladung, M. Schilbach, H. Hoenicka, B. Kersten, and N. A. Muller. Selfing of a single monoecious
430 *Populus tremula* tree produces viable males, females and “supermales”. *Trees Structure and Function*, 33
431 (3):803–816, June 2019. doi: 10.1007/s00468-019-01817-6.
- 432 [64] B. W. Smith. The mechanism of sex determination in *Rumex hastatulus*. *Genetics*, 48:1265–1288, 1963.
- 433 [65] R. Navajas-Perez, R. De la Herran, G. Lopez Gonzalez, M. JAMILENA, R. Lozano, C. Ruiz Rejon,
434 M. Ruiz Rejon, and M. A. Garrido-Ramos. The evolution of reproductive systems and sex-determining
435 mechanisms within rumex (polygonaceae) inferred from nuclear and chloroplastidial sequence data. *Mol.*
436 *Biol. Evol.*, 22(9):1929–1939, September 2005. doi: 10.1093/molbev/msi186.
- 437 [66] D. Crowson, S. C. H. Barrett, and S. I. Wright. Purifying and positive selection influence patterns of
438 gene loss and gene expression in the evolution of a plant sex chromosome system. *Mol. Biol. Evol.*, 34(5):
439 1140–1154, May 2017. doi: 10.1093/molbev/msx064.
- 440 [67] Y. J. Zhao, X. Y. Liu, R. Guo, K. R. Hu, Y. Cao, and F. Dai. Comparative genomics and transcriptomics
441 analysis reveals evolution patterns of selection in the Salix phylogeny. *BMC. Genomics.*, 20(1):253, March
442 2019. doi: 10.1186/s12864-019-5627-z.
- 443 [68] R. Zhou, D. Macaya-Sanz, C. H. Carlson, J. Schmutz, J. W. Jenkins, D. Kudrna, A. Sharma, L. Sandor,
444 S. Shu, K. Barry, G. A. Tuskan, T. Ma, J. Liu, M. Olson, L. B. Smart, and S. P. DiFazio. A willow
445 sex chromosome reveals convergent evolution of complex palindromic repeats. *Genome Biol.*, 21(1):38,
446 February 2020. doi: 10.1186/s13059-020-1952-4.
- 447 [69] P. Almeida, E. Proux-Wera, A. Churcher, L. Soler, J. Dainat, P. Pucholt, J. Nordlund, T. Martin, A. C.
448 Rönnberg-Wästljung, B. Nystedt, S. Berlin, and J. E. Mank. Genome assembly of the basket willow, *Salix*
449 *viminialis*, reveals earliest stages of sex chromosome expansion. *BMC. Biol.*, 18(1):78, June 2020. doi:
450 10.1186/s12915-020-00808-1.
- 451 [70] B. Frick and P. B. Cavers. Aberrations in sex expression in *Silene latifolia*. *Am. Nat.*, 134(4):562–573,
452 October 1989. doi: 10.1086/284997.
- 453 [71] B. Janousek, S. R. Grant, and B. Vyskot. Non-transmissibility of the Y chromosome through the female
454 line in androhermaphrodite plants of *Melandrium album*. *Heredity*, 80:576–583, May 1998.
- 455 [72] P. M. Miller and R. V. Kesseli. A sex-chromosome mutation in *Silene latifolia*. *Sex. Plant Reprod.*, 24(3):
456 211–217, September 2011. doi: 10.1007/s00497-011-0163-2.
- 457 [73] A. S. Papadopoulos, M. Chester, K. Ridout, and D. A. Filatov. Rapid Y degeneration and dosage com-
458 pensation in plant sex chromosomes. *Proc. Natl. Acad. Sci. U.S.A.*, 112(42):13021–13026, October 2015.
459 doi: 10.1073/pnas.1508454112.
- 460 [74] A. Muyle, J. Käfer, N. Zemp, S. Mousset, F. Picard, and G. A. Marais. SEX-DETECTOR: a probabilistic
461 approach to study sex chromosomes in non-model organisms. *Genome Biol. Evol.*, 8(8):2530–2543, August
462 2016. doi: 10.1093/gbe/evw172.
- 463 [75] M. Krasovec, M. Chester, K. Ridout, and D. A. Filatov. The mutation rate and the age of the sex
464 chromosomes in *Silene latifolia*. *Curr. Biol.*, 28(11):1832–1838, June 2018. doi: 10.1016/j.cub.2018.04.069.

- 465 [76] L. L. Soldaat, B. Vetter, and S. Klotz. Sex ratio in populations of *Silene otites* in relation to ve-
466 getation cover, population size and fungal infection. *J. Veg. Sci.*, 8(5):697–702, November 1997. doi:
467 10.2307/3237374.
- 468 [77] H. Martin, F. Carpentier, S. Gallina, C. Gode, E. Schmitt, A. Muyle, G. A. B. Marais, and P. Touzet.
469 Evolution of young sex chromosomes in two dioecious sister plant species with distinct sex determination
470 systems. *Genome Biol. Evol.*, 11(2):350–361, February 2019. doi: 10.1093/gbe/evz001.
- 471 [78] V. Balounova, R. Gogela, R. Cegan, P. Cangren, J. Zluvova, J. Safar, V. Kovacova, R. Bergero, R. Hobza,
472 B. Vyskot, B. Oxelman, D. Charlesworth, and B. Janousek. Evolution of sex determination and het-
473 erogamety changes in section Otites of the genus *Silene*. *Sci. Rep.*, 9(1):1045, January 2019. doi:
474 10.1038/s41598-018-37412-x.
- 475 [79] C. Desfeux, S. Maurice, J. P. Henry, B. Lejeune, and P. H. Gouyon. Evolution of reproductive systems in
476 the genus *Silene*. *Proc. Biol. Sci.*, 263(1369):409–414, April 1996.
- 477 [80] M. K. Chailakhyan. Genetic and hormonal-regulation of growth, flowering, and sex expression in plants.
478 *Am. J. Bot.*, 66(6):717–736, 1979. doi: 10.2307/2442417.
- 479 [81] D. C. Freeman and J. J. Vitale. The influence of environment on the sex-ratio and fitness of spinach. *Bot.*
480 *Gaz.*, 146(1):137–142, 1985. doi: 10.1086/337508.
- 481 [82] J. J. Vitale and D. C. Freeman. Secondary sex characteristics in *Spinacia oleracea* L - quantitative evidence
482 for the existence of at least 3 sexual morphs. *Am. J. Bot.*, 72(7):1061–1066, 1985. doi: 10.2307/2443449.
- 483 [83] N. W. West and E. M. Golenberg. Gender-specific expression of GIBBERELIC ACID INSENSITIVE
484 is critical for unisexual organ initiation in dioecious *Spinacia oleracea*. *New Phytol.*, 217(3):1322–1334,
485 February 2018. doi: 10.1111/nph.14919.
- 486 [84] D. Lewis. The evolution of sex in flowering plants. *Biological Reviews*, 17(1):46–67, January 1942. doi:
487 10.1111/j.1469-185X.1942.tb00431.x.
- 488 [85] S. S. Negi and H. P. Olmo. Sex conversion in a male *Vitis vinifera* L. by a kinin. *Science*, 152(3729):1624,
489 1966. doi: 10.1126/science.152.3729.1624.
- 490 [86] M. Massonnet, N. Cochetel, A. Minio, A. M. Vondras, J. Lin, A. Muyle, J. F. Garcia, Y. Zhou, M. Delle-
491 donne, S. Riaz, R. Figueroa-Balderas, B. S. Gaut, and D. Cantu. The genetic basis of sex determination
492 in grapes. *Nat. Commun.*, 11(1):2902, June 2020. doi: 10.1038/s41467-020-16700-z.
- 493 [87] A. Muyle, N. Zemp, C. Deschamps, S. Mousset, A. Widmer, and G. A. Marais. Rapid de novo evolution
494 of X chromosome dosage compensation in *Silene latifolia*, a plant with young sex chromosomes. *PLoS*
495 *Biol.*, 10(4):e1001308, April 2012. doi: 10.1371/journal.pbio.1001308.
- 496 [88] C Ainsworth. Boys and girls come out to play: The molecular biology of dioecious plants. *Ann. Bot.*, 86
497 (2):211–221, AUG 2000. ISSN 0305-7364. doi: 10.1006/anbo.2000.1201.
- 498 [89] A. Lardon, S. Georgiev, A. Aghmir, G. Le Merrer, and I. Negrutiu. Sexual dimorphism in white campion:
499 complex control of carpel number is revealed by y chromosome deletions. *Genetics*, 151(3):1173–1185,
500 March 1999.
- 501 [90] N. Fujita, C. Torii, K. Ishii, W. Aonuma, Y. Shimizu, Y. Kazama, T. Abe, and S. Kawano. Narrowing down
502 the mapping of plant sex-determination regions using new Y-chromosome-specific markers and heavy-ion
503 beam irradiation-induced Y-deletion mutants in *Silene latifolia*. *G3 (Bethesda)*, 2(2):271–278, February
504 2012. doi: 10.1534/g3.111.001420.
- 505 [91] P. Veltsos and L. F. Delph. The X chromosome is necessary for ovule production in *Silene latifolia*. *PLoS*
506 *One*, 14(5):e0217558, 2019. doi: 10.1371/journal.pone.0217558.
- 507 [92] Amborella Genome Project. The *Amborella* genome and the evolution of flowering plants. *Science*, 342
508 (6165):1241089, December 2013. doi: 10.1126/science.1241089.
- 509 [93] B. Grosse-Veldmann, N. Korotkova, B. Reinken, W. Lobin, and W. Barthlott. *Amborella trichopoda* –
510 cultivation of the most ancestral angiosperm in botanic gardens. *Sibbaldia*, 9:143–155, 2011.
- 511 [94] A. Manichaikul, J. C. Mychaleckyj, S. S. Rich, K. Daly, M. Sale, and W. M. Chen. Robust relationship
512 inference in genome-wide association studies. *Bioinformatics*, 26(22):2867–2873, November 2010. doi:
513 10.1093/bioinformatics/btq559.

- 514 [95] M. Buzgo, P. S. Soltis, and D. E. Soltis. Floral developmental morphology of *Amborella trichopoda*
515 (Amborellaceae). *Int. J. Plant Sci.*, 165(6):925–947, November 2004. doi: 10.1086/424024.
- 516 [96] J. Wang, J. K. Na, Q. Yu, A. R. Gschwend, J. Han, F. Zeng, R. Aryal, R. VanBuren, J. E. Murray,
517 W. Zhang, R. Navajas-Perez, F. A. Feltus, C. Lemke, E. J. Tong, C. Chen, C. M. Wai, R. Singh, M. L.
518 Wang, X. J. Min, M. Alam, D. Charlesworth, P. H. Moore, J. Jiang, A. H. Paterson, and R. Ming.
519 Sequencing papaya X and Yh chromosomes reveals molecular basis of incipient sex chromosome evolution.
520 *Proc. Natl. Acad. Sci. U.S.A.*, 109(34):13710–13715, August 2012. doi: 10.1073/pnas.1207833109.
- 521 [97] H. Badouin, A. Velt, F. Gindraud, T. Flutre, V. Dumas, S. Vautrin, W. Marande, J. Corbi, E. Sallet,
522 J. Ganofsky, S. Santoni, D. Guyot, E. Ricciardelli, K. Jepsen, J. Käfer, H. Berges, E. Duchêne, F. Picard,
523 P. Hugueney, R. Tavares, R. Bacilieri, C. Rustenholz, and G. A. B. Marais. The wild grape genome
524 sequence provides insights into the transition from dioecy to hermaphroditism during grape domestication.
525 *Genome Biol.*, 21(1):223, September 2020. doi: 10.1186/s13059-020-02131-y.
- 526 [98] O. V. Razumova, O. S. Alexandrov, M. G. Divashuk, T. I. Sukhorada, and G. I. Karlov. Molecular
527 cytogenetic analysis of monoecious hemp (*Cannabis sativa* L.) cultivars reveals its karyotype variations
528 and sex chromosomes constitution. *Protoplasma*, 253(3):895–901, May 2016. doi: 10.1007/s00709-015-
529 0851-0.
- 530 [99] J. R. Russell and J. R. Pannell. Sex determination in dioecious *Mercurialis annua* and its close diploid
531 and polyploid relatives. *Heredity (Edinb.)*, 114(3):262–271, March 2015. doi: 10.1038/hdy.2014.95.
- 532 [100] A. Muyle, D. Bachtrog, G. A. B. Marais, and J. M. A. Turner. Epigenetics drive the evolution of sex
533 chromosomes in animals and plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 376(1826):20200124, June
534 2021. doi: 10.1098/rstb.2020.0124.
- 535 [101] G. G. Cossard, J. F. Gerchen, X. Li, Y. Cuenot, and J. R. Pannell. The rapid dissolution of dioecy by
536 experimental evolution. *Curr. Biol.*, 31(6):1277–1283, March 2021. doi: 10.1016/j.cub.2020.12.028.
- 537 [102] J. S. Sánchez Vilas and J. R. Pannell. Plasticity in sex allocation in the plant *Mercurialis annua* is greater
538 for hermaphrodites sampled from dimorphic than from monomorphic populations. *J. Evol. Biol.*, 27(9):
539 1939–1947, September 2014. doi: 10.1111/jeb.12447.
- 540 [103] E. M. Golenberg and N. W. West. Hormonal interactions and gene regulation can link monoecy and
541 environmental plasticity to the evolution of dioecy in plants. *Am. J. Bot.*, 100(6):1022–1037, June 2013.
542 doi: 10.3732/ajb.1200544.
- 543 [104] J. Käfer, G. A. Marais, and J. R. Pannell. On the rarity of dioecy in flowering plants. *Mol. Ecol.*, 26(5):
544 1225–1241, March 2017. doi: 10.1111/mec.14020.
- 545 [105] G. S. Van Doorn. Evolutionary transitions between sex-determining mechanisms: a review of theory. *Sex.*
546 *Dev.*, 8(1-3):7–19, 2014. doi: 10.1159/000357023.
- 547 [106] D. H. Palmer, T. F. Rogers, R. Dean, and A. E. Wright. How to identify sex chromosomes and their
548 turnover. *Mol. Ecol.*, 28(21):4709–4724, November 2019. doi: 10.1111/mec.15245.
- 549 [107] M. Tomaszewicz, P. Medvedev, and K. D. Makova. Y and W chromosome assemblies: Approaches and
550 discoveries. *Trends Genet.*, 33(4):266–282, April 2017. doi: 10.1016/j.tig.2017.01.008.