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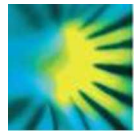
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Plant contributions to our understanding of sex chromosome evolution

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1 Tansley review for *New Phytologist*

2

3

4 Title: **Plant contributions to our understanding of sex chromosome evolution**

5 Deborah Charlesworth

6

7 Address for correspondence:

8 Institute of Evolutionary Biology, University of Edinburgh,

9 Ashworth Lab. King's Buildings, W. Mains Rd., Edinburgh EH9 3FL, UK

10 Phone: 0131 650 5751

11 Email: Deborah.Charlesworth@ed.ac.uk

12

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14 Key words: Sex chromosomes, recombination suppression, partial sex linkage, sex
15 determination, genetic degeneration

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24 **Summary**

25 A minority of angiosperms have male and female flowers separated in distinct
26 individuals (dioecy), and most dioecious plants do not have cytologically different
27 (heteromorphic) sex chromosomes. Plants nevertheless have several advantages
28 for studying sex chromosome evolution, as genetic sex determination has evolved
29 repeatedly and is often absent in close relatives. I review sex-determining regions
30 in non-model plant species, which may help us understand when and how (and,
31 potentially, test hypotheses about why) recombination suppression evolves within
32 young sex chromosomes. I emphasise high throughput sequencing approaches that
33 are increasingly being applied to plants to test for non-recombining regions. These
34 data are particularly illuminating when combined with sequence data that allow
35 phylogenetic analyses, and estimates of when these regions evolved. Together
36 with comparative genetic mapping, this has revealed that sex-determining loci and
37 sex-linked regions evolved independently in many plant lineages, sometimes in
38 closely related dioecious species, and often within the past few million years. In
39 reviewing recent progress, I suggest areas for future work, such as using
40 phylogenies to allow informed choice of outgroup species suitable for inferring
41 the directions of changes, including testing whether Y chromosome-like regions
42 are undergoing genetic degeneration, a predicted consequence of losing
43 recombination.

44

45 **Introduction: Advantages of plants for studying sex chromosome evolution**

46 Most flowering plants have hermaphroditic flowers, and only a minority have
47 separate male and female flowers (monoecy or dioecy). Among dioecious plants, with
48 male and female flowers separated in distinct individuals, some species have
49 environmental, not genetic, control of sex determination (Policansky, 1981;
50 Zimmerman, 1991; Pannell, 1997), and those with genetic sex determination often do
51 not have cytologically differentiated sex chromosomes (Westergaard, 1958; Ming *et*
52 *al.*, 2011; Renner, 2014). In contrast, separate sexes and heteromorphic sex
53 chromosomes are common in many familiar animal groups (Bachtrog, 2012).
54 Nevertheless, plants have several advantages for research on sex chromosomes,
55 because genetic sex determination has evolved repeatedly among angiosperms, and
56 independently in different families (Charlesworth, 1985; Ming *et al.*, 2011; Renner,
57 2014). Compared with the best-studied animal systems (Bellott *et al.*, 2014; Cortez *et*
58 *al.*, 2014; Zhou *et al.*, 2014), many flowering plant sex chromosomes probably
59 evolved very recently (Marais *et al.*, 2011; Renner, 2014), yet, as will be illustrated
60 below, similarities with animal systems are striking. Table 1 summarises the main
61 advantages of using dioecious plants to study sex chromosome evolution, and test
62 hypotheses about sex chromosome evolution derived from theoretical modelling.

63

64

Table 1 about here

65

66 There is now too much published work on plant sex chromosomes and their
67 evolution to include in a single article. I therefore focus on recombination
68 suppression, the defining characteristic of sex chromosomes, which leads to the
69 evolution of sex chromosomes' other unusual characteristics, genetic degeneration
70 and accumulation of repetitive sequences on the sex chromosomes, which I mention
71 only briefly. I review progress that has come through genetic and molecular
72 evolutionary studies, illustrating how this has involved combining approaches,
73 including DNA sequencing, and resequencing of multiple individuals of the same sex
74 and species for genetic and population genetic tests of sex-linkage, together with
75 sequencing for phylogenetic studies, and to place events in well-established time
76 frames.

77

78

79 **Sex chromosomes and estimating their ages from sequence divergence**

80 I define plant sex chromosomes as genome regions of these species that carry
81 the “SEX” locus that controls individuals' sexes, and that do not recombine. Rather
82 than using the term sex chromosomes, I shall often use “fully sex-linked regions” (and
83 full sex linkage), because of the diversity among plants with genetically controlled
84 dioecy — some have extensive non-pairing regions that show heteromorphism
85 between the sexes, like many animal sex chromosomes, but many have no detectable
86 cytological differences (recently reviewed by Renner, 2014). *Silene latifolia* is an
87 example of sex chromosome heteromorphism. It has an XY system, and males are the
88 heterozygous sex, and, as in mammals (Bellott *et al.*, 2014; Cortez *et al.*, 2014). The
89 Y is largely non-recombining, with XY pairing only in a small pseudo-autosomal
90 region (PAR) region at one tip (Westergaard, 1958; Filatov *et al.*, 2008); mapping of
91 genic markers suggests a single PAR (Bergero *et al.*, 2013), though an AFLP map
92 suggests two (Scotti & Delph, 2006). However, unlike many animal Y chromosomes,
93 the fully sex-linked region still carries hundreds of genes (Bergero & Charlesworth,
94 2011; Chibalina & Filatov, 2011; Muyle *et al.*, 2012). In contrast, the fully Y-linked
95 region in papaya is only about 10% of chromosome 1 (Liu *et al.*, 2004; Wang *et al.*,
96 2012). Some diploid plants have ZW systems, in which females are ZW heterozygotes
97 and males are ZZ homozygotes (Westergaard, 1958), as in birds (Zhou *et al.*, 2014)
98 and Lepidoptera (Suetsugu *et al.*, 2013); these include *Fragaria* (strawberry) species
99 (Spigler *et al.*, 2008; Goldberg *et al.*, 2010) and *Salix* (Alstrom-Rapaport *et al.*, 1998).
100 Other systems, including those in haploid plants, will be described below.

101 The time when recombination stopped can be estimated using DNA sequence
102 divergence between genes present on the Y as well as the X, together with a
103 “molecular clock” for synonymous or silent site divergence per year. Higher X-Y
104 divergence values correspond to greater times since recombination suppression. In
105 both humans (Lahn & Page, 1999) and the plant *S. latifolia* (Bergero *et al.*, 2007),
106 divergence increases with the distance from the PAR (in X chromosome genetic or
107 physical maps; (as these Y chromosomes are extensively rearranged, distances on the
108 Y are not informative Skaletsky *et al.*, 2003; Bergero *et al.*, 2008). Therefore,
109 suppressed recombination must have spread from an early non-recombining region,
110 the oldest "evolutionary stratum" (Lahn & Page, 1999), towards younger “strata”

111 closer to the current PAR. X-Y divergence in the older *S. latifolia* stratum is similar to
112 that in the youngest of the five strata in humans (Skaletsky *et al.*, 2003), and the
113 *Silene* XY pair probably evolved about 5-10 MYA (Nicolas *et al.*, 2005).

114 [Figure 1 about here]

115 A sex chromosome system may be older than its oldest stratum, because
116 recombination suppression in a sex-determining region usually takes time to evolve
117 (see below). On the other hand, recombination suppression may pre-date evolution of
118 separate sexes. In several well-studied plants, results from combining genetic and
119 physical mapping reveal large genome regions with infrequent crossing over
120 surrounding the centromeres, with crossovers restricted to the ends of chromosomes,
121 for example in maize (Rodgers-Melnicka *et al.*, 2015). These regions may include
122 substantial proportions of genes; in barley, for example, about 20% of genes are
123 estimated to be located in such regions (Baker *et al.*, 2014). If sex-determining loci
124 evolve in such a region, the oldest stratum will be contemporaneous with the sex-
125 determination system (Figure 1).

126 In what follows, I stress the importance of estimating ages of non-recombining
127 regions for understanding several important aspects of sex chromosome evolution.
128 Young sex chromosome systems are well suited for studying the early stages of
129 evolution of recombination suppression and the evolution of these characteristics; in
130 older animal systems, these processes can only be studied over a coarse time scale
131 that cannot reveal much detail. Young evolutionary strata in plant sex chromosomes
132 are also of interest for studying the time course of genetic degeneration, including
133 gene losses from Y chromosomes.

134

135 **Which plants have sex chromosomes?**

136 Genetic maps can detect the presence of sex-linked regions in dioecious species.
137 In papaya, for example, a large set of AFLP molecular variants was first mapped in a
138 full-sib family (Liu *et al.*, 2004). The completely sex-linked region is small, making
139 BAC sequencing of the region possible, which showed that the X-linked region
140 includes only 3.5 Mb (it is flanked by much larger PARs), and carries about 50 genes
141 with apparently functional Y-linked copies (Wang *et al.*, 2012). Assembly of the
142 physical map of the homologous Y-like region suggests that part of the sex-linked

143 region is probably in a pericentromeric region (Yu *et al.*, 2007; Zhang *et al.*, 2008).
144 Silent site divergence values for XY pairs suggests that inversions occurred,
145 suppressing recombination, about 7 and 1.7 million years ago, implying that a new
146 recombination-suppressed region has formed since the older stratum evolved (Figure
147 1).

148 Ideally, sex-linkage in a family should be confirmed by showing that Y-linked
149 variants are found only in males in a wider sample of genotypes (from natural
150 population samples, or from multiple cultivars of crop species) to exclude partially
151 sex-linked genes in the PAR that did not yield recombinants in the particular cross
152 studied. This is unnecessary for papaya, because, although this is clearly a young
153 system, the sequence divergence across part of the X-Y region is around 7% for silent
154 sites, much higher than between alleles in recombining regions of the genome,
155 including the collinear regions adjoining the fully sex-linked sequences (Wang *et al.*,
156 2012). This strongly suggests complete sex-linkage.

157 Now that large numbers of genetic markers can be developed in non-model
158 organisms, using high-throughput approaches, it will be possible to discover how
159 many other plant cases like papaya exist, without major cytologically detectable sex
160 chromosome heteromorphism, but with fully sex-linked regions carrying multiple
161 genes, and assess how many plants evolved dioecy so recently that their sex-
162 determining loci have not yet evolved non-recombining sex chromosome-like regions.
163 I next outline other approaches that have demonstrated that sex-linked regions have
164 evolved in dioecious plants.

165 When divergence data are not available, genetic mapping in related species can
166 help test whether recombination suppression has evolved in dioecious plants. If the
167 SEX locus of a dioecious species is in a genome region of suppressed recombination,
168 but the homologous region of a non-dioecious relative recombines, this would suggest
169 that recombination suppression evolved following the evolution of genetically
170 controlled dioecy, rather than being the ancestral state (unless the SEX locus is in a
171 pericentromeric region whose extent or location has changed between the species). A
172 dioecious close relative of papaya, *Vasconcellea parviflora*, has been shown to have a
173 homologous SEX locus, based on cytogenetic detection of heterochromatin in the
174 centromere-proximal regions of the homologous chromosomes of the two species.
175 This result also shows that the papaya and *V. parviflora* sex chromosomes are not
176 truly homomorphic (Iovene *et al.*, 2015) — their heteromorphism is minor, but

177 detectable with refined modern cytological methods, consistent with the sequencing
178 results showing that the papaya Y--linked region is larger than the X region (Wang *et*
179 *al.*, 2012).

180 These examples illustrate the value of genetic mapping within families.
181 However, studies of wider population samples can also be used to discover sex-linked
182 regions and establish which is the heterozygous sex. In an XY system, all males
183 should be heterozygotes for fully sex-linked alleles, and population surveys can reveal
184 such male-specific variants (which can be confirmed by data on segregation patterns
185 within families). This approach has established that the date palm (*Phoenix*
186 *dactylifera*) has at least several fully sex-linked genes (Cherif *et al.*, 2012). Neither
187 the physical size of the SEX region, nor the age of this system has yet been estimated.
188 It is a heteromorphic XY system (Siljak-Yakovlev *et al.*, 1996) that may be ancient,
189 as many other species in the palm family are also dioecious (Renner, 2014).

190

191 **Haploid plants**

192 Many haploid plants have sex chromosomes, as determined either from
193 morphology differences in the karyotypes of male and female gametophytes (Bull,
194 1983; Ming *et al.*, 2011), or from the existence of one or more sex-linked genetic
195 markers (Immler & Otto, 2015). The male- and female-determining chromosomes of
196 haploids are now often called V and U, respectively (Bachtrog *et al.*, 2011), to
197 emphasize that the SEX region is never homozygous and can therefore never
198 recombine; until physical maps are produced, it is not possible to relate sequence
199 divergence to the genetic map position in the sex-linked region. The older literature,
200 including studies in *Marchantia polymorpha*, called them X/Y systems (Okada *et al.*,
201 2001; Yamato *et al.*, 2007). *M. polymorpha* has highly heteromorphic sex
202 chromosomes, and divergence between alleles of the few sex-linked gene pairs
203 studied is extremely high, indicating an ancient system.

204 In contrast, genetic mapping in the moss *C. purpureus* found that, as in papaya,
205 only markers in the middle of the linkage group with the SEX locus show full sex-
206 linkage (McDaniel *et al.*, 2007). Sequence data for all site types in coding plus
207 (predominantly) non-coding regions of U- and V- linked allele sequences suggests
208 that evolutionary strata may exist in *C. purpureus*. Divergence between four of 8 U-V
209 gene pairs studied is only around 1-3%, but two genes have divergence of almost 7%

210 for (McDaniel *et al.*, 2013); one of them is long enough to reliably suggest high
211 divergence, either indicating a longer time since recombination stopped between the
212 U and V regions, or a higher mutation rate and/or lesser selective constraint
213 (divergence from the related species is also high for the high U-V divergence gene,
214 consistent with the two latter possibilities). Even the highest silent site divergence
215 currently found suggests, however, that this is not an ancient system (although genes
216 with higher divergence may be discovered when more genes are analysed).

217

218 **Plants with very small, or no, non-recombining regions.**

219 Genetic mapping (or related methods that can detect such regions even if they
220 are small, such as bulk segregant analysis) has yet to be applied in many plants with
221 genetic sex determination, and they could reveal non-recombining regions in many
222 plants not currently classified as having sex chromosomes. Indeed, a major currently
223 unanswered question is whether the number of plants with sex-linked regions is
224 currently under-estimated. Such studies are, however, limited by marker density, and
225 very small non-recombining regions may be missed due to insufficient marker
226 density. Indeed, in several plants, genetic sex determination has been established, and
227 a SEX locus controlling gender has been mapped, but no fully sex-linked marker has
228 been found. In kiwifruit (*Actinidia chinensis*), for example, mapping with 644
229 microsatellite markers still failed to detect any fully sex-linked markers. Other species
230 where the recombination status of the SEX locus is currently uncertain include
231 spinach (Khattak *et al.*, 2006), asparagus (Telgmann-Rauber *et al.*, 2007), and
232 *Populus* species (Yin *et al.*, 2008; Pakull *et al.*, 2011). Such species may, of course,
233 truly lack non-recombining SEX regions. They may either not yet have evolved fully
234 sex-linked regions, or may be single gene systems, which can evolve when a new
235 gene takes over control of flower sex determination after dioecy has become
236 established, replacing an existing sex-determining gene (Bull, 1983; van Doorn &
237 Kirkpatrick, 2007; Vuilleumier *et al.*, 2007; Blaser *et al.*, 2013); Figure 1C illustrates
238 such an event. Takeovers are known in several animal taxa, including insects
239 (Wilkins, 1995; Beye *et al.*, 2003) and fish (Ross *et al.*, 2009; Myosho *et al.*, 2012).

240 To map SEX loci, high throughput methods including RAD-Seq (Baird *et al.*,
241 2008) or RNA-Seq transcriptome sequencing (Bergero & Charlesworth, 2011;
242 Chibalina & Filatov, 2011; Muyle *et al.*, 2012; Hough *et al.*, 2014) can now generate

243 large numbers of markers, overcoming the problem of low marker density.
244 Alternatively, given the large resources sometimes available in crop plants, high
245 density linkage maps can be obtained by “target-sequence capture” (Mamanova *et al.*,
246 2010). In *Fragaria vesca*, for example, a map was made by first obtaining a low
247 coverage genome sequence, then identifying polymorphisms, and then using an
248 enrichment approach to obtain short sequences (200bp) surrounding each
249 polymorphism. This allowed genotyping of 5417 genes in a mapping family
250 (Tennessen *et al.*, 2013). Another recently developed approach that may be helpful in
251 plants ascertains fully sex-linked sequences by searching short read genome sequence
252 data from multiple individuals for k -mers (short sequences of length k) that appear
253 only in one sex (Carvalho & Clark, 2013). In the section on sex-determining genes
254 below, I outline how this approach has ascertained Y-linked sequences in persimmon
255 (*Diospyros lotus*), in the Ebenaceae (Akagi *et al.*, 2014).

256 A major difference between ancient animal sex chromosomes and the Y-linked
257 regions of the plants just discussed (with the possible exception of *M. polymorpha*) is
258 the minor extent of gene loss in plants (see section on genetic degeneration below). In
259 plants, sex-linked genes therefore cannot be ascertained by genome sequencing, using
260 their lower coverage in the heterogametic sex. Moreover, assembly of short-read
261 sequences will be difficult, due to sequence divergence and accumulation of repetitive
262 sequences, which occur in non-recombining genome regions (Charlesworth *et al.*,
263 1994), including plant sex chromosomes (Kubat *et al.*, 2014). Assemblies of the
264 human and papaya Y chromosomes involved deep sequencing of single males,
265 avoiding variants that might confuse assembly, and are restricted to non-
266 heterochromatic regions (Hughes *et al.*, 2010; Wang *et al.*, 2012).

267 Finally, multiple individuals of each sex are needed to determine sex-specific
268 sequences and distinguish fully and partially sex-linked regions. Even with large
269 family sizes, lack of recombinants does not exclude rare recombination. Population
270 genetic approaches can, however, detect recombination in past generations, even if it
271 occurs very rarely. In papaya, for example, the SEX region adjoins a “collinear region”
272 where the sequenced X and Y chromosomes appear to have the same genes in the
273 same order, unlike the older Y-linked strata, whose assembly includes many
274 rearrangements (Wang *et al.*, 2012). Divergence between the single X and Y
275 sequences so far available is low in the collinear region, indicating that recombination
276 must have continued after it had stopped in the two strata defined by the Y region

277 inversions described above. Sequence differences between the single X- and Y-linked
278 region so far sequenced may merely be variants that happens to be carried on those
279 particular chromosomes, and not sex-linked in the species as a whole, so this region
280 may prove to be partially sex-linked.

281 An approach that does include multiple individuals is bulk segregant analysis.
282 This has recently been successful in the grape vine (*V. vinifera*), an XY system. The
283 fully sex-linked genome region is only about 150 kb, and includes only a few fully
284 sex-linked genes, so recombination suppression has clearly not yet extended across
285 any substantial genome region (Picq *et al.*, 2014), consistent with the lack of
286 chromosomal heteromorphism.

287 The observation that much of the chromosome carrying the SEX locus
288 recombines does not necessarily mean that it is a young system. Instead, it may be an
289 ancient system whose non-recombining region has remained limited to just a part of
290 the chromosome. This is the case in the Ratite birds (Pigozzi, 2011; Vicoso *et al.*,
291 2013; Zhou *et al.*, 2014). In *Vitis*, most wild species are dioecious, suggesting that
292 dioecy is ancestral, so this could be a plant example of an old-established system that
293 has not evolved recombination suppression. However, divergence between *Vitis* sex-
294 linked genes has not yet been estimated, so the time when the sex-determining region
295 evolved is not yet known.

296

297 **Comparative genetic mapping**

298 Genetic mapping is also important for detecting differences in the chromosome
299 carrying the SEX region. This can occur when genetic sex-determination evolves
300 independently (or re-evolves after loss of dioecy). For example, genes that are sex-
301 linked in *S. latifolia* do not show sex-linkage in *Silene otites* and *S. colpophylla*
302 (Mrackova *et al.*, 2008). Phylogenetic analyses of sex systems in *Silene* (Mrackova *et*
303 *al.*, 2008; Marais *et al.*, 2011) suggest that these species do not have a dioecious
304 common ancestor, so dioecy probably evolved *de novo* in the two lineages, and
305 involved different genes.

306 Events in which a new gene takes over control of gender, can also cause the
307 SEX loci of related species to be on non-homologous chromosomes (Figure 1C), or to
308 a new location on the same chromosome, as in some animal cases of takeovers (Uno
309 *et al.*, 2008). In *Populus*, as in papaya, the SEX loci appear to be within

310 pericentromeric regions (Geraldes *et al.*, 2011), but, although these regions map to the
311 same linkage group, their locations differ greatly in the physical maps of different
312 *Populus* species (Yin *et al.*, 2008; Pakull *et al.*, 2011). If confirmed, this suggests a
313 takeover event by a new sex-determining gene on the same chromosome. Independent
314 evolution of separate sexes in different *Populus* lineages is not yet excluded, however,
315 even though almost all Salicaceae are dioecious. Data on the ages of the systems,
316 phylogenetic analysis, and genome sequencing, should help to distinguish between the
317 possibilities. Takeovers or independent evolution both predict that different species
318 should have different sets of genes at their SEX loci, unlike a chromosome
319 rearrangement. Independent evolution predicts that the times since recombination
320 stopped should differ (though this might not be detectable if all species have young
321 systems), while takeover events generating single-locus systems may not have been
322 followed by recombination suppression in the surrounding genome region.

323

324 **Why does suppressed recombination evolve?**

325 The repeated evolution of regions without crossing over between sex
326 chromosomes strongly suggests a causal connection with the evolution of sex-
327 determining regions (only the centromeric and pericentromeric regions of autosomes
328 generally have suppressed crossing over). The evolutionary strata of sex
329 chromosomes discussed above prove that suppressed recombination often evolves
330 after a sex-determining system is established. Some disadvantage to recombinant
331 genotypes must clearly be involved. Such situations probably occur both during the
332 initial evolution of dioecy, and also later, as males and females evolve in the absence
333 of constraints imposed by the other sex functions. Briefly, as illustrated in Figure 2,
334 separate sexes in plants probably often evolved from hermaphroditic or monoecious
335 ancestors, often called “cosexual” species (Lloyd, 1982).

336 The change from cosexuality to dioecy probably involves a mutation creating
337 females (a male-sterility mutation in an initially hermaphroditic species, or a mutation
338 suppressing some or all female flowers in an initially monoecious species, or
339 replacing them with male flowers), and then one or more female-suppressing
340 mutations, creating males or male-biased plants (Westergaard, 1958; Charlesworth &
341 Charlesworth, 1978). (I can find no cases where dioecy in plants evolved from
342 environmental sex determination, though this seems possible in principle).

343

344

[Figure 2 about here]

345

346 In this scenario, male-promoting mutations (suppressing femaleness) clearly
347 reduce females' fitness, and are therefore most likely to spread if linked to the gene
348 causing femaleness, which minimises the conflict. If a two-gene polymorphism
349 results, selection against recombinants will generate linkage disequilibrium (with the
350 X associated with male-sterility and the Y with female-suppressor alleles, see Figure
351 2). Suppressed recombination is therefore favoured, and may evolve (Charlesworth &
352 Charlesworth, 1980; Bull, 1983), creating a male-determining Y chromosome.

353 If however, a male-specific female-suppressing mutation occurs, no harm is
354 caused to females; if sufficiently advantageous in males, such a mutation can spread,
355 even if unlinked to the femaleness gene, yielding a single gene sex-determining
356 system (Figure 2A), and no selection for closer linkage with the gene causing
357 femaleness (Muller, 1932; Charlesworth & Charlesworth, 1978).

358 Much genetic evidence supports the two-gene model for plant sex-
359 determination, rather than one with sex-specific gene actions. First, in several species
360 (or intercroses of dioecious plants with close non-dioecious relatives) three allelic
361 types at the sex-determining locus control whether individuals are (i) females, (ii)
362 males, or (iii) hermaphrodites or monoecious functional hermaphrodites
363 (Westergaard, 1958). Second, in papaya and grape vine, humans have selected
364 individuals that have Y-linked regions that do not suppress female functions (Wang *et al.*,
365 2012; Picq *et al.*, 2014). Similarly, in *Silene latifolia*, deletions detectable through
366 loss of Y-linked markers can create hermaphrodites and neuter plants (Fujita *et al.*,
367 2011 and references therein). These plants' Y-linked regions must therefore carry
368 suppressors of female functions whose loss does not affect male functions, and
369 distinct maleness factor(s) elsewhere on the chromosome. Thirdly, in the strawberry
370 species *Fragaria virginiana*, two closely, but not completely, linked genes with the
371 expected phenotypes have been found (Spigler *et al.*, 2008), while a related *Fragaria*
372 species has suppressed recombination (Goldberg *et al.*, 2010). These species' sex-
373 determining regions probably evolved independently (Goldberg *et al.*, 2010), but the
374 results nevertheless suggest recombination suppression evolving in response to a two-
375 locus polymorphism.

376 The evolution of dioecy probably often involves further sexually antagonistic
377 mutations, leading to further selection to suppress recombination, and potentially
378 generating younger strata. For example, dioecy has often evolved from monoecy
379 (Renner, 2014), and full maleness may involve successive increases in the proportion
380 of investment in male flowers (Figure 2B), each involving sexually antagonistic
381 “trade-offs”, because each must decrease the proportion invested in female flowers.
382 Variable degrees of maleness are indeed seen in the monocotyledon *Sagittaria*
383 *latifolia*, in the Alismataceae (Dorken & Barrett, 2004), *Spinacia oleracea* in the
384 Chenopodiaceae (Onodera *et al.*, 2011) and *Urtica dioica* (Glawe & Jong, 2009).
385 Similarly, when the ancestral state is hermaphroditism, evolution of dioecy often
386 involves “inconstant males” with partial female function (for example, producing
387 some fruits in favourable conditions). Species where genetic variation in male
388 functions seems likely include *Antennaria dioica* in the Asteraceae (Ubisch, 1936)
389 and *Euonymus europaeus* in the Celastraceae (Webb, 1979), but these have not been
390 investigated using genetic markers to map the factors. Even after complete
391 unisexuality has evolved, male and female functioning may be sub-optimal, and
392 improvements to each sex may often reduce functions of the other. In *S. latifolia*, for
393 example, female fecundity is enhanced by making large flowers, but fertility is
394 highest for males with many small flowers (Delph & Herlihy, 2012). Just as outlined
395 above for sterility mutations, a mutation benefitting one sex at the expense of the
396 other is most likely to invade but not spread throughout the population; if such a
397 polymorphism is established, it creates selection for reduced recombination with the
398 sex-determining locus, if it arises at a locus closely linked to the SEX locus (Rice,
399 1987; Jordan & Charlesworth, 2012).

400 Testing for the trade-offs and conflicts assumed in these scenarios, and for
401 involvement of sexually antagonistic polymorphisms in the PAR regions of sex
402 chromosomes is clearly a major task for future work. An approach that can potentially
403 detect sexually antagonistic variation is QTL analysis within the two sexes separately,
404 as proposed and implemented in *Silene latifolia* (Scotti & Delph, 2006; Delph *et al.*,
405 2010). This detected several autosomal and PAR QTLs, and, interestingly, the latter
406 appeared only in the analysis of males, implying that their phenotypic effects are not
407 expressed in females. Such male-specific expression is consistent with a past conflict
408 between the sexes that has been resolved in later evolution, as seems to have occurred
409 for some sexually selected male coloration genes in the PAR of a fish, the guppy,

410 *Poecilia reticulata* (Lindholm & Breden, 2002). Male benefit alleles with male-
411 specific expression no longer harm females, and will spread throughout the
412 population; some other selection is therefore required to maintain the QTL variation,
413 perhaps environmental differences (Scotti & Delph, 2006). In *S. latifolia*, for example,
414 thin leaves appear to be disadvantageous to males only in dry years (Delph *et al.*,
415 2011). The *S. latifolia* QTL analysis used dominant AFLP markers, but codominant
416 markers now available in this plant's PAR, and obtainable in other plants, will permit
417 future analyses of variation in natural populations. This may detect factors whose
418 conflict has not been resolved, corresponding to the situation that creates selection for
419 reduced recombination in the theoretical models of sexually antagonistic PAR genes.

420

421 **Recombination suppression: mechanisms**

422 Non-recombining regions may eventually evolve to encompass a large region of
423 the chromosome carrying the sex-determining loci or locus. Studies of young plant
424 sex chromosomes may be valuable for studying the mechanistic basis of
425 recombination suppression, and whether it generally involves infrequent, large-scale
426 events like inversions, or smaller shifts in the position of the PAR boundary.

427 If chromosome inversions cause recombination suppression in SEX regions
428 (Lahn & Page, 1999), the region will often include many non-sex-determining genes.
429 In papaya, two Y chromosome inversions indeed seem to be involved (including 10
430 genes with both X and Y copies in the older stratum includes, and 16 in the newer
431 one, Wang *et al.*, 2012). In closely related dioecious *Vasconcellea* species, alleles of
432 several papaya fully sex-linked genes are not associated with gender (Gschwend *et al.*,
433 2011). Unlike the *Silene* situation described above, this probably does not reflect
434 independent evolution of dioecy in *Carica* and *Vasconcellea*, as BAC-FISH
435 experiments found sex-linked regions including several homologous sequences in
436 similar locations on the largest chromosome of both species (Iovene *et al.*, 2015).
437 Recombination suppression has therefore probably remained restricted in *V.*
438 *parviflora* to a genome region near the SEX locus, whereas it has spread across a
439 wider region in *C. papaya*. This is testable by sequencing to ask whether *V. parviflora*
440 genes homologous to *C. papaya* genes in the older sex-linked stratum have distinct X
441 and Y haplotypes like those of papaya. The alternative that the *V. parviflora* long arm
442 has become a new recombination-suppressed stratum seems unlikely, because the

443 chromosomal positions of all nine relevant BACs in an outgroup, *Jacaratia spinosa*,
444 were found to be similar to those in *V. parviflora*, so the inversions probably occurred
445 in the *C. papaya* lineage.

446 Recombination suppression mechanisms other than inversions may, however,
447 exist, including modifiers controlling the number of crossover events, restricting them
448 to certain genome regions, or restricting crossing over to only one sex. In one of the
449 two human PARs, for example, crossovers are localised very differently in male and
450 female meiosis (Hinch *et al.*, 2014). Some young sex chromosome systems may still
451 be in the process of undergoing recombination suppression. If recombination varies
452 between individuals, or between closely related species that can be interbred, genetic
453 studies can potentially identify the factors involved. In some populations of frog
454 species, male-specificity of microsatellite alleles differs between populations,
455 implying that the XY pair shows suppressed recombination only in some populations
456 (Dufresnes *et al.*, 2014). Apparently similar variation was inferred for an anonymous
457 sequence marker within the plant species *Bryonia dioica* (Oyama *et al.*, 2009), which
458 should be studied further. In *Silene latifolia*, recombination suppression appears to
459 vary between families for several genes (Bergero *et al.*, 2013).

460

461 **Old-established sex chromosome systems**

462 Old sex chromosomes also exist among plants, for example among liverworts
463 (Okada *et al.*, 2001), but have been less studied than young plant systems. As
464 molecular approaches and phylogenetic analyses are extended to studying more plant
465 sex chromosome systems, it will be interesting to include taxa with high proportions
466 of dioecious species, such as the palm, Vitaceae and Ebenaceae (including *Diospyros*
467 *lotus*, see below) families, to test whether dioecy is ancestral and estimate the time
468 when recombination stopped, or has evolved several times (as may be the case in the
469 Salicaceae discussed above).

470 Old systems are particularly interesting for investigating genetic degeneration
471 and repetitive sequence accumulation, which occur over large evolutionary
472 timescales. The potentially large range of ages of dioecious plant sex chromosome
473 systems will allow the time-course of sex chromosome evolution to be studied. Old
474 plant systems may also help us understand why recombination suppression sometimes
475 fails to evolve.

476 The evidence for old-established systems is currently incomplete, and age
477 estimates based on sequence divergence are lacking. There are currently no dense
478 genetic maps for plants that seem likely to have old XY systems, and, so far, genetic
479 mapping in these systems has largely used non-genic markers such as AFLPs and
480 microsatellites. These are excellent for testing for a non-recombining (sex
481 chromosome-like) region, determining which is the heterozygous sex, and estimating
482 the proportion of the chromosome that is fully sex-linked. However, as explained
483 above, estimating the age of a sex chromosome system, and the time when
484 recombination stopped, requires X-Y sequence divergence estimates, based on
485 ascertaining sex-linked genes and sequencing them.

486 In the absence of divergence data, the observation that a sex chromosome
487 system is heterochromatic and heteromorphic might be thought to suggest that it is
488 old-established, especially in plant families that include distantly related dioecious
489 species, such as date palms (Al-Mahmoud *et al.*, 2012). For example, *Rumex acetosa*
490 belongs to a clade that may have been dioecious for 15–16 MYA (Navajas-Pérez *et al.*,
491 2005), but X-Y divergence has not been estimated. Its Y chromosomes are
492 heterochromatic (Shibata *et al.*, 2000; Mariotti *et al.*, 2008), unlike those of other
493 cytologically well-studied plants such as *Silene latifolia* and *S. dioica* (Grabowska-
494 Joachimiak & Joachimiak, 2002; Kubat *et al.*, 2014), which are estimated to be
495 younger (see above). However, heterochromatin can evolve rapidly, as in papaya.
496 Another example is *Coccinia grandis*, within a wholly dioecious genus of 27 species
497 (in the Cucurbitaceae, another family with many dioecious species, often with XY
498 heteromorphism). Its male genome C-value is 10% larger than that of females,
499 indicating that the Y chromosome is much larger than the X, and the entire Y is
500 heterochromatic (Sousa *et al.*, 2012), yet phylogenetic analysis suggests that these
501 characteristics evolved recently (Holstein & Renner, 2011).

502 Sex chromosome heteromorphism can also arise in young systems, for example
503 through fusions with autosomes, as in *Rumex hastatulus* (Smith, 1964) and possibly
504 spinach (Araratjan, 1939). The systems in *Cannabis sativa* (Peil *et al.*, 2003;
505 Sakamoto *et al.*, 2005) and *Humulus lupulus* (hops) in the Cannabaceae, whose Y
506 chromosome is heterochromatic (Westergaard, 1958) are probably much older.

507 Studies of old systems are also needed to test the prediction that other sex-
508 determining system are derived from XY systems (Charlesworth & Charlesworth,
509 1978). Again plants may be very helpful, as systems with male-determining Y

510 chromosomes probably evolve first, as outlined above, but ZW systems also exist, and
511 it can be tested whether the frequencies of such systems increases over time. X-
512 autosome balance systems are also probably derived from XY systems (and
513 potentially allow loss of the Y chromosome, and evolution of an X0 male genotype).
514 However, it has again not yet been demonstrated that such species tend to be older
515 than other plant sex-determining systems. Absence of carpel development in males or
516 stamen development in females, as in hops, may also indicate an ancient system (but
517 might simply be due to a long history of unisexual flowers, for example because
518 dioecy has evolved from monoecy); so far, only one fully sex-linked genetic marker
519 locus has been found in hops (Jakse *et al.*, 2008).

520

521 **Genetic degeneration: the need for empirical data in a phylogenetic setting**

522 Ancient systems are also of great interest for studying genetic degeneration
523 (gene loss or loss of function). In diploid organisms, only the Y chromosomes are
524 predicted to degenerate, because X chromosomes recombine in the XX females,
525 whereas Y-linked regions do not, and are subject to several processes that allow
526 detrimental mutations to increase in frequency in the population of Y-linked alleles,
527 or even to become fixed in this population, as recently reviewed (Bachtrog, 2008). In
528 haploid plants, however, the complete lack of recombination across the entire sex-
529 linked region predicts similar degeneration of both U and V chromosomes (Bull,
530 1983). Genes affecting non-sex functions should not degenerate or become lost, so the
531 female-determining U region should lose only male function genes, and the male-
532 determining V region only female function genes (Figure 3C).

533

534

Figure 3 about here

535

536 Haploid plants with separate sexes of gametophytes are ideal for studying this
537 prediction. In *Marchantia polymorpha*, a species whose sex chromosomes carry
538 highly diverged sequences, the V has been studied in detail, but analysis of the U
539 chromosome is currently incomplete (Okada *et al.*, 2001). In the brown alga,
540 *Ectocarpus siliculosus*, however, about 24 genes were found in the fully sex-linked
541 regions (either the U or V regions, or both), of which 7 were not detected in the V and
542 9 in the U (Ahmed *et al.*, 2014). This is in apparent agreement with Bull's prediction;

543 however, without an outgroup, gene movements onto one sex chromosome, but not
544 the other, cannot be excluded. To determine whether suitably close non-dioecious
545 relatives exist (and avoid species that might have reverted from dioecy to a non-
546 dioecious state), phylogenetic relationships of the species must be known. This is
547 often difficult for closely related species, a frequent situation relevant to the evolution
548 of sex chromosomes. Nevertheless, among plants, sets of species should exist with
549 good phylogenies well suited for future work estimating ancestral character states, and
550 changes in states, (Maddison & Leduc-Robert, 2013).

551 Diploid dioecious plants also have extended haploid life cycle stages, which
552 may also cause genetic degeneration of non-recombining sex chromosomal regions to
553 be minor (Figure 3B). Around 2/3 of plant genes are expressed in male gametophytes
554 of angiosperms (Tanksley *et al.*, 1981; Gorla *et al.*, 1986; Honys & Twell, 2003).
555 Therefore, only genes with no important pollen functions should be lost from plant
556 SEX regions, or lose their functions; the limited evidence so far about loss of genes
557 from the *S. latifolia* Y chromosome is consistent with this expectation (Guttman &
558 Charlesworth, 1998; Chibalina & Filatov, 2011). Degeneration might be thus
559 restricted to around 1/3 of genes (or possibly somewhat higher, if expression of some
560 pollen-expressed genes is not important, and purifying selection maintaining their
561 functions is consequently weak). The few current estimates, from the unrelated plants
562 *S. latifolia* and *Rumex hastatulus*, suggest that fewer than 30% of Y-linked genes have
563 lost expression (Bergero & Charlesworth, 2011; Chibalina & Filatov, 2011; Hough *et*
564 *al.*, 2014). In contrast, such regions are almost completely degenerated in the best
565 studied animals, such as species of *Drosophila* (Muller, 1950), mammals (Skaletsky
566 *et al.*, 2003) and those birds that have extensive fully W-linked regions (Zhou *et al.*,
567 2014), and possibly in part of the much younger Y chromosome of the threespine
568 stickleback (Ross & Peichel, 2008; Yoshida *et al.*, 2014). Large genome regions that
569 stopped recombining recently and carry many genes driving the degeneration
570 processes, such as the neo-Y chromosome of *Drosophila miranda*, have quickly lost
571 functions of large fractions of genes (Bachtrog *et al.*, 2008). However, the regions of
572 the two plants so far studied that recently became fully sex-linked probably include
573 many fewer genes than the *D. miranda* region, so that the small extent of gene losses
574 in these young systems is not surprising. It will be interesting to study older plant
575 systems.

576 Genetic degeneration in young plant sex chromosomes, and in young
577 evolutionary strata in older systems, is also of interest. The first step after
578 recombination is suppressed between Y- and X-linked regions may be accumulation
579 of repetitive sequences, including transposable elements. Such insertions may
580 decrease expression of Y-linked alleles, even before mutations in the coding regions,
581 or in non-coding regions that control the gene's expression. This appears to be the
582 case in *Drosophila albomicans* (Zhou & Bachtrog, 2012).

583 However, plants with sex-determining loci within rarely recombining
584 pericentromeric regions, such as papaya and *Populus* species, are not well suited for
585 studying genetic degeneration, because accumulation of maladaptive sequence
586 changes and of repetitive sequences are also expected in pericentromeric genome
587 regions (Charlesworth *et al.*, 1986). It will therefore be difficult to detect extra effects
588 of the evolution of sex-determining genes in the region. For example, in papaya, gene
589 density is low in the sex-linked region, but this is not wholly due to loss of genes;
590 accumulation of repeated sequences has also reduced gene density (Wang *et al.*, 2012).

591

592 *Dosage compensation*

593 In sex chromosome systems where Y-linked gene expression is reduced, or Y-
594 linked genes have been lost, dosage compensation has sometimes evolved, and it is
595 therefore interesting to test whether X-linked alleles of plant genes whose Y-linked
596 copies have lost function are expressed at higher levels in males than females. There
597 is currently no clear evidence that this occurs in *Silene latifolia* or *Rumex hastatulus*,
598 but partial compensation cannot yet be excluded (Chibalina & Filatov, 2011; Muyle *et al.*,
599 *al.*, 2012; Hough *et al.*, 2014; Bergero *et al.*, 2015).

600

601 **Plant sex-determining loci**

602 To identify sex-linked regions and determine whether males or females are the
603 heterozygous sex, it is not necessary to find the gene(s) controlling male or female
604 development. As explained above, it suffices to find genetic markers, even
605 anonymous ones, such as AFLPs, that co-segregate with sex. However, plant sex-
606 determining loci are interesting in several ways, including for identifying the
607 hypothesised two or more genes causing male- and female-sterility during the
608 evolution of dioecy. If sex-determining genes can be discovered, sequence divergence

609 between their sex-linked alleles may also help estimate the time when recombination
610 first stopped. With the possibility of dense marker development and genome
611 sequences, renewed efforts are being made to identify plant sex-determining genes,
612 and progress can be expected in the next few years.

613 The approach of testing known flower development genes has been largely
614 superseded by high-throughput sequencing methods. Searches have found MADs-box
615 and ABC(DE) genes involved in flower whorl development on the sex chromosomes
616 of *Silene latifolia* and *Asparagus officinalis* (Matsunaga *et al.*, 2003; Park *et al.*, 2003;
617 Cegan *et al.*, 2010; Nishiyama *et al.*, 2010; Penny *et al.*, 2011). However, genes that
618 control floral organ identity are not generally promising candidates. They might be
619 involved in species with complete absence of one sex organs (“Type I” of Mitchell &
620 Diggle, 2005; Ramos *et al.*, 2014). In many dioecious plants, however, both male and
621 female floral organs are initiated in flowers of both sexes, and the development of
622 opposite sex organs is later interrupted.

623 Alternative approaches also encounter difficulties due to the numerous
624 candidates whose loss of function can produce male or female sterility. For example,
625 as mentioned above, deletion mapping of the *S. latifolia* Y chromosome has
626 established that separate loci exist whose deletion causes abortion or incomplete
627 development of stamens, or removes the suppression of pistils that occurs in wild-type
628 males, creating hermaphrodite flowers (Farbos *et al.*, 1999; Lardon *et al.*, 1999;
629 Zluvova *et al.*, 2005; Bergero *et al.*, 2008; Fujita *et al.*, 2011). However, these
630 deletions probably involve loss of many fully sex-linked genes other than the ones
631 causing these phenotypes, and this is supported by the observation that pollen
632 carrying deleted Y chromosome regions often has low ability to fertilise ovules
633 (Lardon *et al.*, 1999). When the sex-linked region is large, it will be difficult to
634 identify the genes responsible for the evolution of dioecy unless small deletions can
635 be generated and identified using dense mapping of sequences lost from deleted
636 genotypes.

637 This problem also hinders attempts to identify genes involved in gender
638 determination using mutations, including mutations induced by EMS or irradiation
639 (Ohnishi, 1985; Christensen *et al.*, 1998; Honys & Twell, 2004; Wellmer *et al.*, 2006;
640 Chang *et al.*, 2011), or by studying genes with different expression in flower buds of
641 the two sexes. Moreover, many genes have stamen- or pistil-specific expression, and
642 will be non-expressed in buds of one sex purely because the relevant structures are

643 absent. Distinguishing such downstream acting genes from the sex-determiners
644 themselves requires establishing sex linkage. If, however, the fully sex-linked region
645 includes many genes, the problem of having too many candidates with suitable
646 function is not eliminated. In addition, expression differences may not be involved
647 (for example, male sterility can involve mutations in coding sequences, and the
648 mutant alleles may be present in mRNA).

649 Small sex-linked regions may offer the best prospects for identifying the sex-
650 determining genes, because fewer candidates need to be considered. A candidate Y-
651 linked gene has been proposed in persimmon (Akagi *et al.*, 2014). This study started
652 by identifying sex-linked genes, using pools of males and females from a full-sib
653 family, and their sex-linkage was confirmed in samples of unrelated males and
654 females. Efforts were made to ensure that most Y-linked genes present in transcripts
655 were detected, by employing RNA-Seq, and 22 expressed sequences were identified.
656 The total length of sex-specific sequences was only 1Mb, suggesting a small fully
657 sex-linked region. One candidate for involvement in sex determination was found.
658 This gene (named *OGI*) is expressed only in male flower buds. *OGI* is a duplication
659 onto the Y-linked region of an autosomal gene called *MeGI* that expresses a male-
660 suppressing regulatory RNA in females. Because no X copy exists, X-Y divergence
661 cannot be estimated, but divergence from the presumed autosomal progenitor is high,
662 and Y-linked *OGI* sequences were detected in other species of Ebenaceae, suggesting
663 an old-established Y-linked duplication. Low divergence was found between the X-
664 and Y-linked alleles of other sex-linked genes (silent site divergence of 12 XY allele
665 pairs was below 2%), suggesting that a younger stratum evolved recently.

666 The proposed scenario for sex-determination in persimmon is that the Y-linked
667 *OGI* gene opposes *MeGI*'s male-suppressing action. This form of gene action that
668 could act in the heterozygous state, and should increase male functions, and the
669 processes in the two sexes may indeed conflict, as proposed for the female suppressor
670 in the two-gene model outlined in Figure 2A above. It is currently unclear how
671 females evolved. The *MeGI* male suppressing factor is autosomal, and is therefore
672 unlikely to represent the male-sterility gene in the two-gene model. *OGI* could
673 therefore be an example of a single-locus sex-determining gene that evolved by a
674 take-over event, if searches fail to find a femaleness factor.

675 Although, as mentioned already, reversals and re-evolution of dioecy can
676 complicate comparative studies and hinder inferences of the ages of the origins of

677 dioecy, they may also be very helpful in revealing the genetic basis of dioecy
678 (Westergaard, 1958), and molecular studies of such hermaphrodites could help
679 identify plant sex-determining genes. In papaya and *Vitis*, hermaphrodites are
680 commercially successful crop plants. The Y chromosomes in these hermaphrodites do
681 not suppress female functions, but their sequences are very similar to those of males
682 (Picq *et al.*, 2014; Van Buren *et al.*, 2015), and they probably have no large deletions,
683 making them ideal for identifying the gene whose loss causes reversion to
684 hermaphroditism, a good candidate for the female suppressor involved in the
685 evolution of dioecy.

686 The hypothesised X-linked genes responsible for the male sterility of females in
687 dioecious plant species are likely to be even harder to identify, but this may be
688 possible in systems where suppressed recombination has not yet evolved. If two
689 incompletely linked sex determining genes exist, hermaphrodite recombinants, and
690 recombinants with the male sterility allele of females and the female suppressor of
691 males, should arise. With the modern ability to identify the region, and genotype
692 closely linked markers, as in *Fragaria* species (Tennessen *et al.*, 2013), it should be
693 possible to check that these phenotypes are indeed associated with recombination, and
694 to pinpoint both genes.

695 Once the genes are identified in some plant species, this will open the way for
696 testing whether the same genes are sex-linked in other dioecious plants. Given that
697 large numbers of genes affect flower and inflorescence development, different genes
698 may be involved in different angiosperm lineages, rather than the same genes being
699 repeatedly involved. If so, plants will differ from major animal groups such as insects,
700 which share sex-determination pathways across major taxa (Saccone *et al.*, 2002;
701 Beye *et al.*, 2003; Pomiankowski *et al.*, 2004; Pane *et al.*, 2005). In plants, sterility
702 factors may have to be identified, and their actions investigated, in individual genera
703 and species. Moreover, it should not be assumed that the sex-determining genes
704 necessarily function during flower development, or cause sterility. In monoecious
705 plants, a state that is ancestral to many dioecious species (Renner & Ricklefs, 1995;
706 Renner & Won, 2001), they might instead control the proportions of male and female
707 flowers, perhaps at developmental stages before flower parts are initiated (Figure 2B).
708 Unisexuality may be much more ancient than dioecy, and early, complete abortion of
709 male or female parts may be ancestral.

710

711 **Conclusions**

712 It is now technically feasible to use young sex chromosomes in non-model
 713 plants to test hypotheses about the initial evolution of suppressed recombination, and
 714 to study the time course of later evolution of sex chromosomes in older systems, as
 715 has been initiated in some animal systems (Bachtrog *et al.*, 2009). Young plant sex-
 716 linked systems should also be suitable for studying the earliest adaptations to dioecy,
 717 which have so far been little studied. The change from cosexuality to unisexuality
 718 may be accompanied by considerable expression changes, if unisexuality is released
 719 from conflicts between the two sex functions, so that changes can occur to optimise
 720 expression in each sex. For example, the non-dioecious *S. vulgaris* appears suitable as
 721 an outgroup for studying the evolution of changes in expression in the dioecious
 722 species *S. latifolia* (Marais *et al.*, 2011). Because hermaphrodite *S. vulgaris*
 723 individuals have both stamens and pistils, differences in unisexual individuals of the
 724 dioecious species that are caused directly by loss of these structures should be
 725 distinguishable from changes in expression of genes that are expressed in non-sex-
 726 specific structures. Such studies can potentially discover genes that can be expressed
 727 in both sexes, but that change when dioecy evolves, and evaluate whether, as has been
 728 predicted, the sex chromosomes, including the PAR, carry unexpectedly large
 729 numbers of such genes (Vicoso & Charlesworth, 2006; Vicoso *et al.*, 2013).

730

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733

734 **References**

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1167 **Figure legends**

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1169

1170 **Figure 1.** Possible histories of sex-linked regions. A. Sex-determining genome region
1171 with no prior history of suppressed recombination, in which recombination
1172 suppression evolves in response to a polymorphism at two sex-determining
1173 loci. B. Pericentromeric region with suppressed recombination, in which
1174 sex-determining loci evolve. The sex-linked region may later become
1175 extended by evolution of a further region of suppressed recombination,
1176 perhaps due to the establishment of a sexually antagonistic polymorphism
1177 on the same chromosome (see text). C. A take-over event in which a new
1178 male-determining gene arises in a genome region not previously involved
1179 in sex-determination; the figure illustrates the case when the new sex-
1180 determining gene is on a chromosome (indicated by a dashed line) different
1181 from the previously existing one, but a changed location on the same
1182 chromosome is also possible.

1183

1184 **Figure 2.** Evolution of sex-determining and sex-linked genome regions. A. Evolution
1185 of sex-determining genes in a genome region starting from an
1186 hermaphrodite ancestor, and of suppressed recombination in the region,
1187 forming a sex chromosome-like region, showing disadvantageous
1188 recombinants between the proto-Y and the proto-X chromosomes (the
1189 reciprocal recombinant would be hermaphroditic, and is not shown). If the
1190 female suppressor has male-specific expression (or evolves expression
1191 restricted to males), it can spread through the entire population, and create a
1192 single-gene sex-determining system (bottom left). B. Evolution of sex-
1193 determining genes in a monoecious ancestor.

1194

1195 **Figure 3.** Loss of genes from sex-linked regions. A. Chromosome before gene loss.
1196 The region that will evolve sex linkage includes four genes essential for
1197 vegetative functions in both sexes (green), and other genes essential only in
1198 males (blue) or females (pink), or affecting both sex functions (both
1199 colours). B. Loss of female function genes from a Y chromosome in a
1200 diploid plant, and loss of the third gene essential vegetative functions

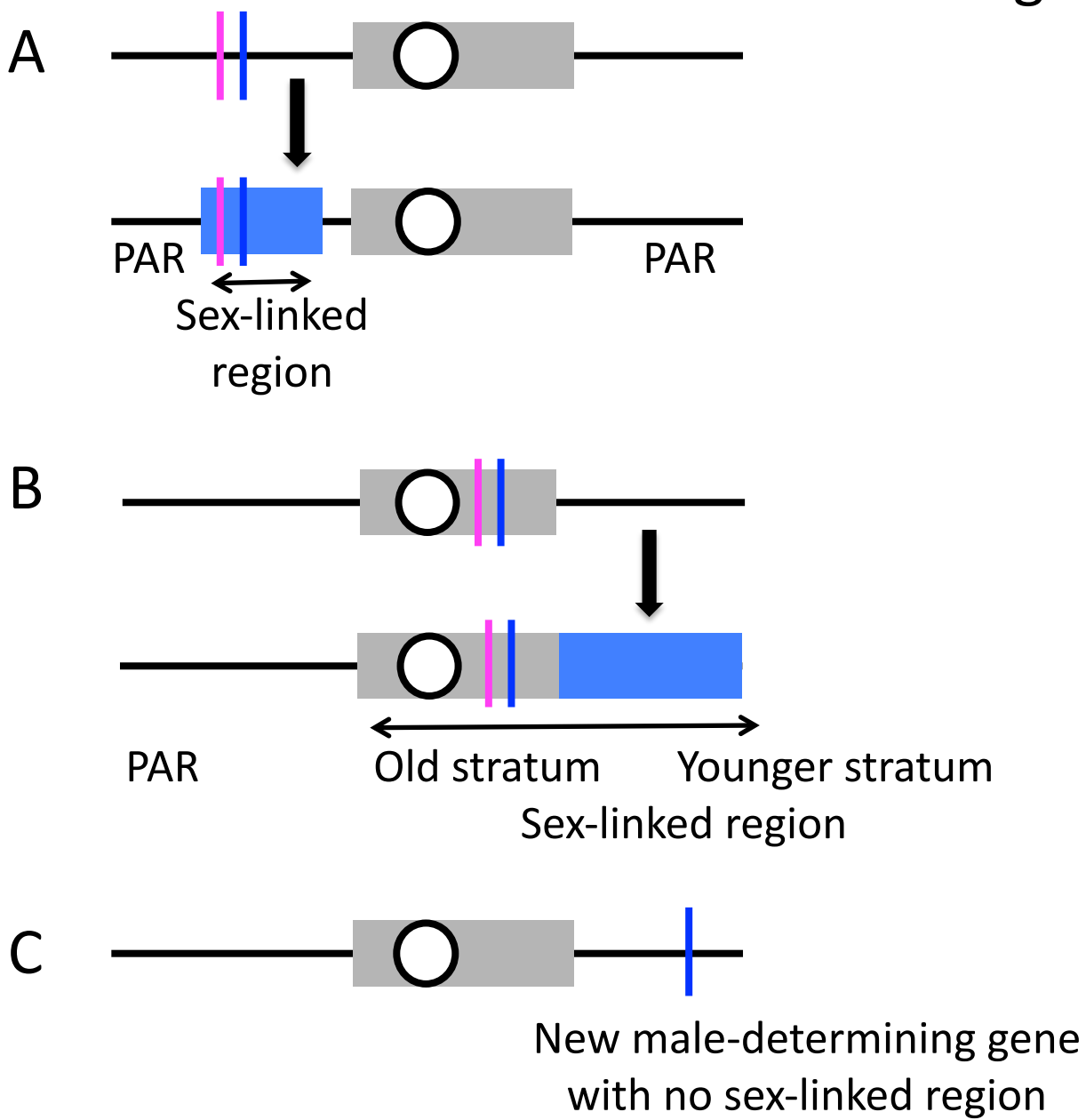
1201 (which is non-lethal due to the presence of the copy on the X chromosome).
1202 C. Loss of female and male function genes from male- and female-
1203 determining chromosomes in a haploid plant. Some genes with functions in
1204 the diploid vegetative stage could also be lost from either the U or the V.
1205

For Peer Review

Table 1. Some characteristics favourable for studying the genetics and evolution of plant sex chromosomes. The main text provides examples from plant studies.

Characteristic	Advantages	Specific evolutionary questions
A range of sex chromosome ages exist, including recently evolved ones and old-established systems	The time when recombination stopped can be estimated using a molecular clock, as it is often not long, and sequence differences will not be saturated, but will reflect times when recombination was suppressed	1. Which species without cytologically visible heteromorphism have sex-linked regions that include genes other than the sex-determining genes?
		2. Does recombination suppression always evolve, even in old-established systems, or does it sometimes fail to evolve (and, if so, why)?
	The earliest stages of sex chromosome evolution can be studied	1. Is there a tendency for chromosomal heteromorphism, heterochromatinisation, ZW systems, and X-autosome balance systems to be associated with older-established systems?
		2. How did recombination suppression evolve (gradually, or in distinct recombination suppression events affecting genome regions with many genes), and how often do such events happen?
Closely related non-dioecious outgroup species often exist	The directions of changes during sex chromosome evolution can be studied	3. Did repetitive sequences accumulate before genes started to lose functions, or does their accumulation contribute to loss of functions?
		1. Have plant X and/or Y chromosomes adapted to the new dioecious state?
Dioecy evolved repeatedly	The directions of changes during sex chromosome evolution can be studied	2. Have plant Y chromosomes degenerated genetically? If so, what is the time course?
		In taxa with many dioecious species, is dioecy ancestral or has it evolved several times; if the latter, are the same sex-determining genes involved, or different genes in different species?

Figure 1



KEY



Centromere



Sex determining genes

Regions with suppressed recombination



Pericentromeric region



Male-specific region

Figure 2

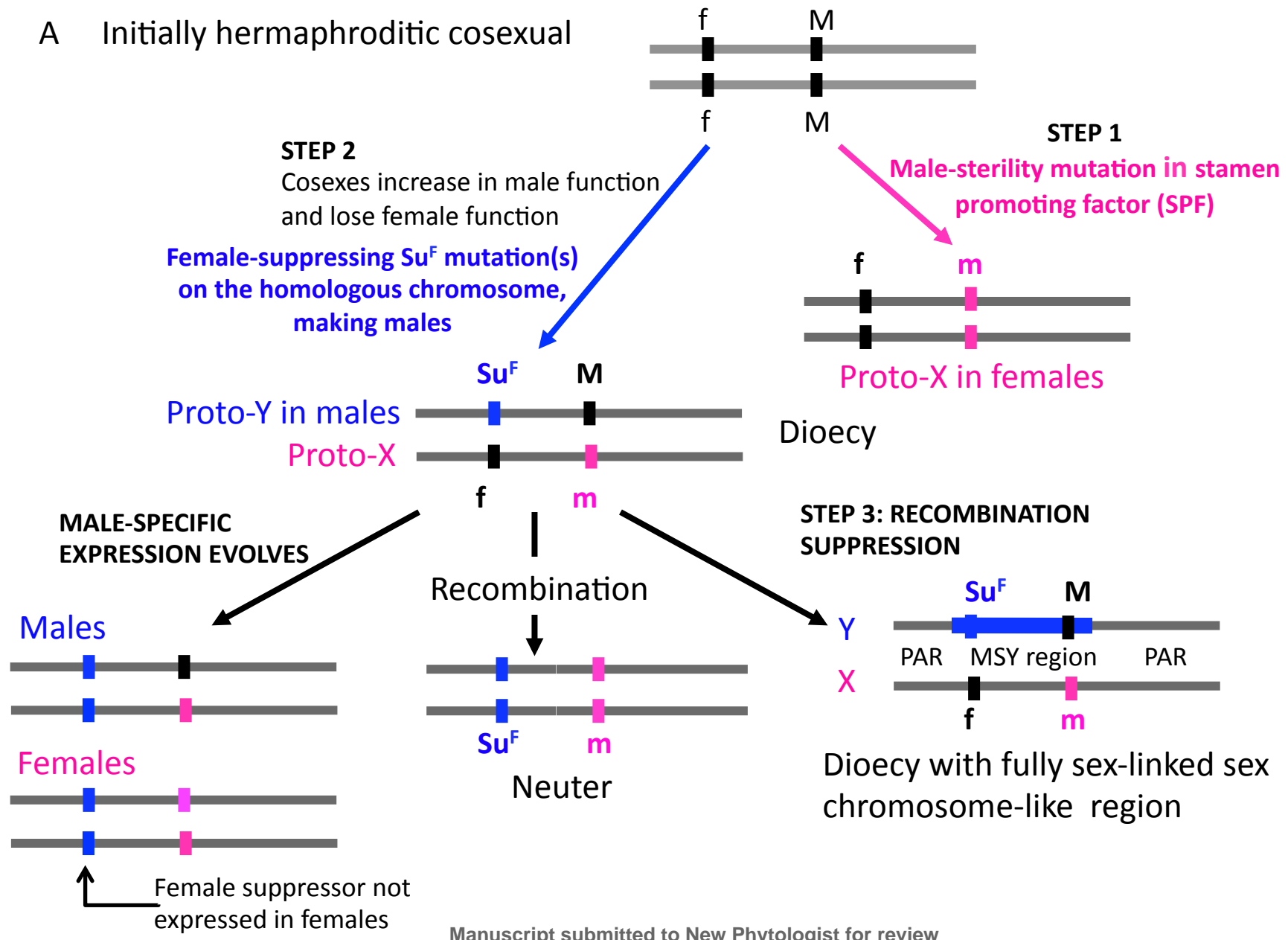


Figure 2, continued

B Initially monoecious cosexual

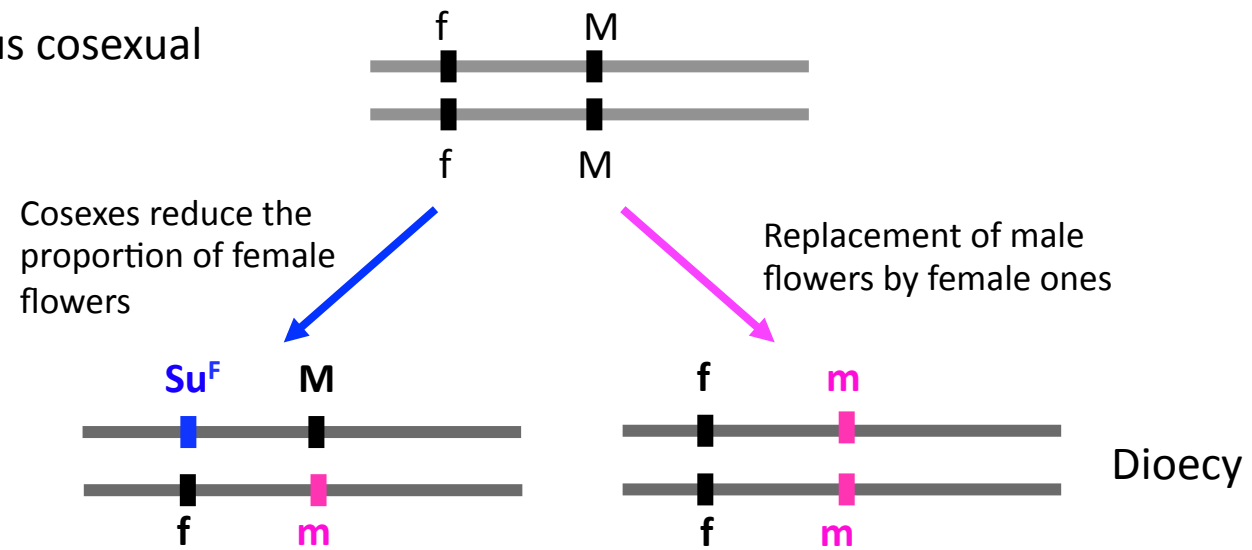
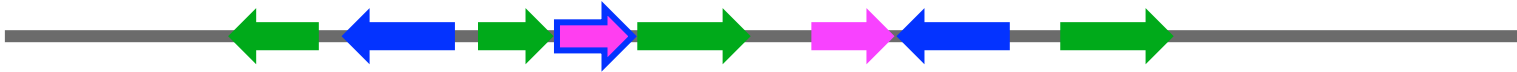
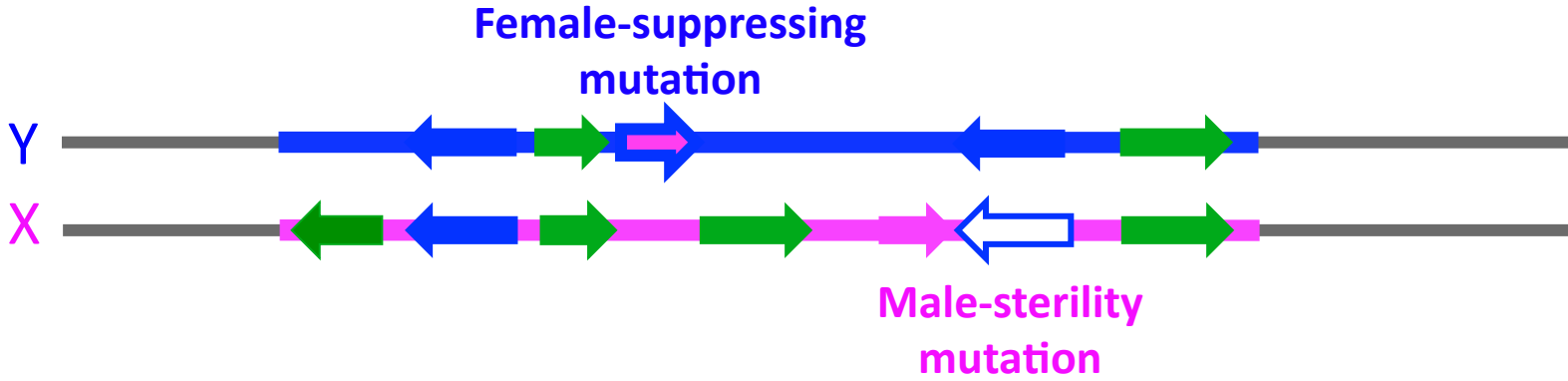


Figure 3

A



B



C

