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Citation for published version:

Charlesworth, D 2019, 'Young sex chromosomes in plants and animals', *New Phytologist*.
<https://doi.org/10.1111/nph.16002>

Digital Object Identifier (DOI):

[10.1111/nph.16002](https://doi.org/10.1111/nph.16002)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

New Phytologist

Publisher Rights Statement:

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1 **Young sex chromosomes in plants and animals**

2

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11

12 **Total word count** for the main body of the text (Introduction, Materials and Methods,
13 Results, Discussion, and Acknowledgements): 5,944

14

15

16 **Word counts for each section**

17 **1. Introduction:** 1,238 words

18 **2. Properties of initial sex-determining mutations:** 2,259 words

19 **3. Has close linkage evolved in response to a sex-determining locus, or did a sex-**
20 **determining mutation arise in a pre-existing non-recombining region?** 1,432 words

21 **4. Are single-gene systems derived?** 840 words

22 **5. The ages of plant and fish sex chromosomes:** 280 words

23 **6. Conclusions:** 532 words

24

25

26 Number of figures (indicating which figures should be published in colour): 3 (all with
27 colours)

28 Number of tables: 0

29 There is no supporting information

30

31

32 **Abstract (196 words)**

33

34 A major reason for studying plant sex chromosomes is that they may often be “young”
35 systems. There is considerable evidence for the independent evolution of separate sexes
36 within plant families or genera, in some cases showing that the maximum possible time
37 during which their sex-determining genes have existed must be much shorter than those of
38 several animal taxa. Consequently, their sex -linked regions could either have evolved soon
39 after genetic sex determination arose, or considerably later. Plants therefore include species
40 with both young and old systems. I review several questions about the evolution of sex-
41 determining systems and sex chromosomes that require studies of young systems, including
42 (i) the kinds of mutations involved in the transition to unisexual reproduction from
43 hermaphroditism or monoecy (a form of functional hermaphroditism), (ii) the times when
44 they arose, and (iii) the extent to which the properties of sex-linked regions of genomes
45 reflect responses to new selective situations created by the presence of a sex-determining
46 locus. I also evaluate which questions are best studied in plants, versus other suitable
47 candidate organisms. Studies of young plant systems can help understand general
48 evolutionary processes that are shared with the sex chromosomes of other organisms.

49

50 **Keywords in alphabetical order:** Flower development, gene duplications, sex chromosomes,
51 sex linkage, turnover events, Y chromosomes

52 1. Introduction

53 Although plant sex chromosomes are often stated to be “young”, the reasons for studying
54 young systems are less often explained. I here examine several questions concerning the
55 early evolution of sex-linked regions and sex chromosomes, and discuss the organisms in
56 which they can be studied, to clarify the advantages of studying sex chromosomes of plants.
57 As the box outlines, sex chromosomes, broadly defined, are surprisingly varied, and,
58 although their ages can only be defined relatively to those of such chromosome systems in
59 other organisms, and not in absolute terms, both young and old systems are present among
60 plants, which therefore include examples of most of the range of different sex chromosome
61 types possible.

62 I concentrate on the initiation and evolution of separate sexes (called gonochory by
63 zoologists, and dioecy here, following botanical usage), and genetic sex-determination. I
64 also consider the evolution of genome regions that carry sex-determining genes. In species
65 in which males are the heterozygous sex (male heterogamety), the fully sex-linked regions,
66 with no crossing over between the regions determining the two sexes are called Y- and X-
67 linked, and males are denoted by XY, whereas in species with female heterogamety, females
68 are heterozygous for Z- and W-linked regions and males are ZZ. Sex chromosomes often also
69 include regions that undergo genetic recombination, called “pseudo-autosomal” regions, or
70 “PARs”. Even if the fully sex-linked region is very small, for example, a single gene, and most
71 of it is a partially sex-linked PAR, the chromosome carrying the Y- (or W-) linked region may
72 still be called a sex chromosome. It is important to understand that such a chromosome will
73 often not have the characteristics of the most familiar ancient sex chromosomes, such as
74 those of mammals or *Drosophila* or birds, with major gene losses from the Y or W
75 chromosomes (genetic degeneration), causing hemizyosity in one sex (summarised in the
76 box).

77 In order to concentrate on the evolution of young sex chromosomes, I will not discuss the
78 interesting evolutionary consequences that follow from the evolutionary processes affecting
79 fully sex-linked regions in detail, including loss of functions of genes, or deletion of Y- or W-
80 linked genes, known as “genetic degeneration”. These have been reviewed recently (e.g.
81 Bachtrog, 2012), and have been detected in plants (Hough *et al.*, 2014), and are summarised
82 in the box. An interesting question, reviewed recently (Lorenzo *et al.*, 2018), concerns the

83 relative importance of deleterious mutations becoming fixed in the Y (or W) chromosome
84 population, versus accumulation of repetitive sequences, which are predicted to accumulate
85 in non-recombining regions (Charlesworth *et al.*, 1994). Transposable elements are a major
86 proportion of repetitive sequences in plant genomes, and their accumulation can account
87 for low gene densities in sex-linked regions (Wang *et al.*, 2012). They may also contribute to
88 genetic degeneration, as their transposition can cause mutations, including deletions and
89 chromosome rearrangements. Genome processes that evolved to suppress their
90 transposition can also suppress expression of nearby genes (Hollister & Gaut, 2009),
91 perhaps initiating Y chromosome degeneration (Zhou & Bachtrog, 2012).

92 Studying young systems requires evidence supporting their recent evolutionary origins. One
93 definition of young systems is that they have not yet undergone major degeneration, for
94 example the Y-linked region in *Asparagus officinalis* (Harkess *et al.*, 2017b), or the guppy, a
95 fish whose Y chromosome appears to carry all genes found on the X (Bergero *et al.*, 2019).
96 However, these are rough measures of age, as degeneration rates depend on many
97 organism-specific properties (see box). Estimating the rate of degeneration requires having
98 reliable age estimates, including ages relative to those of other sex chromosome systems, as
99 outlined in the box. I shall not discuss approaches for estimating the ages of sex
100 chromosomes in detail, but note here that each of the two main approaches has advantages
101 and disadvantages. Phylogenetic studies can identify clades with genetic sex determination,
102 and the clade's absolute age in generations or years can be estimated using divergence for
103 synonymous or silent sites in DNA sequences, preferably using multiple nuclear gene
104 orthologues in different species (such divergence can also be used to assess relative ages,
105 ideally taking account of substitution rate differences). This might not identify the origin of
106 the system correctly, because it is difficult to distinguish between multiple recent
107 evolutionary changes, and the existence of an ancestral system that has reverted several
108 times and lost dioecy. Figure 1 illustrates different evolutionary histories for a set of species
109 with some dioecious and some hermaphrodite taxa, starting from an ancestrally functionally
110 hermaphrodite state (including monoecy in a plant).

111 Alternatively, estimates can be based on divergence between sequences of genes in the sex-
112 linked region (if one exists). Divergence between X and Y sequences of a fully sex-linked
113 gene estimates the time when this "male-specific Y", or MSY, region stopped recombining

114 with its X counterpart (and similarly for W-Z divergence). However, different sex-linked
115 genes may stop recombining at different times in the system's evolution. So-called
116 "evolutionary strata", each representing a chromosome region that stopped recombining at
117 a given time, and differing in divergence from other strata, have been found in mammals
118 (Lahn & Page, 1999), several plants (Bergero *et al.*, 2007; Wang *et al.*, 2012), and a fish, the
119 threespine stickleback (Schultheiß *et al.*, 2015; White *et al.*, 2015).

120 In addition to helping assign time of origin of sex chromosome systems, strata can help
121 identify the sex-determining locus, as this is expected to lie within the oldest sex-linked
122 region. The discovery of such strata is also important because their presence on sex
123 chromosomes tells us that recombination stopped in events subsequent to the initial
124 establishment of a sex-determining region on the chromosome, suggesting that
125 recombination suppression is an evolved response to sex-specific adaptation (see the box
126 and the sections 2 and 3 below).

127 However, the existence of strata is still difficult to test, as exemplified by uncertainties in sex
128 chromosomes of fish such as the Atlantic cod (Kirubakaran *et al.*, 2019) and some cichlid
129 species (Gammerdinger & Kocher, 2018a). Strata are most likely to be detectable in
130 moderately young sex chromosomes. Detecting strata requires a reliable physical map of
131 the chromosome carrying the sex-determining genes. The X chromosome genetic map is
132 often used as a proxy for the physical arrangement of the sequences used. Sex-linkage can
133 be identified if variants with X-linked inheritance are discovered, or genome regions are
134 found with lower representation in one sex than the other, indicating hemizygoty in males
135 in XY systems (or females in ZW systems). Chromosome level genome assembly methods
136 are now becoming reliable enough to avoid the need for laborious genetic mapping and
137 directly obtain physical arrangements for both X and Y chromosomes. However, several
138 difficulties in detecting strata remain. First, Y-X divergence estimates require Y-linked
139 sequences. Genes may be very rare in ancient systems' degenerated old strata (see Box).
140 Assigning a time of origin of the sex chromosome requires divergence estimates for
141 sequences from the region that stopped recombining longest ago; unless the oldest stratum
142 is included, the age will be under-estimated. It may also be impossible to estimate enough
143 sequence divergence values to detect clear differences between different sex chromosome
144 regions, given these estimates' high variances. Moreover, sequence divergence between the

145 two sex chromosomes may be saturated in old strata, further obscuring any differences
146 defining strata. The small number of species where sex chromosome strata have been
147 identified should increase as sequence information become available from more species,
148 especially as knowledge of systems ages accumulates, and suitable ages can be targeted for
149 genome sequencing.

150

151 **2. Properties of initial sex-determining mutations**

152 One reason for studying young sex chromosomes is to discover the genes that may have
153 been involved in the change from an ancestral functionally hermaphrodite state, including
154 (in plants) monoecy, to two separate sexes. Discovering the genes involved will reveal
155 whether the mutations actually involved have the properties assumed in these models.
156 Different routes to dioecy can be imagined, involving at least two initial mutations, one to
157 produce each sex (Charlesworth, B & Charlesworth, D, 1978). Because transitions from
158 functional hermaphroditism to dioecy have occurred many times in flowering plants
159 (Charlesworth, 1985; Renner, 2014), they are particularly good for studying which of these
160 possible routes has, in fact, been involved.

161 A mutational model that seems plausible for natural transitions to dioecy from
162 hermaphroditism (rather than requiring intervention by plant geneticists, see below), seems
163 likely most often to involve a major male-sterility mutation, creating females and producing
164 a gynodioecious population, followed by one or more mutations converting the
165 hermaphrodite individuals to more male-like forms (Westergaard, 1958). This genetic
166 pathway can apply to an ancestor with hermaphrodite flowers or a monoecious ancestor. In
167 the simplest case, the second mutation converts (functional) hermaphrodites to full males,
168 with complete loss of female functions. Males created by a female-suppressor mutation can
169 be advantageous once females are established in a population, but female fitness is
170 inevitably decreased, and the mutation is sexually antagonistic. Both loci will become
171 polymorphic in the population (since neither male- nor female-sterility can become fixed),
172 generating selection for closer linkage between the two mutations (see next section).

173

174 *Sterility through loss-of-function mutations and through suppressive mutations*

175 Genetic data show that mutations in distinct genes are involved in several plants where
176 relevant information can be obtained. Examples where the ancestor was probably
177 monoecious include *Ecballium* (Cucurbitaceae) and spinach (Amaranthaceae), *Sagittaria*
178 *latifolia* (Alismataceae), and the date palm genus *Phoenix* (Westergaard, 1958; Dorken &
179 Barrett, 2004; Nadot *et al.*, 2016; Torres *et al.*, 2018; Wadlington & Ming, 2018). There is
180 also good evidence for the involvement of two distinct genes in at least two species where
181 dioecy appears to have evolved from hermaphroditism, *Silene latifolia*, based on the
182 phenotypic effects of deletions of Y-linked regions (Westergaard, 1958; Kazama *et al.*,
183 2016), and *Asparagus officinalis*, in which genome sequencing yielded candidate male-
184 sterility and female-suppressing genes (Harkess *et al.*, 2017b; Harkess *et al.*, 2018). Many
185 loci in plants can generate loss-of-function mutations causing male sterility (Ohnishi, 1985;
186 Timofejeva *et al.*, 2013), and such mutations are generally recessive, as assumed in the
187 model above. The resulting female homozygotes could have high-fitness if inbreeding
188 depression disfavors individuals with both male and female functions (Charlesworth, B &
189 Charlesworth, D, 1978). It is important to evaluate the evidence for the two gene model in
190 as many plants as possible, because, as illustrated in Figure 2, it predicts that recombinant
191 combinations of alleles will be disfavoured, favouring close linkage and subsequent
192 suppression of recombination, between the two genes involved (Charlesworth, B &
193 Charlesworth, D, 1978). This model therefore potentially explains the repeated loss of
194 recombination that appears to have evolved repeatedly between members of sex
195 chromosome pairs (Bull, 1983). If the two locus hypothesis can be excluded in an organism
196 with a sex-linked region where empirical testing is possible, other evolutionary causes must
197 be sought for the evolution of its sex-linked region, as will be discussed below. It should be
198 noted, however, that it is currently unclear how many cases of the evolution of dioecy have
199 been followed by recombination suppression events (see section 3 below).

200

201 *Trade-offs during reproduction and possible evolution of dioecy by two mutations in a single*
202 *primary sex-determining gene*

203 Mutations in separate genes, need not necessarily always be involved. An important
204 assumption of the model just outlined is that the second mutation is unlikely to be a simple
205 sterility mutation. Population genetic analysis shows that avoidance of inbreeding

206 depression cannot be the sole selective force favouring males, so that considerably
207 increased male fertility, relative to that of the ancestral hermaphrodites, is required to
208 compensate for loss of female fertility (Charlesworth, B & Charlesworth, D, 1978).
209 Furthermore, while femaleness is often recessive (consistent with a loss-of-function
210 mutation creating XX females), the mutation creating males must be expressed in
211 heterozygous (XY) individuals. Partial dominance of the female suppressor suggests that it
212 might control resource allocations during flower development. Re-allocating the functional
213 hermaphrodites' resources towards greater investment in male functions can be
214 advantageous in gynodioecious populations.

215 This idea of a trade-off between male versus female functions further suggests that a single
216 gene could, in principle, undergo two mutations to produce both sexes from an ancestral
217 functionally hermaphrodite state: mutations reducing male functioning should increase
218 female function, and *vice versa*, potentially producing a single sex-determining gene. A gene
219 with such developmental properties has indeed been discovered in a liverwort whose
220 gametophytes are either male or female, *Marchantia polymorpha* (Hisanaga *et al.*, 2019). It
221 appears to encode an MYB class transcription factor protein whose loss (or loss-of-function),
222 converts female gametophytes to males that produce non-functional sperm (they
223 presumably lack sex-linked genes essential for producing functional sperm). An anti-sense
224 transcript expressed from the same gene acts to repress the transcription factor, and its
225 loss-of-function mutations convert male gametophytes into non-functional female ones. A
226 function in directing development into male or female pathways is therefore likely.
227 However, this gene is not sex-linked in *M. polymorpha*, and is therefore not the primary sex-
228 determining gene, but must act downstream of the primary control.

229 A situation in which partial (rather than complete) loss of male or female functions is
230 plausible involves male-female flower ratios in monoecious plants, although mutations
231 reducing the proportion of male flowers are less likely to be advantageous, compared with
232 sterility mutations, as the rate of self-fertilisation might often be only slightly reduced
233 (Charlesworth, D & Charlesworth, B, 1978). However, the assumptions of this theoretical
234 model have been little tested, and more studies of the relationship between male/female
235 flower numbers and selfing rates in monoecious plants would be helpful. Furthermore, if
236 the argument above is correct that such mutations should generally show intermediate

237 dominance, the recessivity of one sex observed in many plants and animals is puzzling: even
238 in plant systems that are probably young, male heterogamety, with femaleness being
239 recessive, is common (Westergaard, 1958).

240 In a species that has recently evolved dioecy, new, secondary mutations improving the
241 functioning of one sex may quickly become incorporated in genome regions closely linked to
242 the sex-determining gene(s), and will be hard to distinguish from the initial mutations. The
243 genome region closely linked to the sex-determining locus of *Asparagus officinalis* includes
244 several genes likely to affect development of flower parts (Harkess *et al.*, 2017a; Murase *et al.*,
245 2017). Genes with sex differences in expression are also found unexpectedly often in the
246 14.5 Mb apparently fully sex-linked region of the *Mercurialis annua* genome (Veltsos *et al.*,
247 2018) and a 2 Mb pericentromeric region on the *Salix purpurea* chromosome, 15, carrying
248 this species' ZW system's sex-determining locus (Carlson *et al.*, 2017). Gender-specific
249 expression is, of course, also found for genes unlinked to such regions (West & Golenberg,
250 2018), reflecting either effects in development downstream of the sex-determination step,
251 or adaptations to the separate sexed condition. The extent to which newly dioecious plants
252 adapt to unisexual functioning is not yet clear, but gene expression changes can suggest
253 possible adaptive changes. For example, female *Silene vulgaris* (a gynodioecious species in
254 the Caryophyllaceae) have been used to estimate the direct effects of loss of androecium on
255 gene expression in flower buds, and show that expression changes in *S. latifolia* females are
256 larger, consistent with evolved changes in this dioecious species (Zemp *et al.*, 2018).

257

258 *Other mutation types that could generate dioecy*

259 Hypotheses for routes to the evolution of dioecy are not restricted to the mutation types
260 outlined above (the two gene model with male-sterility followed by a female suppressor
261 was proposed in order to understand why recombination often becomes suppressed in
262 genome regions carrying sex-determining loci). In persimmon (Ebenaceae, a dioecious
263 system whose age is intermediate between those of many fish discussed below and those of
264 ancient sex chromosomes in mammals and birds) two mutations were involved, with
265 properties differing from those in the model outlined above, as illustrated in Figure 3; the
266 figure is adapted from the model proposed for this system (Akagi *et al.*, 2014) to show a
267 plausible evolutionary pathway. As in the loss-of-function scenario above, a mutation in a

268 gene named *MeGI* probably created females first. In the ancestral hermaphrodite, this gene
269 promotes male function, perhaps by permitting expression of another gene necessary for
270 some male function. The mutant product suppresses the male function gene's expression,
271 producing females; this would not be expected to be fully recessive, but should act even in
272 heterozygotes. Experiments expressing *MeGI* in the hermaphroditic plant *Arabidopsis*
273 *thaliana* suggest that androecium sterilization leads to increased female functions through a
274 trade-off effect, as Darwin (Darwin, 1877) suggested, rather than *MeGI* having a second
275 action directly promoting gynoecium development (Akagi *et al.*, 2014). Like any male-
276 sterility mutation, this mutation might establish a polymorphism for females in the
277 population (provided that deleterious pleiotropic effects do not prevent this).

278 A non-mutant *MeGI* copy duplicated onto a different chromosome, named "*OGI*", then
279 arises and dominantly blocks the expression of the *MeGI* gene, changing the ancestral
280 bisexuals into males (Akagi *et al.*, 2014). In the presence of the duplication, *MeGI*'s wild-type
281 male-promoting action is assumed to be stronger than that of the ancestral allele, so that
282 female functions are suppressed through the trade-off effect proposed above. As in the
283 loss-of-function mutational model outlined above, males could have become advantageous
284 once females became common in the population.

285 In this model, the *MeGI* gene polymorphism can be lost, as all members of the population
286 can carry the *MeGI* mutation that causes femaleness, because this is active only in females
287 (in which its product blocks male functions). The resulting population would then be
288 polymorphic only for the *OGI* duplicate, and the chromosome with this gene would behave
289 as a Y-linked region with a single gene controlling whether the individual is male or female
290 (even though the developmental system involved of course includes several other genes,
291 and the evolution of the present system involved at least two mutations).

292 Note that the term "Y-linked region" here does not imply that suppressed recombination
293 has evolved, but merely that a sex-determining locus is present. However, it is worth noting
294 that the insertion of the *OGI* duplicate into an ancestral autosome (indicated as a black line
295 in Figure 3), creates a region that is hemizygous in males, and might prevent pairing with its
296 homologue. If so, the surrounding genome region might include several co-segregating
297 genes with no involvement in sex-determination, resembling a miniature version of the
298 situation in sex chromosomes that have evolved suppressed recombination. In the

299 persimmon, an approximately one megabase region shows Y linkage (Akagi *et al.*, 2014).
300 Suppressed recombination near such an inserted male-determining gene is not an evolved
301 response to the presence of a sex-determining gene, and certainly not to a situation with
302 two partially linked polymorphic genes selecting against recombinant haplotypes.

303 This complicated model is just one possibility for systems in which a single gene controls
304 male versus female development. It was long thought that “originally there must have been
305 just one gene that played the critical role in the sex-deciding process” (1932), and separate
306 sexes can indeed be produced in the normally monoecious maize, by combining recessive
307 mutations in two maize genes (reviewed by Jones 1934). Homozygotes for the silkless
308 mutation are males, due to failure of female flowers, but homozygosity for the tasselseed
309 mutation causes male (terminal) inflorescences to form flowers with female function. In a
310 population of silkless homozygotes, gender can therefore be controlled by segregation of
311 alleles of the single tasselseed gene, with males heterozygous for the wild-type allele,
312 resembling a dominant maleness factor. Unlike the persimmon case, however, the wild-type
313 silkless allele must be deliberately excluded, because the females would be eliminated
314 without artificial intervention, due to low fertility.

315

316 *Predicted lack of pleiotropy*

317 As explained above, sterility mutations involved in the natural evolution of separate sexes
318 must confer some fitness advantage: females avoid producing inbred offspring, and males
319 can optimize their resource allocation in response to the presence of females. There is
320 evidence for both these effects in plants evolving dioecy, for example in *Wurmbea species*
321 (Liliaceae) (Barrett, 1992; Ramsey *et al.*, 2006). Also, it is obvious that mutations with
322 detrimental pleiotropic effects on other tissues should rarely be involved in the evolution of
323 dioecy: sterility mutations in plant genes that act in the anthers or their development will be
324 eliminated unless female functions are unaffected (or enhanced), and vice versa. With
325 modern developmental approaches and understanding of flower development, it may be
326 possible to test this prediction. For instance, transgenic experiments in hermaphrodite
327 species could be used to test genes whose loss of function causes male or female sterility, to
328 discover which genes have such side effects, and whether candidate primary sex-
329 determining genes identified on actual sex chromosomes do not. Hermaphrodites closely

330 related to dioecious species could also be used to test for the expected resource re-
331 allocation. Given the shared flower developmental systems of angiosperms, even unrelated
332 species could potentially be used in testing for pleiotropy or its absence. An experiment
333 expressing the persimmon *MeGI* feminization factor in the distantly related hermaphrodite
334 plant *Nicotiana tabacum*, using *N. tabacum* promoters, indeed found few pleiotropic
335 effects, though high expression of *MeGI* in *Arabidopsis thaliana* led to sterile androecia (as
336 expected), and normal carpels, but also stunted growth (Akagi *et al.*, 2014).

337

338 **3. Has close linkage evolved in response to a sex-determining locus, or did a sex-**
339 **determining mutation arise in a pre-existing non-recombining region?**

340 Non-recombining completely sex-linked genome regions could arise in several ways. Models
341 of the evolution of dioecy involving mutations in separate genes, and interacting in a
342 manner that creates a disadvantage to recombinants (which, as outlined above, is not
343 always the case), select for closer linkage between the two mutations (Bull, 1983). This
344 could sometimes create sex-linked regions large enough to include many genes. In contrast,
345 as mentioned above, an inserted male-determining gene could directly create a small sex-
346 linked region.

347 *Single gene systems*

348 Selection favouring closer linkage between two mutations occurs only if the mutations
349 initially recombine; if they are within the same gene, they will be closely linked from the
350 time they originated, again forming a small sex-linked region. Single gene systems have
351 been discovered in several fish, including fugu (Kamiya *et al.*, 2012) and Atlantic salmon
352 (Kirubakaran *et al.*, 2019), and could be involved in plants with homomorphic sex
353 chromosomes, which appear to be common among angiosperms (Westergaard, 1958). A
354 single gene system can evolve via two mutations, one of which becomes fixed in the species
355 (as appears to have happened in the persimmon, see above). In the next section, I explain
356 how such systems could evolve by duplications and in other ways involving single genes
357 taking over the sex-determining function. Before doing so, I note that extensive fully sex-
358 linked regions can arise if new sex-determining genes evolve in genome regions that lack
359 recombination.

360 *Evolution of a new sex-determining gene in a non-recombining genome region*

361 Another situation in which existence of a fully sex-linked region does not imply selection
362 driven by the presence of sex-determining genes is when such genes evolve within an
363 already non-recombining genome region. Current information about the relationship
364 between physical and genetic maps is scanty, as both well-assembled genome sequences
365 and genetic maps are required. However, it is now possible to get relevant data if a
366 chromosome level assembly of a species' genome sequence can be obtained and combined
367 with genetic mapping of families. Clearly, this is still difficult, as large genomes with
368 substantial repeat content, such as those of many organisms, are difficult to assemble and
369 will currently inevitably include some errors. Obtaining families from species of interest for
370 sex chromosome evolution is also often difficult. Among angiosperms, dioecious species
371 tend to be trees or shrubs (Darwin, 1877; Renner, 2014), rather than species with short
372 generation times suitable for genetic studies. Moreover, many such plants have large
373 genome sizes that are difficult to assemble, and some have undergone whole genome
374 duplications (Pellicer *et al.*, 2018).

375 Nevertheless, many dioecious plants have non-dioecious relatives that may allow
376 comparisons to test whether a sex-determining region evolved its non-recombining state in
377 the newly dioecious lineage. Currently, few comparisons of recombination patterns have
378 been published, and it is rarely known whether recombination changed after a species
379 evolved separate sexes, or after a new male-determining factor has appeared on a
380 chromosome. In the ninespine stickleback, a maleness factor has evolved in a region that
381 rarely recombines (due to a polymorphic inversion); this case is an example where the lack
382 of recombination pre-dates its sex-determining role (Natri *et al.*, 2019).

383 It may be common for a sex-determining locus to evolve within a pericentric genome region
384 in which recombination is rare. Genetic maps in animals and plants, pooled for male and
385 female meiosis, are revealing that (for evolutionary reasons that are not well understood)
386 such regions can represent large proportions of chromosomes' physical sizes, including in
387 maize (Kianian *et al.*, 2018) and *A. thaliana* (Giraut *et al.*, 2011). In barley (*Hordeum*
388 *vulgare*), crossovers are localised to small end regions of the physically huge chromosomes,
389 based on genotyping single pollen grains (Dreissig *et al.*, 2015; Dreissig *et al.*, 2017).

390 Although the central regions have low gene density, some genes are present and sex-

391 determining loci could evolve in these regions. In *Petunia*, the genes controlling self-
392 incompatibility types, which requires maintaining suitable combinations of alleles of at least
393 two genes (similar to the two-gene model for the evolution of separate sexes), evolved in a
394 pericentric region that recombines rarely (TenHoopen *et al.*, 1998). Sex-linkage from the
395 moment a sex-determining locus arises, for this reason, has been proposed for the oldest Y-
396 linked region of papaya (*Carica papaya*) (Zhang *et al.*, 2008). The sex-linked regions in
397 spinach species (a genus in which dioecy probably evolved from a monoecious ancestral
398 state) may also have arisen in this way. The homomorphic XY chromosome pair exhibits
399 extensive synteny with sugar beet chromosomes 4 and 9, and protein-coding genes closely
400 linked to the male-determining locus in spinach are located in the putative pericentromeric
401 and centromeric regions of these *Beta vulgaris* chromosomes (Takahata *et al.*, 2016), while
402 much of the pair recombines. High-density mapping identified an 18 Mb region of linkage
403 group 4, with 143 genes, as fully sex linked (Qian *et al.*, 2017); its location is consistent with
404 a pericentromeric region, as is the low gene density of the region (Kudoh *et al.*, 2018). This
405 may be a young system, as a rare viable YY homozygote has been observed, suggesting that
406 there has not been enough time for loss of essential genes from the Y (Wadlington & Ming,
407 2018). Interestingly, the homologous chromosome (carrying sequences that show sex-
408 linkage in different *Spinacia* species) is heteromorphic in one species, probably due to a
409 pericentric inversion that changed the arm ratio by altering the centromeric position (Fujito
410 *et al.*, 2015). It is not yet known whether this event expanded a fully sex-linked region, or, if
411 so, whether the expansion was favoured by the appearance of a mutation affecting sexual
412 dimorphism. However, intriguingly, inflorescence dimorphism is more pronounced in the
413 species with the expanded MSY, and mutations in a gene a few centiMorgans away lead to a
414 highly staminate monoecious phenotype (Onodera *et al.*, 2011).

415 Similarly, if recombination occurs only in one sex, any chromosome acquiring a sex-
416 determining factor will immediately become sex-linked. I am not aware of any plant with
417 such extreme sexual dimorphism in crossing over, but more analyses are needed of male
418 and female meiosis separately, in species with separate sexes. Highly sexually dimorphic
419 recombination patterns have recently been found in fish, for example a salmonid
420 (Sutherland *et al.*, 2017). In another fish, the guppy (*Poecilia reticulata*), cytogenetic
421 visualisation of crossovers in male meiosis suggests localisation near the tips of all the

422 acrocentric chromosomes (Lisachov *et al.*, 2015). Genetic mapping results (Bergero *et al.*,
423 2018) suggest that this sex difference in crossing over accounts for the large regions of the
424 chromosome carrying the sex-determining locus that do not recombine in male meiosis.
425 Genetic maps of related species should help distinguish between (i) a non-recombining state
426 pre-dating the present sex-determining locus evolved, versus (ii) selection against X-Y
427 recombination after this locus appeared on chromosome 12, leading to evolution of
428 genome-wide chiasma localisation in male meiosis.

429 Why recombination patterns or rates often differ between the sexes remains unresolved.
430 Hypotheses include differences between male and female meiosis (which can account for
431 differences observed in hermaphrodite species), or selection that is related to sex-linked
432 regions (Lenormand, 2003; Lenormand & Dutheil, 2005). The genetic control of localisation
433 patterns is also not currently clearly understood in any species, though genetic differences
434 in genomic patterns of recombination events, as well as rates, have been documented.,
435 including a difference in female meiosis between two closely related *Drosophila* species
436 (Brand *et al.*, 2018); males of almost all *Drosophila* species lack crossing over.

437

438 *Gene duplications in fish and plant sex determination*

439 Non-recombining regions may also arise through insertion of a duplicate gene copy into a
440 chromosome (see above). An insertion/deletion difference between the two alleles at the
441 distyly locus in plants in the genus *Primula* (Huu *et al.*, 2016; Li *et al.*, 2016), may have
442 prevented recombination between the two, and the asparagus sex-determining region
443 appears to lack recombination for the same reason (Harkess *et al.*, 2017b; Harkess *et al.*,
444 2018).

445 In a fish, the medaka (*Oryzias latipes*), the chromosome carrying the sex-determining locus
446 is cytologically homomorphic, but has an insertion of a duplicate of a member of a gene
447 family with involvement in male-determination in other species; this initial 43-kb has
448 subsequently accumulated 137 kb of repetitive sequences (Kondo *et al.*, 2009).

449 Recombination is suppressed near the sex determining region, presumably due to the
450 absence of a homologous sequence with which to pair, while the rest of the chromosome
451 recombines and appears to be pseudo-autosomal (Kondo *et al.*, 2001); a small inserted

452 region creates hemizyosity that may prevent recombination in the region, and in flanking
453 regions that also cannot pair. Overall, however, this species' total genetic map lengths are
454 similar in both sexes (Kimura *et al.*, 2006).

455

456 **4. Are single-gene systems derived?**

457 As described above, the genes involved in the initial evolution of separate sexes sometimes
458 have properties that can lead to establishment of two-gene polymorphisms and will
459 generate selection for closer linkage. However, if single gene systems prove to be common,
460 an important question is whether they represent the loci involved in the initial evolution of
461 separate sexes, or a derived state that has replaced an ancestral system. In either case, the
462 sex chromosomes evolve from autosomes, but *de novo* evolution of sex-determining genes
463 in an ancestral hermaphrodite should be distinguished from the appearance of a new sex-
464 determining locus in a lineage that had already evolved separate sexes.

465 Changes of the second kind are well documented in several organisms, particularly in fish.
466 Two types of event are possible. First, a new gene may take control of the sex determination
467 system (bottom part of Figure 2). This must involve a gene in the system that controls
468 development of the sex structures (Wilkins, 1995), or interacts with such a gene, for
469 example in the rainbow trout (Bertho *et al.*, 2018). Many examples are known in fish where
470 the sex-determining loci of related species are on different chromosomes. Examples are
471 known in the genus *Oryzias* (Myosho *et al.*, 2015), and among cichlids, in which, among only
472 14 species that evolved in the past approximately 45 MY, 11 different chromosomes carry
473 the sex-determining locus (Gammerdinger & Kocher, 2018a). Sometimes the new region
474 carrying the locus is physically small, and includes no candidate sex-determining genes
475 shared between the species. A change to a new location on the same chromosome is less
476 easily detectable, but fine-scale genetic mapping has revealed an example in the pufferfish
477 genus *Takifugu* (Ieda *et al.*, 2018). Transitions are known both from XY to ZW systems, and
478 vice versa (Gammerdinger & Kocher, 2018a).

479 It is difficult to distinguish between "take-over" events, versus movement of an existing sex-
480 determining gene to a new genome location. Both types of movement seem to have
481 occurred in house flies (Meisel *et al.*, 2017). Plants with young sex chromosomes may also
482 have undergone such events, and may be excellent species for studying them and

483 understanding the situations that promote such changes. The sex-determining factors in
484 several closely related species of section *Otites* in the genus *Silene* map to different
485 chromosomes, all non-homologous with the XY pair in *S. latifolia* and its close relatives
486 (Balounova *et al.*, 2019). Both male and female heterogamety are found among these
487 species, yet the sex chromosomes in this section evolved very recently, and the non-
488 recombining region of the *S. otites* W-chromosome carries few genes. One hypothesis,
489 involving mutational load (Blaser *et al.*, 2013) favours a new (or re-located) sex-determining
490 locus if a sex-linked region is old enough to have undergone genetic degeneration, but has
491 not yet evolved dosage compensation. This probably does not explain the changed sex-
492 determining regions in section *Otites*, but it may explain changes in other taxa that
493 repeatedly evolved new sex-determining loci. Other classes of models to account for such
494 “turnover” events are reviewed by Blaser *et al.* (reviewed in Blaser *et al.*, 2013). They
495 include sex ratio selection, including responses to sex ratio distortion due to meiotic drive
496 (Scott *et al.*, 2018), a polymorphism for sexually antagonistic alleles making a new sex-
497 determining mutation advantageous if it arises in a closely linked region (vanDoorn &
498 Kirkpatrick, 2007), escape from aneuploidy caused by chromosomal inversions associated
499 with non-recombining sex-linked regions (Blackmon & Brandvain, 2017). Selectively neutral
500 changes can also lead to changed sex determination in a population, because dominant
501 alleles are predicted to replace ones with lesser dominance (Veller *et al.*, 2017; Saunders *et al.*
502 *et al.*, 2018).

503 No case is yet known of a plant with a different sex-determining locus replacing an
504 established one, but movements of a pre-existing sex-determining factor have been
505 uncovered in octoploid species in the plant genus *Fragaria* (Tenessen *et al.*, 2018). Fine-
506 scale genetic mapping, combined with sequencing, identified similar genes and their
507 flanking sequences in different locations at which sex-determining factors map. This plant
508 seems to have undergone transposition events very similar to those of the region containing
509 the ~ 4kb sex-determining locus that is inferred to have moved between different
510 chromosomes in Atlantic salmon, and its close relatives (Woram *et al.*, 2003; Faber-
511 Hammond *et al.*, 2015; Lubieniecki *et al.*, 2015); recent results are reviewed in Kijas *et al.*
512 (2018). The insertions may prevent recombination in the small *Fragaria* genome regions
513 affected (Tenessen *et al.*, 2018), as in the case of the insertion in the medaka fish.

514 However, extensive degenerated non-recombining regions are not created, and therefore
515 the movements probably again did not occur due to selection created by mutational load. It
516 is currently unknown how such transpositions occur; they could involve recombination
517 between homeologous chromosomes of these polyploid species, or movements by
518 transposable elements (TEs), some of which have the potential to move host genes, for
519 example in maize (Morgante *et al.*, 2005; Dong *et al.*, 2011). TEs have contributed to
520 duplicative movements of nucleotide-binding site (NBS)-encoding disease resistance (NBS-R)
521 in the grapevine genome, and defence genes in Brassicaceae (van-den-Bergh *et al.*, 2016).
522 Multiple gene fragments are often found within single maize helitrons, indicating that
523 several sequential transpositions have happened during their history, as proposed in
524 *Fragaria*.

525

526 **5. The ages of plant and fish sex chromosomes**

527 Turnover events of either of the two kinds just described can create single gene sex-
528 determining loci, creating young sex-linked systems in species with a different ancestral
529 state. Another way in which new sex-linked genome regions can evolve, potentially creating
530 extensive, multi-gene sex-linked regions if new sex-determining loci evolve within non-
531 recombining regions. This could be the situation in *Rumex*, where the sex chromosomes of
532 two species groups are non-homologous, and one appears to be old (with a highly
533 heteromomorphic XY pair carrying a high proportion of genes with high sequence
534 divergence), and the other with much less XY differentiation, and therefore probably
535 recently evolved (Crowson *et al.*, 2017). It will be important to understand whether, in
536 plants, turnover events replacing older systems by new ones are as common as the loss of
537 dioecy and its re-evolution.

538 Determining ages of systems will be important in relation to the question of whether single
539 gene systems reflect *de novo* evolution involving two mutations in a single gene. If dioecy is
540 shared by several related species, it could initially have evolved long ago, but successive
541 turnovers could have occurred, persisting too briefly to evolve large non-recombining
542 regions and chromosome heteromorphism (Rodrigues *et al.*, 2018). Overall, it is currently
543 unknown what determines why a large fully sex-linked region sometimes evolves, or why a
544 turnover event sometimes occurs before this has occurred. Plants with homomorphic sex

545 chromosomes could be valuable for such studies, as closely related outgroup species may be
546 available to infer changes.

547

548 **6. Conclusions**

549 I have argued here that some of the properties of sex chromosomes may not have evolved in
550 response to the presence of a sex-determining locus. I have concentrated on the concept
551 that a lack of recombination need not reflect suppression of crossing over between two
552 genes involved in sex-determination, or one sex-determining gene and a gene that mutates
553 to an allele that benefits only one sex and is disadvantageous in the other sex (i.e. is sexually
554 antagonistic). Young systems may be ideal for testing whether recombination rates have
555 changed, similarly to testing whether the molecular evolution of the lineages leading to
556 extant Y (or W) chromosomes indicates genetic degeneration of sequences carried on the
557 ancestral chromosome that evolved into the sex chromosome pair (and which are still
558 present on the X or Z).

559 A similar question relates to sex chromosome evolution after an extensive sex-linked region
560 has evolved and has undergone genetic degeneration. In the Y-linked region in an XY system,
561 the lower number of functional copies of sex-linked genes in males than females may lead to
562 disadvantageously low expression of many genes, and natural selection should then favour
563 dosage compensation to restore the diploid level. However, it is important to test, not only
564 whether dosage compensation occurs in a species that has evolved a sex-linked region, but
565 also whether it evolved in response to genetic degeneration. The alternative is the existence
566 of cellular homeostasis systems that can up-regulate expression for a gene that is
567 heterozygous for a mutant or deleted copy (reviewed by Malone *et al.*, 2012; Birchler, 2013).

568 Although the time-course of gene losses from young Y chromosomes, and deterioration of
569 function, are rarely known, and probably differ greatly in systems with different sized non-
570 recombining regions, these processes occur gradually, so that compensation might initially
571 evolve independently for each gene affected. Moreover, chromosome-wide dosage
572 compensation is likely to be disadvantageous in young sex chromosomes, as this would
573 result in males over-expressing any genes still present on the Y, like a triploid aneuploid for
574 this chromosome. Furthermore, increased expression is unlikely initially to be restricted to

575 males, but might evolve if the advantage of correcting low male expression outweighs this
 576 disadvantage, adding a further disadvantage of over-expression in females (Charlesworth,
 577 1996). In *Silene latifolia*, a special form of imprinting causing increased expression from the
 578 maternal X chromosome in both sexes, compared with autosomal genes, has been
 579 proposed; this would avoid overexpression in males, but not in females (Muyle *et al.*, 2018).
 580 This species, and other plants, are likely to be excellent systems for evaluating the time
 581 course of evolution of dosage compensation in sex chromosomes, and whether the relative
 582 ages of sex chromosome systems can be inferred from the existence, or not, of a dosage
 583 compensation system, and whether individual genes show compensation, or a
 584 chromosome-level system has evolved (see box). Currently, expression estimates remain
 585 difficult and errors of the expression are high, as exemplified by differing conclusions about
 586 dosage compensation for the same species, such as the old (degenerated) stratum of the
 587 threespine stickleback (Schultheiß *et al.*, 2015; White *et al.*, 2015). Clearly, more work is
 588 needed in the future, to understand many of the puzzles outlined here.

589

590 **Acknowledgements:** I am grateful to the ERC for funding my research by an ERC Advanced
 591 grant, ERC grant number 695225 (GUPPYSEX).

592 References

- 593 **Akagi T, Henry IM, Ohtani H, Morimoto T, Morimoto K, Beppu K, Kataoka I, Tao R.**
 594 **2018.** A Y-encoded suppressor of feminization arose via lineage-specific duplication
 595 of a cytokinin response regulator in kiwifruit. *Plant Cell* **30**: 780–795.
- 596 **Akagi T, Henry IM, Tao R, Comai L. 2014.** A Y-chromosome–encoded small RNA acts as
 597 a sex determinant in persimmons. *Science* **346**: 646-650.
- 598 **Bachtrog D. 2008.** The temporal dynamics of processes underlying Y chromosome
 599 degeneration. *Genetics* **179**(3): 1513-1525.
- 600 **Bachtrog D. 2012.** Y-chromosome evolution: emerging insights into processes of Y-
 601 chromosome degeneration. *Nature Reviews Genetics* **14**(2): 113 -124.
- 602 **Balounova V, Gogela R, Cegan R, Cangren P, Zluvova J, Safar J, Kovacova V, Bergero**
 603 **R, Hobza R, Vyskot B, et al. 2019.** Evolution of sex determination and heterogamety
 604 changes in section *Otites* of the genus *Silene*. *Scientific Reports* **9**(1): 1045.
- 605 **Barrett SCH. 1992.** Gender variation in *Wurmbia dioica* (Liliaceae) and the evolution of
 606 dioecy. *Journal of Evolutionary Biology* **5**: 423-444.
- 607 **Bergero R, Forrest A, Kamau E, Charlesworth D. 2007.** Evolutionary strata on the X
 608 chromosomes of the dioecious plant *Silene latifolia*: evidence from new sex-linked
 609 genes. *Genetics* **175**: 1945-1954.

- 610 **Bergero R, Gardner J, Bader B, Yong L, Charlesworth D. 2019.** Exaggerated
611 heterochiasmy in a fish with sex-linked male coloration polymorphisms. *Proceedings*
612 *of the National Academy of Sciences of the United States of America* **116**(14): 6924-
613 6931.
- 614 **Bertho S, Herpin A, Branthonne A, Jouanno E, A Yano, Nico B, Muller T, Pannetier M,**
615 **Pailhous E, Miwa M, et al. 2018.** The unusual rainbow trout sex determination gene
616 hijacked the canonical vertebrate gonadal differentiation pathway. *Proceedings of the*
617 *National Academy of Sciences of the United States of America* **115**(50): 12781-12786.
- 618 **Beukeboom LW, Perrin N. 2014.** *The Evolution of Sex Determination*. Oxford: Oxford
619 University Press.
- 620 **Birchler J. 2013.** Aneuploidy in plants and flies: The origin of studies of genomic imbalance.
621 *Seminars in Cell and Developmental Biology* **24**(3): 315-319.
- 622 **Blackmon H, Brandvain Y. 2017.** Long-term fragility of Y chromosomes is dominated by
623 short-term resolution of sexual antagonism. *Genetics* **207**: 1621-1629.
- 624 **Blaser O, Grossen C, Neuenschwander S, Perrin N. 2013.** Sex-chromosome turnovers
625 induced by deleterious mutation load. *Evolution* **67**(3): 635-645.
- 626 **Brand CL, Cattani MV, Kingan SB, Landeen EL, Presgraves DC. 2018.** Molecular
627 evolution at a meiosis gene mediates species differences in the rate and patterning of
628 recombination. *Current Biology* **28**(8): P1289-1295.E1284.
- 629 **Bull JJ. 1983.** *Evolution of Sex Determining Mechanisms*. Menlo Park, CA:
630 Benjamin/Cummings.
- 631 **Carlson C, Choi Y, Chan A, Serapiglia M, Town C, Smart L. 2017.** Dominance and
632 sexual dimorphism pervade the *Salix purpurea* L. transcriptome. *Genome Biology and*
633 *Evolution* **9**(9): 2377-2394.
- 634 **Charlesworth B. 1996.** The evolution of chromosomal sex determination and dosage
635 compensation. *Current Biology* **6**: 149-162.
- 636 **Charlesworth B, Charlesworth D. 1978.** A model for the evolution of dioecy and
637 gynodioecy. *Amer. Nat.* **112**: 975-997.
- 638 **Charlesworth B, Sniegowski P, Stephan W. 1994.** The evolutionary dynamics of repetitive
639 DNA in eukaryotes. *Nature* **371**: 215-220.
- 640 **Charlesworth D 1985.** Distribution of dioecy and self-incompatibility in angiosperms. In:
641 Greenwood PJ, Slatkin M eds. *Evolution - Essays in Honour of John Maynard Smith*.
642 Cambridge: Cambridge University Press, 237-268.
- 643 **Charlesworth D, Charlesworth B. 1978.** Population genetics of partial male-sterility and
644 the evolution of monoecy and dioecy. *Heredity* **41**: 137-153.
- 645 **Crowson D, Barrett SCH, Wright SI. 2017.** Purifying and positive selection influence
646 patterns of gene loss and gene expression in the evolution of a plant sex chromosome
647 system. *Molecular Biology and Evolution* **34**(5): 1140-1154.
- 648 **Darwin CR. 1877.** *The Different Forms of Flowers on Plants of the Same Species*. London:
649 John Murray.
- 650 **Dong Y, Lu X, Song W, Shi L, Zhang M, Zhao H, Jiao Y, Lai J. 2011.** Structural
651 characterization of helitrons and their stepwise capturing of gene fragments in the
652 maize genome. *BMC Genomics* **12**: 609.
- 653 **Dorken ME, Barrett SCG. 2004.** Sex determination and the evolution of dioecy from
654 monoecy in *Sagittaria latifolia* (Alismataceae). *Proceedings of the Royal Society of*
655 *London B.* **271**: 213-219.
- 656 **Dreissig S, Fuchs J, Capal P, Kettles N, E Byrne, Houben A. 2015.** Measuring meiotic
657 crossovers via multi-locus genotyping of single pollen grains in barley. *PLOS ONE*
658 **10**(9): e0137677.

- 659 **Dreissig S, Fuchs J, Himmelbach A, Mascher M, Houben A. 2017.** Sequencing of single
660 pollen nuclei reveals meiotic recombination events at megabase resolution and
661 circumvents segregation distortion caused by postmeiotic processes. *Frontiers in*
662 *Plant Science* **8**: 1620
- 663 **Faber-Hammond J, Phillips R, Brown K. 2015.** Comparative analysis of the shared sex-
664 determination region (SDR) among Salmonid fishes. *Genome Biology and Evolution*
665 **7(7)**: 1972–1987.
- 666 **Fujito S, Takahata S, Suzuki R, Hoshino Y, Ohmido N, Onodera Y. 2015.** Evidence for a
667 common origin of homomorphic and heteromorphic sex chromosomes in distinct
668 *Spinacia* species *G3* **5**: 1663-1673.
- 669 **Gammerdinger W, Kocher T. 2018a.** Unusual diversity of sex chromosomes in African
670 Cichlid fishes. *Genes* **9(10)**: 480.
- 671 **Gammerdinger W, Kocher T. 2018b.** Unusual diversity of sex chromosomes in African
672 Cichlid fishes. *Genes* **9**: 480.
- 673 **Giraut L, Falque M, Drouaud J, Pereira L, Martin O, Mezard C. 2011.** Genome-wide
674 crossover distribution in *Arabidopsis thaliana* meiosis reveals sex-specific patterns
675 along chromosomes. *PLoS Genetics* **7(11)**: Article Number: e1002354.
- 676 Gschwend, A. R., Yu, Q., Tong, E. J., Zeng, F., Han, J., VanBuren, R., . . . Ming, R. (2012).
677 Rapid divergence and expansion of the X chromosome in papaya. *Proceedings of*
678 *the National Academy of Sciences of the United States of America*, **109(34)**, 13716-
679 13721. doi:10.1073/pnas.1121096109
- 680 **Harkess A, Huang K, Hulst RVd, Tissen B, Caplan JL, Koppula A, Batish M, Meyers BC,**
681 **Leebens-Mack JH. 2018.** Sex chromosome evolution via two genes. *bioRxiv*. bioRxiv
682 494112; doi: <https://doi.org/10.1101/494112>
- 683 **Harkess A, Zhou J, Xu C, Bowers J, Hulst RVd, Ayyampalayam S, Mercati F, Riccardi**
684 **R, McKain M, Kakrana A, et al. 2017a.** The asparagus genome sheds light on the
685 origin and evolution of a young Y chromosome. *Nature Communications* **8**: 1279.
- 686 **Harkess A, Zhou J, Xu C, Bowers JE, Hulst RVd, Ayyampalayam S, Mercati F,**
687 **Riccardi P, McKain MR, Kakrana A, et al. 2017b.** The asparagus genome sheds
688 light on the origin and evolution of a young Y chromosome. *Nature Communications*
689 **8**: 1279.
- 690 **Hisanaga T, Okahashi K, Si Yamaoka, Kajiwara T, Nishihama R, Shimamura M,**
691 **Yamato K, Bowman JL, Kohchi T, Nakajima K. 2019.** A cis-acting bidirectional
692 transcription switch controls sexual dimorphism in the liverwort. *EMBO Journal* **38**:
693 e100240 DOI: 10.15252/embj.2018100240
- 694 **Hollister JD, Gaut BS. 2009.** Epigenetic silencing of transposable elements: A trade-off
695 between reduced transposition and deleterious effects on neighboring gene
696 expression. *Genome Research* **19**: 1419-1428.
- 697 **Hough J, Hollister JD, Wang W, Barrett SCH, Otto SP. 2014.** Genetic degeneration of
698 old and young Y chromosomes in the flowering plant *Rumex hastatulus*. *Proceedings*
699 *of the National Academy of Sciences of the United States of America* **111(21)**: 7713–
700 7718.
- 701 **Huu C, Kappel C, Keller B, Sicard A, Takebayashi Y, Breuninger H, Nowak MD,**
702 **Bäurle I, Himmelbach A, Burkart M, et al. 2016.** Presence versus absence of
703 CYP734A50 underlies the style-length dimorphism in primroses. *Elife* **5**: e17956.
- 704 **Ieda R, Hosoya S, Tajima S, Atsumi K, Kamiya T, Nozawa A, Y Aoki, Tasumi S,**
705 **Koyama T, Nakamura O, et al. 2018.** Identification of the sex-determining locus in
706 grass puffer (&ITTakifugu niphobles&IT) provides evidence for sex-chromosome
707 turnover in a subset of Takifugu species. *PLOS ONE* **13(1)**: e0190635.

- 708 **Kamiya T, Kai W, Tasumi S, Oka A, Matsunaga T, Mizuno M, Fujita M, Suetake H,**
 709 **Suzuki S, Hosoya S, et al. 2012.** A trans-species missense SNP in *amhr2* is
 710 associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (Fugu).
 711 *PLoS Genetics* **8**(7): e1002798.
- 712 **Kazama Y, Ishii K, Aonuma W, Ikeda T, Kawamoto H, Koizumi A, Filatov D,**
 713 **Chibalina M, Charlesworth D, Bergero R, et al. 2016.** A new physical mapping
 714 approach refines the sex-determining gene positions on the *Silene latifolia* Y-
 715 chromosome. *Scientific Reports* **6**: 18917.
- 716 **Kianian P, Wang M, Simons K, Ghavam F, ..., Mudge J, Chen C, Kianian S, Pawlowski**
 717 **W. 2018.** High-resolution crossover mapping reveals similarities and differences of
 718 male and female recombination in maize. *Nature Communications* **9**(1): 2370.
- 719 **Kijas J, McWilliam S, Sanchez MN, Kube P, King H, Evans B, Nome T, Lien S, Verbyla**
 720 **K. 2018.** Evolution of sex determination loci in atlantic salmon. *Scientific Reports* **8**:
 721 5664.
- 722 **Kimura T, Yoshida K, Shimada A, Jindo T, M. Sakaizumi HM, Kiyoshi Naruse,**
 723 **Hiroyuki Takeda, Hidetoshi Inoko, Gen Tamiya and Minori Shinya. 2006.**
 724 Genetic linkage map of medaka with polymerase chain reaction length
 725 polymorphisms. *Gene* **363**: 24-31.
- 726 **Kirubakaran T, Andersen Ø, Rosa MD, Andersstuen T, Hallan K, Kent M, Lien S.**
 727 **2019.** Characterization of a male specific region containing a candidate sex
 728 determining gene in Atlantic cod. *Scientific Reports* **9**(2): 116.
- 729 **Kondo M, Nagao E, Mitani H, Shima A. 2001.** Differences in recombination frequencies
 730 during male and female meioses of the sex chromosomes of the medaka, *Orzyias*
 731 *latipes*. *Genetical Research* **78**: 23-30.
- 732 **Kondo M, Nanda I, Schmid M, Scharl M. 2009.** Sex determination and sex chromosome
 733 evolution: insights from Medaka. *Sexual Development* **3**: 88-98.
- 734 **Krasovec M, Chester M, Ridout K, Filatov D. 2018.** The mutation rate and the age of the
 735 sex chromosomes in *Silene latifolia*. *Current Biology* **28**(11): e4.
- 736 **Kudoh T, Takahashi M, Osabe T, Toyoda A, Hirakawa H, Y Suzuki, Ohmido N,**
 737 **Onodera Y. 2018.** Molecular insights into the non-recombining nature of the spinach
 738 male-determining region. *Molecular Genetics and Genomics* **293**(2): 557-568.
- 739 **Lahn BT, Page DC. 1999.** Four evolutionary strata on the human X chromosome. *Science*
 740 **286**: 964-967.
- 741 **Lenormand T. 2003.** The evolution of sex dimorphism in recombination. *Genetics* **163**(2):
 742 811-822.
- 743 **Lenormand T, Dutheil J. 2005.** Recombination difference between sexes: A role for haploid
 744 selection. *PLoS Biology* **3**(3): 396-403.
- 745 **Li J, Cocker JM, Wright J, Webster MA, McMullan M, Dyer S, Swarbreck D, Caccamo**
 746 **M, Oosterhout Cv, Gilmartin PM. 2016.** Genetic architecture and evolution of the S
 747 locus supergene in *Primula vulgaris*. *Nature Plants* **2**: 16188.
- 748 **Lisachov A, Zadesenets K, Rubtsov N, Borodin P. 2015.** Sex chromosome synapsis and
 749 recombination in male guppies. *ZEBRAFISH* **12**(2): 174-180.
- 750 **Liu Z, Moore PH, Ma H, Ackerman CM, Ragiba M, Pearl HM, Kim MS, Charlton JW,**
 751 **Yu Q, Stiles JI, et al. 2004.** A primitive Y chromosome in Papaya marks the
 752 beginning of sex chromosome evolution. *Nature* **427**: 348-352.
- 753 **Lorenzo J, Hobza R, Vyskot B. 2018.** DNA methylation and genetic degeneration of the Y
 754 chromosome in the dioecious plant *Silene latifolia*. *BMC Genomics* **19**: 540.
- 755 **Lubieniecki K, Lin S, Cabana E, Li J, Lai Y, Davidson W. 2015.** Genomic instability of
 756 the sex-determining locus in Atlantic Salmon (*Salmo salar*). *G3-Genes Genomes*
 757 *Genetics* **5**(11): 2513-2522.

- 758 **Malone J, Cho D, Mattiuzzo N, Artieri C, Jiang L, Dale R, Smith H, McDaniel J,**
759 **Munro S, Salit M, et al. 2012.** Mediation of *Drosophila* autosomal dosage effects
760 and compensation by network interactions. *Genome Biology* **13**: R28.
- 761 **Meisel RP, Gonzales CA, Luu H. 2017.** The house fly Y Chromosome is young and
762 minimally differentiated from its ancient X Chromosome partner. *Genome Research*
763 **27**: 1417-1426.
- 764 **Morgante M, Brunner S, Pea G, Fengler K, Zuccolo A, Rafalski A. 2005.** Gene
765 duplication and exon shuffling by helitron-like transposons generate intraspecies
766 diversity in maize. *Nature Genetics* **37**: 997 - 1002.
- 767 **Muller HJ. 1932.** Some genetic aspects of sex. *The American Naturalist* **66**: 118-138.
- 768 **Murase K, Shigenobu S, Fujii S, K Ueda, T Murata, Sakamoto A, Wada Y, Yamaguch**
769 **K, Osakabe Y, Osakabe K, et al. 2017.** MYB transcription factor gene involved in
770 sex determination in *Asparagus officinalis*. *GENES TO CELLS* **22**(1): 115-123.
- 771 **Muyle A, Zemp N, Deschamps C, Mousset S, Widmer A, Marais G. 2012.** Rapid de novo
772 evolution of X chromosome dosage compensation in *Silene latifolia*, a plant with
773 young sex chromosomes. *PLoS Biology* **10**(4): e1001308.
- 774 **Muyle A, Zemp N, Fruchard C, Cegan R, Vrana J, Deschamps C, Tavares R, Hobza R,**
775 **Picard F, Widmer A, et al. 2018.** Genomic imprinting mediates dosage
776 compensation in a young plant XY system. *Nature Plants* **4**(9): 677-680.
- 777 **Myosho T, Takehana Y, Hamaguchi S, Sakaizumi M. 2015.** Turnover of sex
778 chromosomes in celebensis group medaka fishes. *G3-Genes Genomes Genetics* **5**(12):
779 2685-2691.
- 780 **Nadot S, Alapetite E, Baker WJ, Tregear JW, Barfod SS. 2016.** The palm family
781 (*Arecaceae*): a microcosm of sexual system evolution. *Botanical Journal of the*
782 *Linnean Society* **182**(2): 376–388.
- 783 **Nanda I, Schories S, Tripathi N, Dreyer C, Haaf T, Schmid M, Scharl M. 2014.** Sex
784 chromosome polymorphism in guppies. *Chromosoma* **123**(4): 373-383.
- 785 **Natri H, Merilä J, Shikano. 2019.** The evolution of sex determination associated with a
786 chromosomal inversion. *Nature Communications* **10**(1): 145.
- 787 **Norup M, Petersen G, Burrows S, Bouchenak-Khelladi Y, Leebens-Mack J, Pires J,**
788 **Linder H, Seberg O. 2015.** Evolution of *Asparagus* L. (*Asparagaceae*): Out-of-
789 South-Africa and multiple origins of sexual dimorphism. *Molecular Phylogenetics*
790 *and Evolution* **92**: 25–44.
- 791 **Ohnishi O. 1985.** Population genetics of cultivated buckwheat, *Fagopyrum esculentum*
792 Moench. III. Frequency of sterility mutants in Japanese populations. *Japanese*
793 *Journal of Genetics* **60**: 391-404.
- 794 **Ohyama K, Takemura M, Oda K, Fukuzawa H, Kohchi T, Nakayama S, Ishizaki K,**
795 **Fujisawa M, Yamato K. 2001.** Gene content, organization and molecular evolution
796 of plant organellar genomes and sex chromosomes - Insights from the case of the
797 liverwort *Marchantia polymorpha*. *PROCEEDINGS OF THE JAPAN ACADEMY*
798 *SERIES B-PHYSICAL AND BIOLOGICAL SCIENCES* **85**(3): 108-124.
- 799 **Onodera Y, Itaru Y, Hiroki M, Tanaka A, Niikura S, Yamazaki S, Mikami T. 2011.**
800 Mapping of the genes for dioecism and monoecism in *Spinacia oleracea* L.: evidence
801 that both genes are closely linked. *Plant Cell Reports* **30**(6): 965-971.
- 802 **Papadopulos AST, Chester M, Ridout K, Filatov DA. 2015.** Rapid Y degeneration and
803 dosage compensation in plant sex chromosomes. *Proceedings of the National*
804 *Academy of Sciences of the United States of America* **112**(42): 13021–13026.
- 805 **Pellicer J, Hidalgo O, Dodsworth S, Leitch I. 2018.** Genome size diversity and its impact
806 on the evolution of land plants. *Genes (Basel)* **9**(2): 88.

- 807 **Qian W, G Fan, Liu D, Zhang H, Wang X, Wu J, Xu Z. 2017.** Construction of a high-
 808 density genetic map and the X/Y sex-determining gene mapping in spinach based on
 809 large-scale markers developed by specific-locus amplified fragment sequencing
 810 (SLAF-seq). *BMC Genomics* **18**: 276.
- 811 **Ramsey M, Vaughton G, Peakall R. 2006.** Inbreeding avoidance and the evolution of
 812 gender dimorphism in *Wurmbea biglandulosa* (Colchicaceae). *Evolution* **60**(3): 529–
 813 537.
- 814 **Renner S. 2014.** The relative and absolute frequencies of angiosperm sexual systems:
 815 Dioecy, monoecy, gynodioecy, and an updated online database. *American Journal of*
 816 *Botany* **101**: 1588-1596.
- 817 **Rodrigues N, Studer T, Dufresne C, Perrin N. 2018.** Sex-chromosome recombination in
 818 common frogs brings water to the fountain-of-youth. *Molecular Biology and*
 819 *Evolution* **35**(4): 942-948.
- 820 Saunders, P., Neuenschwander, S., & Perrin, N. (2018). Sex chromosome turnovers and
 821 genetic drift: A simulation study. *Journal of Evolutionary Biology*, **31**(9), 1413-1419.
 822 doi:10.1111/jeb.13336
- 823 **Schultheiß R, Viitaniemi H, Leder E. 2015.** Spatial dynamics of evolving dosage
 824 compensation in a young sex chromosome system. *Genome Biology and Evolution*
 825 **7**(2): 581–590.
- 826 **Scott MF, Osmond M, Otto SP. 2018.** Haploid selection, sex ratio bias, and transitions
 827 between sex-determining systems. *PLoS Biology* **6**(6): e2005609.
- 828 **Sutherland B, Rico C, Audet C, Bernatchez L. 2017.** Sex chromosome evolution,
 829 heterochiasmy and physiological QTL in the salmonid Brook Charr *Salvelinus*
 830 *fontinalis*. *G3: GENES, GENOMES, GENETICS* **7**: 2749-2762.
- 831 **Takahata S, Yago T, Iwabuchi K, H H, Suzuki Y, Onodera Y. 2016.** Comparison of
 832 spinach sex chromosomes with sugar beet autosomes reveals extensive synteny and
 833 low recombination at the male-determining locus. *Journal of Heredity* **107**(7): 679-
 834 685.
- 835 **TenHoopen R, Harbord RM, Maes T, Nanninga N, Robbins TP. 1998.** The self-
 836 incompatibility (S) locus in *Petunia hybrida* is located on chromosome III in a region,
 837 syntenic for the Solanaceae. *Plant Journal* **16**: 729-734.
- 838 **Tenessen J, Wei N, Straub S, Govindarajulu R, Liston A, Ashman T. 2018.** Repeated
 839 translocation of a gene cassette drives sex-chromosome turnover in strawberries.
 840 *PLoS Biology* **16**(8): e2006062.
- 841 **Timofejeva L, Skibbe D, Lee S, Golubovskaya I, Wang R, Harper L, Walbot V, Cande**
 842 **W. 2013.** Cytological characterization and allelism testing of anther developmental
 843 mutants identified in a screen of maize male sterile lines. *G3-Genes Genomes*
 844 *Genetics* **3**(2): 231-249.
- 845 **Torres M, LS Mathew, Ahmed I, Al-Azwani I, Krueger R, Rivera-Nuñez D, Mohamoud**
 846 **Y, Clark A, Suhre K, Malek J. 2018.** Genus-wide sequencing supports a two-locus
 847 model for sex-determination in Phoenix. *Nature Communications* **9**(1): 3969.
- 848 **van-den-Bergh E, Hofberger E, Johannes A, Schranz M. 2016.** Flower power and the
 849 mustard bomb: Comparative analysis of gene and genome duplications in
 850 glucosinolate biosynthetic pathway evolution in Cleomaceae and Brassicaceae.
 851 *American Journal of Botany* **103**(7): 1212-1222.
- 852 **vanDoorn G, Kirkpatrick M. 2007.** Turnover of sex chromosomes induced by sexual
 853 conflict. *Nature* **449**: 909-912.
- 854 **Veller C, Muralidhar P, Constable G, Nowak M. 2017.** Drift-induced selection between
 855 male and female heterogamety. *Genetics* **207**: 711–727

- 856 **Veltsos P, Cossard G, Beaudoin E, Beydon G, CRoux, González-Martínez S, Pannell**
857 **JR. 2018.** The size and content of the sex-determining region of the Y chromosome in
858 dioecious *Mercurialis annua*, a plant with homomorphic sex chromosomes. *Genes*
859 (*Basel*)(9): 6.
- 860 **Vicoso B, Bachtrog D. 2009.** Progress and prospects toward our understanding of the
861 evolution of dosage compensation. *Chromosome Research* **17**(5): 585-602.
- 862 **Wadlington W, Ming R. 2018.** Development of an X-specific marker and identification of
863 YY individuals in spinach. *Theoretical and Applied Genetics* **131**(9): 1987-1994.
- 864 **Wang J, Na J, Yu Q, Gschwend AR, Han J, Zeng F, Aryal R, VanBuren R, Murray JE,**
865 **Zhang W, et al. 2012.** Sequencing papaya X and Yh chromosomes reveals molecular
866 basis of incipient sex chromosome evolution. *Proceedings of the National Academy of*
867 *Sciences of the United States of America* **109**(34): 13710-13715.
- 868 **West N, Golenberg E. 2018.** Gender-specific expression of GIBBERELLIC ACID
869 INSENSITIVE is critical for unisexual organ initiation in dioecious *Spinacia*
870 *oleracea*. *New Phytologist* **217**(3): 1322-1334.
- 871 **Westergaard M. 1958.** The mechanism of sex determination in dioecious plants. *Advances*
872 *in Genetics* **9**: 217-281.
- 873 **White M, Kitano J, Peichel C. 2015.** Purifying selection maintains dosage-sensitive genes
874 during degeneration of the threespine stickleback Y chromosome. *Molecular Biology*
875 *and Evolution* **32**(8): 1981-1995.
- 876 **Wilkins A. 1995.** Moving up the hierarchy: a hypothesis on the evolution of a genetic sex-
877 determination pathway. *Bioessays* **17**(1): 71-77.
- 878 **Woram RA, Gharbi K, Sakamoto T, Hoyheim B, Holm L-E, Naish K, McGowan C,**
879 **M.M.Ferguson, Phillips RB, Stein J, et al. 2003.** Comparative Genome Analysis of
880 the Primary Sex-Determining Locus in Salmonid Fishes. *Genome Research* **13**(2):
881 272-280.
- 882 **Zemp N, Charlesworth D, Widmer A. 2018.** Has adaptation occurred in males and females
883 since separate sexes evolved in the plant *Silene latifolia*? *Proceedings of the Royal*
884 *Society B: Biological Sciences* **285**: 20172824.
- 885 **Zhang W, Wang X, Yu Q, Ming R, Jiang J. 2008.** DNA methylation and
886 heterochromatinization in the male-specific region of the primitive Y chromosome of
887 papaya. *Genome Research* **18**(12): 1938-1943.
- 888 **Zhou Q, Bachtrog D. 2012.** Chromosome-wide gene silencing initiates Y degeneration in
889 *Drosophila*. *Current Biology* **22**: 522–525.
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893 **Figure legends**

894

895 **Figure 1.** Two possible ways in which separate sexes might arise in related species, with
 896 changes in the sex system shown as dotted lines in the tree. **A** shows the case when dioecy
 897 evolved in an ancestor of three species, one of which has reverted to functional
 898 hermaphroditism. The phylogeny could potentially reveal the evolutionary events, provided
 899 that a fully sex-linked sequence in the oldest part of the sex chromosome pair can be
 900 identified: for example, in an XY system, the divergence between the alleles in either
 901 dioecious species will then date back to the dioecious common ancestor, and should exceed
 902 divergence of either species from the hermaphrodite species. It may be possible to infer
 903 whether the hermaphrodite evolved by a change in the Y or the X. For example, papaya
 904 hermaphrodites carry a Y chromosome that probably arose by deletion of a Y-linked female
 905 suppressor (Wang *et al.*, 2012). A related species, *Vasconcellea monoica*, has reverted to
 906 functional hermaphroditism, and orthologues of papaya sex-linked genes have almost
 907 identical divergence from papaya X and Y alleles.; loss of dioecy therefore occurred before,
 908 or soon after, these genes stopped recombining in papaya (Gschwend *et al.*, 2011). **B** shows
 909 a case where the evolutionary history is less clear. Dioecy could have evolved independently
 910 in the two dioecious species at different times, so that XY sequence divergence differ in the
 911 two, reflecting the different times. However, similar divergence differences can arise if the
 912 true evolutionary history is the one shown in part A, but the sequences being compared are
 913 in a younger evolutionary stratum and started diverging recently. If the hermaphrodite is
 914 extinct, one might conclude that dioecy is the ancestral state, unless X-Y divergence is
 915 known

916

917 **Figure 2.** Diagram to illustrate how a non-recombining region may evolve in a chromosome
 918 carrying a sex-determining locus, and later disappear, for the case of male heterogamety.
 919 Chromosomes are shown as horizontal lines. At the top, an ancestral chromosome with a
 920 single sex-determining gene is assumed. Unless the presence of the gene directly causes
 921 suppressed recombination (see Figure 3), sex linkage will be confined to this gene, and the
 922 chromosome pair will be homomorphic (only the “Y” is shown, as its homologue will be the
 923 same, apart from the maleness factor or male-sterility mutation, either a mutation in a
 924 coding sequence of a gene essential for male function, or a change preventing expression of

925 such a gene). If a sexually antagonistic (SA) mutation occurs in the region and establishes a
926 polymorphism, then, because the mutation is disadvantageous in females, this generates
927 selection for close linkage with the sex-determining gene, or male-specific expression of the
928 male-benefit trait (Outcomes 1 and 2, respectively). An extensive Y-linked region may
929 evolve, including both these gene and many others in the region (wide blue bar), creating
930 true X- and Y-linked regions. An inversion might cause suppressed recombination, directly
931 producing morphologically different X and Y chromosomes, or heteromorphism may evolve
932 later, as well as genetic degeneration. The bottom diagram shows a possible later stage,
933 when the male-determining factor is replaced by a new one taking control of sex-
934 determination; sex-determination is then again under single gene control and the sex
935 chromosome pair is again homomorphic.

936

937 **Figure 3.** An evolutionary scenario incorporating the findings in the persimmon (see
938 explanations in the main text). Three different chromosomes are symbolised by horizontal
939 pink, green and black lines, and the genes in the model are coloured to match their
940 chromosomes, except that the maleness factor on the black chromosome is in blue. The
941 diagram shows the effects of the two mutations involved in the evolution of females (first)
942 and then males; each of the mutations acts by affecting expression of a different gene. Note
943 the insertion that created the female suppressor, and the effect of the insertion in creating
944 a region of non-pairing in this chromosome region (grey), and potentially in the region
945 flanking the insertion, so that recombination is suppressed creating XY-linked regions (red).

Box: The stages in sex chromosome evolution, illustrated for the case of an XY system

The stages illustrate (i) that the age of a sex chromosome system is best understood in relation to the different kinds of events and changes from the ancestral state that can, but need not inevitably, occur, and (ii) the potentiality for a lineage with an old system to change back to a new and younger state. Ages are not given in terms of years, for two reasons. First, the number of generations, not years, is most relevant to the stage that will be reached, including the extent of sequence divergence between Y- and X-linked sequences and of genetic degeneration. Second, such processes occur at different rates per generation, depending on factors including the species' effective population size (Bachtrog, 2008). However, some estimates in years are mentioned, to provide rough information; these values illustrate that a later stage can be found in a species at an earlier stage, as there is no necessary progression from stages 1 to 2 and onwards. For example, the persimmon case is listed among young systems, because a single-gene system will be young when it first evolves, and may not lead to the establishment of diverged X- and Y-linked regions, even if it is maintained for a long evolutionary time.

Stage	Evolutionary event	Possible consequences of the event	Examples (plant examples in bold)	References
1: early stage of young systems	Evolution of a sex-determining gene or genes on a chromosome that was not formerly a sex chromosome	In a single-gene system (see below, and the main text, for situations in which such systems can arise), the sex-determining region may retain crossing over	Fugu and possibly guppy Persimmon, kiwifruit (20 MYA¹)	(Kamiya <i>et al.</i> , 2012), (Bergero <i>et al.</i> , 2019) (Akagi <i>et al.</i> , 2014), (Akagi <i>et al.</i> , 2018)
2	Evolution of suppressed recombination: if two sex-determining genes are involved, closer linkage may evolve between them, creating a young “proto-Y and X” sex chromosome system ²	a) Sexually antagonistic polymorphism(s) may become established closely linked to a sex-determining locus, promoting evolution of even less recombination in the region b) The genome region in which recombination is suppressed may be a small part of the chromosome, leaving most of the chromosome as a pseudo-autosomal region	Papaya (7 MY) Asparagus (2 MY)³ Several fish, including cichlids	(Liu <i>et al.</i> , 2004) Harkess <i>et al.</i> , 2017 (Gammerdinger & Kocher, 2018b)

¹ Age estimated from divergence of the male-determining factor and the gene from which it appears to have evolved by duplication

² Multiple events suppressing Y-X recombination may occur at different times, creating “evolutionary strata” of Y-X divergence (see main text)

³ Norup M, Petersen G, Burrows S, Bouchenak-Khelladi Y, Leebens-Mack J, Pires J, Linder H, Seberg O. 2015. Evolution of *Asparagus* L. (Asparagaceae): Out-of-South-Africa and multiple origins of sexual dimorphism. *Molecular Phylogenetics and Evolution* 92: 25–44.5

		c) The non-recombining region may extend beyond the region containing the two genes		
3	Divergence between Y-linked and X-linked homologues	Repetitive sequences accumulate and X-Y differences develop (heteromorphism and low gene density) ⁴	Papaya, <i>Silene latifolia</i> (6-10 MY) Threespine stickleback, guppy	(Wang <i>et al.</i> , 2012), (Gschwend <i>et al.</i> , 2011) (White <i>et al.</i> , 2015), (Nanda <i>et al.</i> , 2014)
4	Y-linked genes' functions may degenerate	Closely linked transposable element sequences may affect gene expression	<i>Rumex rothschildianus, Silene latifolia</i> Threespine stickleback	Stephen Wright's article in this volume, (Bachtrog, 2008) (Papadopulos <i>et al.</i> , 2015) (White <i>et al.</i> , 2015)
5: ancient systems	Dosage compensation of genes in non-recombining regions may evolve to restore adequate male gene expression levels (of X-linked copies)	Dosage compensation may act on expression of individual genes, and eventually at the whole chromosome level	Drosophila, mammals (>150 MY), birds <i>Silene latifolia</i>	Reviewed by (Vicoso & Bachtrog, 2009), Bachtrog, 2008 (Muyle <i>et al.</i> , 2012)
6	Y-linked regions lacking functional copies of essential genes may be deleted, leading to hemizyosity in males	a) Possible evolution of a new male-determining gene on a non-Y chromosome, either an autosome or the X, or movement of an existing male-determining gene to a new location on the X or an autosome, creating a single gene sex-determining system, returning the species to "young" state 1 above b) Possible loss of essential male function genes from the Y, creating an XO system	<i>Marchantia polymorpha</i>	(Ohyama <i>et al.</i> , 2001) reviewed in (Beukeboom & Perrin, 2014)

⁴ For example, an inversion may suppress crossovers between them and also across a larger genome region, or crossovers may be suppressed in meiosis of one sex, potentially in the entire genome, as in Dipteran males