Does degeneration or genetic conflict shape gene content on UV sex chromosomes?

Sarah B. Carey[1,§,‡], Leslie M. Kollar[1,*], and Stuart F. McDaniel[1,2]

[1]Department of Biology, University of Florida, Gainesville, FL, USA

[§]Current address: Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL, USA

[‡]Current address: HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA

[*]Current address: Department of Plant Biology, Michigan State University, East Lansing, MI, USA

[2]Email: stuartmcdaniel@ufl.edu

Key words: UV sex chromosomes, bryophytes, algae, genetic conflict, speciation

Words: 5995

Tables: 1

Figures: 3

Abstract

Studies of sex chromosomes have played a central role in understanding the consequences of suppressed recombination and sex-specific inheritance among several genomic phenomena. However, we argue that these efforts will benefit from a more rigorous examination of haploid UV sex chromosome systems, in which both the female-limited (U) and male-limited (V) experience suppressed recombination and sex-limited inheritance, and both are transcriptionally active in the haploid and diploid states. We review the life cycle differences that generate UV sex chromosomes and genomic data showing that ancient UV systems have evolved independently in many eukaryotic groups, but gene movement on and off the sex chromosomes, and potentially degeneration continue to shape the current gene content of the U and V chromosomes. Although both theory and empirical data show that the evolution of UV sex chromosomes is shaped by many of the same processes that govern diploid sex chromosome systems, we highlight how the symmetrical inheritance between the UV chromosomes provide an important test of sex-limited inheritance in shaping genome architecture. We conclude by examining how genetic conflict (over sexual dimorphism, transmission-ratio distortion, or parent-offspring conflict) may drive gene gain on UV sex chromosomes, and highlight the role of breeding system in governing the action of these processes. Collectively these observations demonstrate the potential for evolutionary genomic analyses of varied UV sex chromosome systems, combined with natural history studies, to understand how genetic conflict shapes sex chromosome gene content.

Introduction

On this 50th anniversary of the International Association of Bryology, we look back another five decades to the very beginnings of genetics and a remarkable period of discovery in bryology. During this time, the first sex chromosomes documented in plants were found in the liverwort *Sphaerocarpus donellii* (Allen 1917) and (Heitz 1928) coined the now ubiquitous term "heterochromatin" for the dark-staining, heteromorphic sex chromosomes in mosses (reviewed in (Reski 1998; Anderson 2000; Berger 2019)). More recent work on sex chromosomes has led to key discoveries regarding fundamental genetic processes such as recombination and gene dosage (Muller 1932; Begun & Aquadro 1992). However, nearly all this subsequent foundational work has focused on species with XY and ZW sex chromosomes like in Dipterans, Lepidopterans, Mammals, Birds, and few seed plants, diverse organisms that nevertheless do not reflect the complete diversity of sex chromosomes. Many bryophytes and algae possess UV sex chromosomes, which are shaped by distinct evolutionary processes (reviewed in (Bachtrog *et al.* 2011; McDaniel & Perroud 2012; Renner *et al.* 2017; Coelho *et al.* 2018). Only in the past few years have researchers brought new experimental tools to observations first made more than a century ago to explore the untapped potential of comparative genomic analyses among haploid-dioecious organisms.

The purpose of this review is to highlight the novel insights into genome evolution that can be gained by studying UV sex chromosomes. We first present the two-locus model for the evolution of sex chromosomes in the context of a UV system. Next we describe how the symmetry of U and V sex chromosome transmission alters established expectations for several central molecular population genetic parameters, including recombination, effective population size, dosage, and the accumulation of deleterious mutations, relative to XY or ZW chromosome systems. In the third section, we focus on one of the key unanswered questions in sex chromosome evolution - what are the roles of various forms of genetic conflict, like sexual dimorphism, sex-ratio bias, and parent-offspring conflict, in driving differences in the evolution of

sex chromosome gene content? We conclude by highlighting how this framework provides a clear motivation for combining bryophyte genomics with systematic natural history observations and classical genetic analysis.

The evolution of UV sex chromosomes from a hermaphroditic ancestor

All eukaryotes alternate between haploid and diploid stages in their life cycle. Meiosis reduces the genome to a haploid state, while fertilization restores it to diploid. Fertilization, of course, results from the fusion of two gametes, typically called sperm and egg in anisogamous species. In species with separate males and females, the segregation of a chromosome pair often determines sex. Where in the life-cycle sexual differentiation occurs varies among lineages [BOX 1]. In organisms with dioecy in the diploid stage, the sex chromosomes are referred to as either XY or ZW, depending on which sex is heterogametic, while in haploid systems the sex chromosomes are always UV (Fig.1) (Bachtrog *et al.* 2011). The sex chromosome systems share much in common with one another, but the differences among them can shed light on important evolutionary processes.

Sex chromosomes are widely believed to evolve from ordinary autosomes, and may first evolve in concert with dioecy. The evolution of genetically-determined dioecy from a hermaphroditic ancestor requires linked male and female-sterility mutations (Westergaard 1958; Charlesworth & Charlesworth 1978) (but see (Akagi *et al.* 2014; Müller *et al.* 2020) for alternative routes). In the standard diploid formulation of this model, a recessive, male-sterility mutation (i.e., one that renders a hermaphrodite effectively a female) can increase in frequency in a hermaphroditic population when inbred offspring are only half as fit as outbred offspring. That is, if inbreeding is deleterious, male sterility may be favored because the increase in maternal fitness compensates for the loss of fitness through paternity. This produces a gynodioecious population containing females and hermaphrodites (Fig. 2A). Such populations

72 are widely known in angiosperms (Dufaÿ *et al.* 2014; Renner 2014), but to our knowledge have
73 not been experimentally verified in bryophytes.
74 In a diploid gynodioecious population, a dominant, female suppressor (i.e., a proto-Y
75 chromosome) can increase in frequency, provided that a) it has compensatory effects on male
76 fitness and b) its inheritance is negatively correlated with the male-sterility mutation (now the
77 proto-X chromosome). The fixation of both mutations produces a fully dioecious population with
78 an XY sex chromosome system (Fig. 2A). The conditions under which a female-sterility
79 mutation evolves first, leading to an androdioecious population, are more restrictive. In this case
80 dioecy occurs following the evolution of a dominant, male suppressor (i.e., a proto-W
81 chromosome; Fig. 2A). Recombination between the sterility factors produces some individuals
82 that have both male and female-sterility factors. Because these individuals cannot contribute to
83 subsequent generations, these circumstances favor the evolution of suppressed recombination
84 between the sterility loci, completing the transformation of an autosome into a sex chromosome.
85 We should point out two important differences between the diploid and haploid
86 formulations of the two-locus model. First, both the androdioecy and gynodioecy pathways lead
87 to the evolution of UV sex chromosomes (Fig. 2B). Like diploid systems, we expect that
88 gynodioecious mating systems should be more frequent than androdioecious systems, but we
89 know of no rigorous evaluation of the frequencies of such systems in any haploid mating
90 species. Second, because the sexes are haploid, dioecy in a UV system can evolve without a
91 dominant male or female suppressor, because dominance is irrelevant in a haploid system.
92 Given that the evolution of a UV system does not depend on the occurrence of rare dominant
93 mutations, unlike both XY and ZW systems, UV systems might evolve more readily than their
94 diploid counterparts (McDaniel *et al.* 2013; Villarreal & Renner 2013; Laenen *et al.* 2016).
95 Interestingly, most UV systems that have been studied to date are quite old (Ahmed *et al.* 2014;
96 Bowman *et al.* 2017; Carey *et al.* 2020). This is of course not to say that they have been static -
97 these data do not preclude turnover events in which a new chromosome “captures” the sex-

98 determining locus, or the formation of neo-sex chromosomes through translocations (Vicoso
99 2019) - but they do suggest these lineages have experienced a long, uninterrupted history of
100 dioecy.
101
102 **Symmetry in sex chromosome transmission alters evolutionary patterns**
103 The fundamental differences between XY/ZW and UV sex chromosomes stem from the
104 symmetrical transmission of U and V compared to the asymmetry of X and Y or Z and W (Table
105 1). The most obvious implications of symmetry in transition are in the expected amount of
106 segregating variation on the sex chromosomes. In diploid systems, each mated pair has three
107 copies of an X or Z chromosome compared to four copies of each autosome and only one Y or
108 W. Thus, the baseline expected effective population size (N_e) for a X or Z-linked locus is ¾ of an
109 autosome and a Y or W-linked locus is ¼ (Bachtrog *et al.* 2011). In haploid-dioecious systems,
110 each mated pair has one U and one V for every two autosomes so both the U and the V
111 chromosomes are expected to have ½ N_e of an autosome (McDaniel *et al.* 2013). Thus, in UV
112 systems males and females may have similar amounts of sex-linked genetic variation on which
113 selection can act, while in diploid systems the sexes are expected to have different amounts.
114 We should point out that demographic realities, including differential variance in reproductive
115 success between the sexes (Charlesworth 2009), for example due to deviations from an equal
116 ratio of males to females, or variance in female reproductive output (Bengtsson & Cronberg
117 2009), may dramatically alter the patterns of N_e in sex chromosomes relative to the autosomal
118 expectation.
119 Regions of suppressed recombination, like sex chromosomes, also generally have lower
120 than expected levels of diversity due to selective sweeps and background selection, which
121 remove variation across these linked regions (reviewed in (Sayres 2018)). In XY/ZW systems,
122 suppressed recombination is confined to the male-specific Y or the female-specific W
123 chromosomes. In contrast, in UV systems, suppressed recombination is present on both the

female-specific U and male-specific V chromosome (Table 1). Thus, both the male and female-specific chromosomes are expected to experience an equivalent decrease in nucleotide diversity as consequence of suppressed recombination, barring differences in mutation rate or the strength of selection between the sexes. In addition to reduced genetic variation, suppressed recombination can cause genes on the non-recombining chromosome to respond slower to selection (positive or negative) (Comeron *et al.* 2008; Hough *et al.* 2017). Consequently, such regions may show decreased codon bias (relative to autosomes) and an increase in transposable element (TE) abundance, which in UV systems, is expected to affect both equally, in contrast to the asymmetry in diploid systems (Table 1).

The decreased response to selection in non-recombining regions can promote the accumulation of deleterious mutations and loss of functional genes, a process referred to as degeneration (Charlesworth & Charlesworth 2000). In addition, because Y and W chromosomes are always heterozygous with the X and Z, respectively, recessive deleterious mutations are sheltered from purifying selection. In UV sex chromosomes, because the diploids are always UV (not UU or VV; Fig. 1&2) the exposure to selection is symmetrical between the male and female chromosome (Immler & Otto 2015). Indeed, since the sex chromosomes are both expressed in haploids, UV sex chromosomes overall should degenerate slower than either Y or W chromosomes (Table 1) (Immler & Otto 2015). Of course, a haploid-expressed gene could degenerate on one of the sex chromosomes – for example, a gene specifically involved in sperm production which is incorporated into the UV non-recombining region is likely to experience strong purifying selection in males where its function is critical, but weak purifying selection on the female U chromosome if its function may be superfluous (Table 1). Nevertheless, we have no a priori reason to expect that the U or V sex chromosome should degenerate more than the other. The sparse data for UV systems largely support these predictions. [BOX 2]

149 The lack of degeneration on UV sex chromosomes influences the potential for dosage
150 compensation, a common feature of XY/ZW systems. In diploid systems, the homogametic sex
151 has two copies of either an X or Z, and therefore two copies of sex-linked genes to express. The
152 heterogametic sex, in contrast, may have only one gene copy if the Y or W-linked copies have
153 been lost. The gene expression imbalance for X or Z-linked genes that lack a Y or W homolog,
154 due to degeneration, can cause developmental problems or a variety of genetic disorders. Such
155 problems are averted by various forms of dosage compensation, in which one copy of many X-
156 linked genes is silenced in females or the expression of X-linked genes is doubled in males. In
157 contrast, the sex chromosome composition of all life stages is balanced in the UV system - the
158 haploids are either U or V, and the diploids are homogeneous (Fig. 1). If only sex-specific genes
159 are lost on U and V chromosomes, and these genes are not expressed in the unsexed diploid
160 phase, then dosage compensation is unlikely to evolve. While dosage compensation in diploid
161 sex chromosomes is highly variable, and even may be absent or incomplete (reviewed in (Mank
162 2013)), there is no expectation for any such compensation in haploid systems.
163
164 **The role of genetic conflict in the expansion of UV chromosome systems**
165 The population genetic processes that we have discussed so far suggest that sex chromosome
166 evolution follows a well-circumscribed path. This view masks the diversity in sex chromosome
167 size and gene content that is found among, and even within, many lineages of eukaryotes (Bull
168 1983; Tree of Sex Consortium 2014; Furman *et al.* 2020). Clearly degeneration may not play a
169 major role in the evolution of gene content on UV chromosomes, suggesting that other factors,
170 potentially related to life history, may explain variation in sex chromosome size. We should point
171 out that we are focusing only on the non-recombining, sex-limited portions of the sex
172 chromosomes, which may be flanked by psuedoautosomal regions that also vary in size, and
173 experience a host of related but distinct evolutionary processes (Otto *et al.* 2011).

Surveying the UV species that have been studied so far (Box 2), the proportion of the genome residing on the sex chromosomes ranges from >1% in the brown algae *Ectocarpus*, ~1% in the green algae *Volvox*, ~2% in the liverwort *Marchantia*, and as high as 30% of the genome size in the moss *Ceratodon* (Ferris *et al.* 2010; Ahmed *et al.* 2014; Bowman *et al.* 2017; Carey *et al.* 2020). It is possible that the variation in UV sex chromosome size is simply a consequence of different patterns of degeneration, like the accumulation of TEs. However, based on the data currently available, each of these UV pairs shows a similar proportion of shared versus sex-specific genes (~½ in *Ectocarpus*, *Volvox*, and *Ceratodon*; ~¼ in *Marchantia*; Box 2). If degeneration were the cause of gene number variation, older UV systems should have a greater proportion of sex-specific genes. Instead, the similar proportion of shared genes suggests that a U chromosome that currently possesses more genes overall, for example from *Ceratodon*, also lost more homologs that it used to share with the V, meaning that degeneration does not explain the lower gene number in *Marchantia* or *Ectocarpus*. Instead, the size of the sex-limited portion of the U and V chromosomes within a species shows a remarkable correspondence with potential for new mutations to have different fitness effects in males and females, based on the life history of each species (Fig. 3).

If we consider the fate of a new mutation that increases the fitness of females more than males (i.e., a sexually antagonistic allele), that mutation has a greater chance of increasing in frequency if it is tightly linked to a U or W chromosome compared to an autosome (Rice 1987). Conversely, a male-beneficial allele is more likely to fix in a population if it is linked to a V or Y chromosome. Mutations that tighten the linkage between sexually antagonistic alleles and the sex-determining region, such as chromosomal inversions, translocations, or expansions of heterochromatin, also have a greater chance of fixing. Despite the intuitive appeal of this mechanism to explain the evolution of sex linkage, the forces that drive the expansion of regions of suppressed recombination on sex chromosomes remain poorly understood (Ironside 2010). If sexual antagonism is a major force driving the evolution of non-recombining sex chromosomes,

200 then species that experience more sexual antagonism should have more gene-rich sex
201 chromosomes.
202 Three major forms of genetic conflict can potentially shape the evolution of genes on UV
203 sex chromosomes, including sexual dimorphism, parent-offspring conflict, and meiotic sex-ratio
204 distortion. Some evidence supports the action of each of these forms of conflict in species with
205 UV systems. The prevalence of conflict is predicted to covary with the breeding system and
206 genetic diversity, both of which vary considerably among bryophyte species (Eppley *et al.*
207 2007). Although work on genetic conflict in UV systems is in its infancy, it is clear the forms of
208 conflict may act synergistically, through both increasing outbreeding levels and altering linkage
209 patterns on the sex chromosomes. Here we specifically explore how the prevalence of genetic
210 conflict may drive variation in sex chromosome content in bryophytes and other UV systems.
211
212 *Sexual dimorphism:* Perhaps the most obvious reason for sex chromosomes to grow relates to
213 their role in sexual dimorphism. Males and females achieve fitness through different strategies
214 (Bateman 1948; Robert 1972; Lessells & Parker 1999; Chapman 2006). Certainly, genes
215 related to sex-limited functions like sperm or egg production are likely candidates for genes that
216 could evolve sex linkage, although the number of genes that are directly involved in gamete
217 production may be relatively modest and is probably similar among organisms. Nevertheless,
218 anisogamy can generate selection for sexual dimorphism for a variety of additional traits
219 between the sexes (Parker & Others 1979; Bonduriansky *et al.* 2008).
220 The ubiquity of sexual dimorphism suggests selection frequently favors different trait
221 optima in males and females. For example, females of the moss *C. purpureus* produce
222 abundant volatile organic compounds (VOCs) while males produce much less (Rosenstiel *et al.*
223 2012). Remarkably, the female VOCs attract moss sperm-dispersing microarthopods (mites and
224 springtails) in laboratory choice experiments, suggesting that VOC production is part of a scent-
225 based fertilization syndrome analogous to flowering plant-pollinator mutualisms (Rosenstiel *et*

226 *al.* 2012; Shortlidge *et al.* 2020). Co-cultivating mosses and arthropods can increase both the
227 number of genotypes that reproduce and the overall number of sporophytes produced
228 (Cronberg *et al.* 2006; Shortlidge *et al.* 2020). The fact that males have lower VOC production,
229 however, suggests that scent production may be costly. Thus, a mutation that increases VOC
230 production may be beneficial for females, but deleterious for males. This sexual antagonism can
231 be resolved by evolving either sex-linkage (i.e., moving the genes for VOC production to the sex
232 chromosome, where they can evolve to their sex-specific optima) or sex-biased gene
233 expression, where the genes that control VOC production may be expressed in different ways in
234 males and females (Vicoso & Charlesworth 2006; Ellegren & Parsch 2007). Sex-biased gene
235 expression is very common in animals, but the relative importance of differential autosomal
236 gene expression compared to sex linkage in UV systems is unknown. Although in *C. purpureus*,
237 the abundant sex-linked genes are clearly important between the sexes (Carey *et al.* 2020).
238 Sexual dimorphism is found in a wide variety of other traits in bryophytes, suggesting
239 that sexually antagonistic selection may drive the evolution of sex linkage. For example, leaf
240 size and juvenile growth in *C. purpureus* are sexually dimorphic (McDaniel 2009) as are life
241 history traits in the liverwort *M. introflexa* (McLetchie & Puterbaugh 2000; Fuselier 2008) and the
242 brown algae *Ectocarpus* (Lipinska *et al.* 2015). The links between such trait variation and either
243 male or female fitness are less clear than for VOC production, but it remains a reasonable
244 hypothesis that more dimorphic species, or species with stronger male–male competition or
245 female-mate choice, may possess more sex-linked genes. We should point out that the
246 relationship between dimorphism and sex chromosome gene content may not be entirely
247 straight-forward – for example, several species of mosses, like the genera *Dicranum* and
248 *Homalothecium*, possess an extreme form of sexual dimorphism in which dwarf males grow
249 epiphytically on more typical sized females (Hedenäs & Bisang 2011; Rosengren & Cronberg
250 2014), a form of sexual dimorphism that may actually decrease male-male competition. Detailed

studies relating the breeding system of a species to levels of sexual conflict are fertile areas for research in bryophyte biology.

Parent-offspring conflict over nutrient allocation: The fact that males and females differ in the amount of resources they allocate to reproduction also sets the stage for genomic conflict (Arnqvist & Rowe 2005). Theory suggests conflict is particularly likely for species that provision offspring through vivipary (Zeh & Zeh 2000). In embryophytes (i.e., land plants), the embryo is retained on the haploid, female gametophyte and water and nutrients are provided for a time through specialized transfer cells analogous to a placenta. The males, in contrast, have no role in providing for offspring after fertilization. A better provisioned embryo (sporophyte) is likely to make more and healthier spores, increasing the fitness of both the male and female. However, in species where the female can reproduce more than once, the allocation to one sporophyte may come at the expense of other sibling sporophytes (Stark *et al.* 2009). Therefore, female fitness is maximized by equal allocation to several offspring by modulating the transfer of nutrients across the placenta (Haig 2013). In contrast, a male may not mate with the same female again (nor any other female), so male fitness increases with every additional viable spore that an offspring sporophyte produces. Thus, paternal genes in the embryo are expected to evolve to extract as much of the female's resources as possible to promote spore production (Haig 2013). Consistent with this, studies in *Funaria hygrometrica* showed the maternal calyptra tissue that covers the offspring sporophyte, controls its growth (Bopp 1957; Budke 2019). Moreover, spore production in experimental crosses in *C. purpureus* suggest the genetic variation necessary for parent-offspring conflict is found in this species (Shortlidge *et al.* 2020). The same female crossed to two different males produced sporophytes that varied in spore production, suggesting the paternal genotype influences the transfer of nutrients across the placenta (Shortlidge *et al.* 2020). Interestingly, the difference between the interests of the maternal and paternal contributions becomes more intense as the levels of outbreeding

277 increase (Haig 2010), again highlighting the importance of the breeding system for shaping
278 genetic conflict.
279 We expect that sex chromosomes may be enriched in genes that influence the flow of
280 nutrients across the placenta from mother to offspring. In XY systems, only the degenerate Y
281 chromosome shows sex-limited inheritance, potentially minimizing its role in parent-offspring
282 conflict. However, autosomal genes that influence maternal transfer may have parent-of-origin
283 effects depending upon whether they are transferred from the mother or father, an observation
284 termed genomic imprinting (Reik & Walter 2001). What is striking about UV systems is the
285 unusual pattern of inheritance of the sex chromosomes accomplishes what other systems do by
286 genomic imprinting. Because the U and V chromosomes do not recombine, their interests
287 diverge considerably in the developing UV-heterozygous embryo, providing a highly-favorable
288 environment for the fixation of diploid-acting, sexually antagonistic alleles that influence the flow
289 of nutrients from the maternal (U) gametophyte to the offspring (UV) sporophyte. The U-linked
290 maternal genes within the embryo are genetically identical to its mother, and therefore share a
291 common interest, but the V-linked paternal genes in the embryo may have different interests
292 regarding the amount of offspring provisioning (Haig & Wilczek 2006). Formal tests linking
293 parent-offspring conflict to the expansion of UV sex chromosomes will likely involve a
294 combination of placental transcriptomics, classical genetics, and transgenic approaches.
295
296 *Sex-biased transmission ratio distortion*: A final form of sexual antagonism that is common to all
297 dioecious species with chromosomal sex determination is meiotic sex-ratio distortion. The
298 meiotic sex ratio in a wide variety of eukaryotes deviates from the expected 1:1 segregation of
299 males and females (Lyttle 1993). At meiosis, any allele that preferentially transmits itself to the
300 next generation has an immediate benefit, thus selection on these loci can be very strong
301 because such alleles consistently have higher frequency in offspring. In principle, any
302 heterozygous locus could experience biased transmission (Haig 2010). Distorter alleles may

bias transmission by a wide variety of mechanisms, but in UV systems distorters generally disable meiotic products carrying the alternate allele. Mutations that result in more female offspring are likely to arise on, or be inherited with the U, while mutations that distort the sex ratio in favor of males will be associated with the V.

Most meiotic sex-ratio distorter systems typically consist of multiple distinct genes (Werren & Beukeboom 1998). Distorter systems require a killer locus that halts development or otherwise disables the products of meiosis, and a second locus that protects the distorting chromosome from being disabled (Bravo Núñez *et al.* 2018). Thus, a distorting U chromosome would have a killer allele and an allele at the second locus that disarms the killer, often termed an insensitive-responder allele. The V chromosome, in contrast, would lack the killer and would carry a linked, sensitive-responder allele. Because the killer and insensitive-responder must be inherited together, genomic regions that lack meiotic recombination, like sex chromosomes, may be more likely to evolve distorter systems simply because they contain more genes that can potentially evolve the killing and resistance functions. As a meiotic killer system spreads, one sex could become rare, conditions that favor the evolution of alleles at a third locus that suppress the killer allele, restoring fertility (Tao *et al.* 2007). Although restorer alleles can arise on autosomes, the sex chromosomes are predicted to be hot-spots for the evolution of restorer alleles too (Hall 2004). Thus, because they contain large regions of suppressed recombination that have sex-limited inheritance, sex chromosomes are likely to accumulate multiple genes that influence the outcome of meiosis through either spore killing or fertility restoration.

A key difference between diploid and haploid mating systems is the action of a meiotic distorter in haploids most often will result in spore death (Nauta & Hoekstra 1993). Because each spore directly contributes to forming the next generation of gametophytes, unlike sperm which contribute indirectly through fertilization of an egg, spore death causes a loss of fitness. Thus, the evolution of meiotic sex-ratio distorters is likely to be held in check by the fitness cost caused by spore killing, in addition to the reduction in mating opportunity caused by decreasing

329 the frequency of one sex. Nevertheless, several spore killer systems segregate in fungi (e.g.,
330 (Hammond *et al.* 2012)) and variation in spore death in *C. purpureus* has a genetic basis (Shaw
331 & Gaughan 1993; McDaniel *et al.* 2007; Norrell *et al.* 2014). Numerous questions remain
332 regarding the true fitness costs of meiotic sex-ratio distortion in bryophytes (and other
333 organisms), life-history traits that promote the evolution of meiotic distorters, and the potential
334 interactions among different forms of genetic conflict in shaping the gene content of the male
335 and female sex chromosomes.
336
337 **The central role of natural history in evolutionary genomics**
338 In this review, we have argued that the wide variation in sex chromosome size and gene content
339 may be explained by variation in levels of genetic conflict, and further that bryophytes provide
340 the necessary variation to test this hypothesis. In UV systems, suppressed recombination is not
341 confounded with sex-specific mutational biases, sex-specific inheritance, and sexual selection,
342 making bryophytes well-suited for studying how conflicts affect both male and female sex
343 chromosomes. Clearly this effort will be bolstered by sequencing additional bryophyte genomes,
344 and we anticipate that soon we will have a clearer picture of the gene content and evolutionary
345 history of an increasing number of UV sex chromosome systems. In addition, generating sex-
346 linked markers will open numerous research avenues in many previously genetically intractable
347 systems, enabling us to understand many sex-specific demographic phenomena (e.g.,
348 dispersal, growth, mating success, male-male competition, female-mate choice, biased
349 mortality, or N_e), in addition to more effectively designing studies of sexual dimorphism, parent-
350 offspring conflict, and meiotic sex-ratio variation.
351 Equally important, however, is the role that natural history and phylogenetics can play in
352 contextualizing the results of genomic analyses. Life history features relating to patterns of
353 mating in nature, in particular, may be central to modulating the role of genetic conflict in
354 genome evolution. Bryophytes are one of the few terrestrial groups that contain large

proportions of both hermaphrodites and species with separate sexes. This circumstance makes bryophytes unusually well-suited for linking sex chromosome history and molecular evolution to patterns of diversification. How often is speciation associated with a change in mating system? Are sex chromosomes more likely than autosomes to be exchanged among distant populations, or more likely to host loci that promote reproductive isolation? Does genetic conflict promote elevated rates of speciation (Crespi & Nosil 2013), or extinction (Price *et al.* 2010; Werren 2011), and under what conditions? Critically, bryophytes possess underexplored life history and taxonomic variation that may be used to draw general solutions to key questions regarding the long-term evolution of sex chromosomes across eukaryotes.

BOX 1: UV sex chromosomes are found in haploid-dioecious life cycles

In animals and vascular plants with genetically-determined separate sexes, sexual differentiation occurs in the diploid stage of the life cycle. Therefore, the karyotypic sex of an individual is determined by the genotype at fertilization. In the XY system, the fusion of Y-bearing sperm with an X-bearing egg produces a male zygote (the heterogrametic sex), while the fusion of an X-bearing sperm with an X-bearing egg produces a homogametic, female zygote. In the ZW system, in contrast, the female is the heterogametic sex. In these diploid systems, the non-recombining Y and W chromosomes are sex-specific (i.e., transmitted only through the male or female line, respectively), while the X and Z are shared between both sexes and freely recombine, much like an autosome, in the homogametic sex. In both the XY or ZW systems, sexual differentiation is a diploid phenomenon.

In other multicellular lineages, including the Phaeophytes (brown algae), Rhodophytes (red algae), Chlorophytes (Ulvophyceae and Chlorophyceae green algae), and Bryophytes (mosses, liverworts, and hornworts), sex determination occurs in the haploid stage of the life cycle. As such, only one copy of a sex chromosome is present during sex expression, with

individuals inheriting the U correlating with females and V with males, and making both the U and V sex-specific (Bachtrog *et al.* 2011). Indeed, since the sexes are already haploid, gametes are produced by mitosis not meiosis (Fig. 1). Fertilization of a U-linked egg and a V-linked sperm produces a zygote that is monomorphic (i.e., non-sexed) and heterozygous at the sex determining factor (homozygous UU or VV sporophytes are not formed in typical matings). In the mature diploid individual, the U and V pair at meiosis and segregate independently into females and males (Bachtrog *et al.* 2011).

The U and V chromosomes are in some ways similar to mating-type (MT) loci found in fungi, algae, and other protists (e.g., (Ferris *et al.* 2010; Bazzicalupo *et al.* 2019). In these organisms, however, the gametes bearing alternate MT are not phenotypically distinct from one another (i.e., isogamous) and therefore do not have defined sexes (Fig. 3). Thus, while MT loci provide valuable systems for studying the evolution of suppressed recombination in the absence of anisogamy (Branco *et al.* 2017; Bazzicalupo *et al.* 2019), we restrict our focus here to true UV sex chromosomes.

BOX 2: Genomic analyses of UV systems

A growing number of genomic analyses of UV sex chromosomes systems (reviewed in (Coelho *et al.* 2018)) provide a foundation for comparative analyses between haploid and diploid sex chromosome systems (Table 1). One of the best genomically studied UV systems to date is the brown algae *Ectocarpus sp.* An ancestral-state reconstruction of sexual condition in brown algaes found dioecy evolved near the origins of this lineage (Luthringer *et al.* 2014) and molecular evidence indicates *Ectocarpus* and the kelps (Laminariales) may share a common UV system that evolved at least ~80-110 million years ago (MYA) (Lipinska *et al.* 2017) The U and V chromosomes in *Ectocarpus sp*. are relatively small (0.93 and 0.92 megabases (Mb) of the 205 Mb genome, respectively) (Ahmed *et al.* 2014). The non-recombining portions of the U and V have similar numbers of genes at 22 and 20, respectively, and share 11 homologous genes

(Ahmed *et al.* 2014). Avia et al. (2018) found the *Ectocarpus* U and V-linked loci had an N_e about ½ the autosomal average, suggesting these chromosomes experience levels of selection similar to autosomal loci (Avia *et al.* 2018). As a result of suppressed recombination, the sex chromosomes show decreased codon bias (Lipinska *et al.* 2017) and increased TEs relative to autosomes in both sex-determining and pseudoautosomal regions (Ahmed *et al.* 2014; Luthringer *et al.* 2015). Consistent with the effects of degeneration, Lapinska et al. (2017) found evidence of gene loss from the ancestral brown algae sex chromosome, in addition to gene gain and gene movement to the autosomes (Lipinska *et al.* 2017).

Within the chlorophyte green algae, at least two UV sex chromosome systems evolved independently. Dioecy and MT loci evolved in the ancestor of the volvocine algae, including *Gonium pectorale*, *Volvox carteri,* and *Chlamydomonas reinhardtii* at least 200 MYA (Herron *et al.* 2009; Hamaji *et al.* 2016). Although, strictly speaking, only *Volvox* possess anisogamy and therefore true UV sex chromosomes, we mention the other species because the MT chromosomes and UV sex chromosomes are clearly homologous. The *Volvox* U and V are 1.51 and 1.13 of the 131 Mb genome and also have similar numbers of genes at 55 and 60, respectively, 50 of which are homologs between the two (Ferris *et al.* 2010). Consistent with the effects of suppressed recombination, the *Volvox* sex chromosomes have accumulated TEs (Ferris *et al.* 2010) and comparisons between *Gonium, Chlamydomonas,* and *Volvox* found evidence for gene loss (Hamaji *et al.* 2016). A second UV sex chromosome evolved in the lineage including *Ulva partita* (Yamazaki *et al.* 2017). The 1.5 Mb *Ulva* U (referred to as MT+) and 1.0 Mb V (referred to as MT-) have some asymmetry in gene numbers with 67 and 46 genes, respectively, and share 23 homologs. While the size of the *Ulva partia* genome is currently unknown, the recent *Ulva mutabilis* genome is 98.5 Mb and *U. partia* may be similar (De Clerck *et al.* 2018). The discovery of sex-linked PCR markers in red algae (Guillemin *et al.* 2012), suggests that UV sex chromosomes independently evolved in this group too, but genomic analyses are currently lacking.

Within the bryophytes, more than half of the approximately 20,000 described species are dioecious. Heteromorphic sex chromosomes have been observed using cytological methods in 16 unrelated genera (Allen 1945; Renner *et al.* 2017) and sex-linked molecular markers have been tested in several additional species (McLetchie & Puterbaugh 2000; McDaniel *et al.* 2007; Korpelainen *et al.* 2008; Baughman *et al.* 2017). Ancestral-state reconstructions of sexual condition first showed that bryophyte sex chromosomes may have ancient origins (McDaniel *et al.* 2013; Laenen *et al.* 2016) and emerging phylogenomic data support in liverworts they evolved at least 400 MYA and in mosses 300 MYA (Carey *et al.* 2020).

To date, the best studied bryophyte UV sex chromosomes are in the liverwort *Marchantia polymorpha*. The 4.37 Mb U chromosome contains 74 genes and the 6 Mb V contains 105 genes, with 20 shared homologs between the U and V, suggesting some asymmetry in size and gene number between the sexes (total genome size 225.8 Mb; (Okada *et al.* 2001; Yamato *et al.* 2007; Bowman *et al.* 2017). The gene density in the U and V-linked regions is ~5x lower than the autosomes, consistent with the accumulation of TEs due to suppressed recombination (Bowman *et al.* 2017; Montgomery *et al.* 2020). The U and V sex chromosomes in the moss *C. purpureus* were each ⅓ of the genome (~110 Mb of the 360 Mb genome with ~3,400 genes, ~1,800 of which are homologous), consistent with earlier cytological data (Voglmayr 2000; McDaniel *et al.* 2007; Szövényi *et al.* 2015; Carey *et al.* 2020). Moreover, the sex chromosomes showed a greater abundance of repeats and decreased codon bias than the autosomes, suggesting weaker selection due to suppressed recombination (Carey *et al.* 2020). In contrast to *Ectocarpus (Avia et al. 2018)*, sex-linked genes in *C. purpureus* exhibited lower nucleotide diversity than an panel of autosomal loci (McDaniel *et al.* 2013), suggesting they may have experience recent bouts of natural selection.

Acknowledgements

We thank two anonymous reviewers for helpful feedback on this manuscript. SFM was supported by NSF-DEB grants 1541005 and 1542609.

Figures and Tables

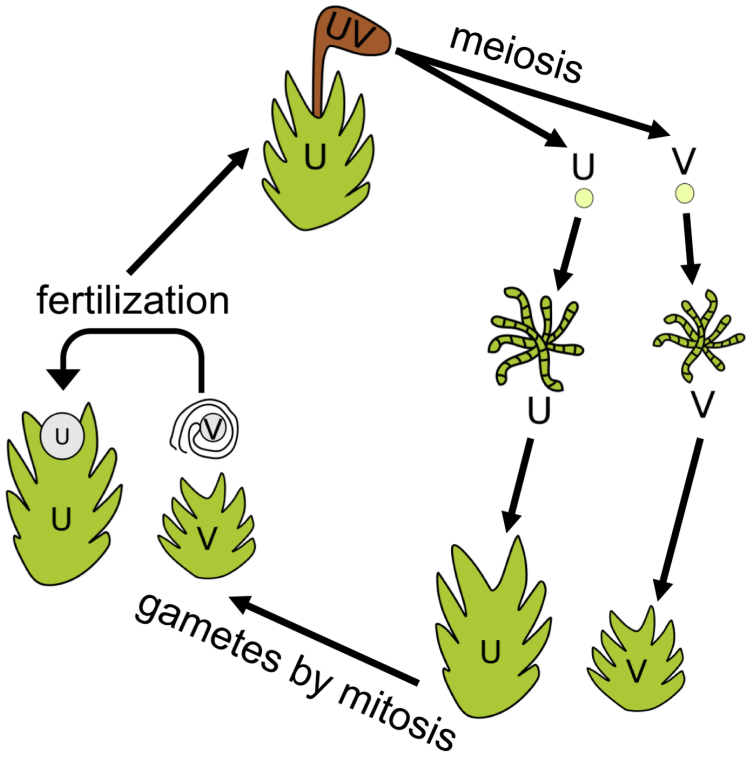
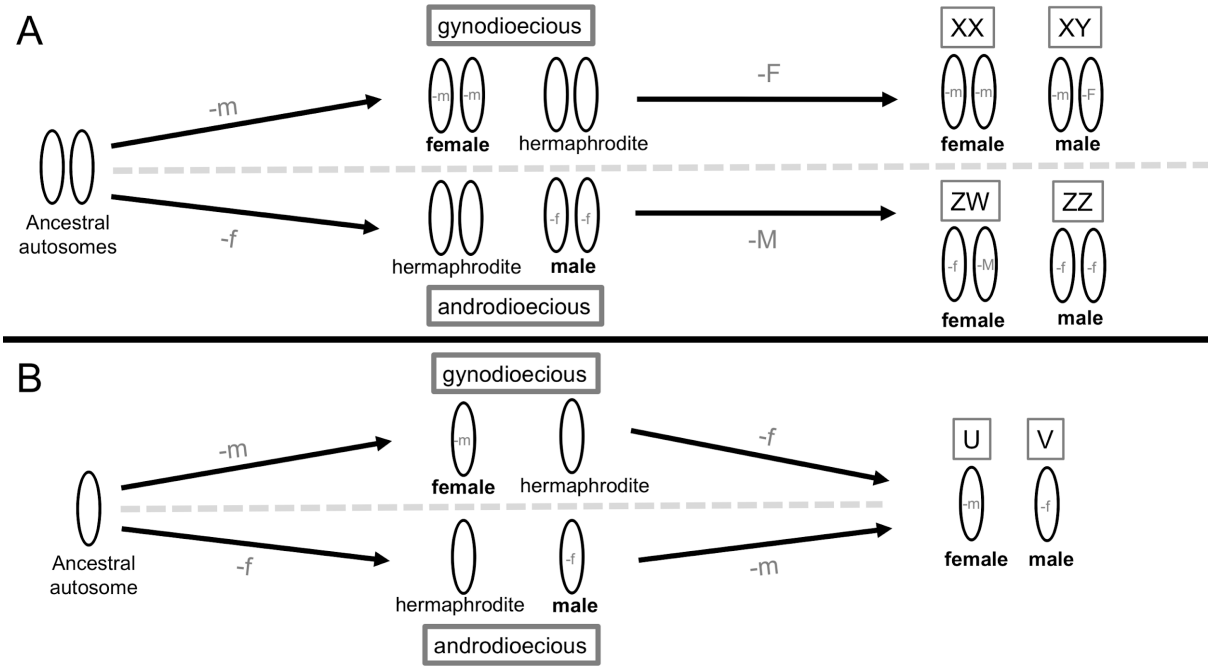


Figure 1. Life cycle of a dioecious moss. In mosses, the products of meiosis are haploid spores. For dioecious species, meiosis also segregates the U and V sex chromosomes in females and males, respectively. The spores germinate and produce protonemal filaments, upon which gametophores will develop. Because they are haploid, the mature male and female gametophytes produce gametes by mitosis. A V-containing sperm fuses with a U-containing egg, undergoing fertilization, and a diploid embryo (sporophyte) will develop on the maternal plant. The sporophyte embryo has both U and V sex chromosomes, but is considered a non-sexed stage, and within which the spores are produced.



478

479 **Figure 2. Two-locus model for the evolution of XY, ZW, and UV sex chromosomes.** For
480 diploid sex chromosomes (A) that originate from an ancestral pair of autosomes, a recessive
481 male-sterility mutation (-m) first evolves, making a gynodioecious population (females and
482 hermaphrodites). From the gynodioecious population a dominant female-sterility mutation (-F)
483 arises making males. Once recombination is suppressed between these sex-determining loci,
484 sex chromosomes are formed. This pathway leads to an XY sex chromosome system. If from
485 an ancestral autosomal pair, a recessive female-sterility mutation (-f) evolves first, making an
486 androdioecious population, followed by a dominant male-sterility mutation (-M), the result is a
487 ZW sex chromosome system. In contrast in haploid sex chromosome evolution (B), if a male or
488 female-sterility mutation evolves first, followed by sterility mutation of the opposite sex, the result
489 is always a UV sex chromosome.

Table 1. Comparison of XY, ZW, and UV characteristics.

Characteristic	X	Y	Z	W	U	V
Inheritance pattern	M and F	M	M and F	F	F	M
Hemizygosity	M	M	F	F	diploid phase	diploid phase
Expected N_e, relative to autosomes	3/4	1/4	3/4	1/4	1/2	1/2
Suppressed recombination	NA	Yes	NA	Yes	Yes	Yes
TE content, relative to autosomes	similar	higher	similar	higher	higher	higher
Gene density, relative to autosomes	similar	lower	similar	lower	lower	lower
Gene-content bias	NA, F, or M-biased	M-biased	NA, F, or M-biased	F-biased	F-biased	M-biased

Males (M); females (F); Effective population size (N_e); Transposable elements (TE)

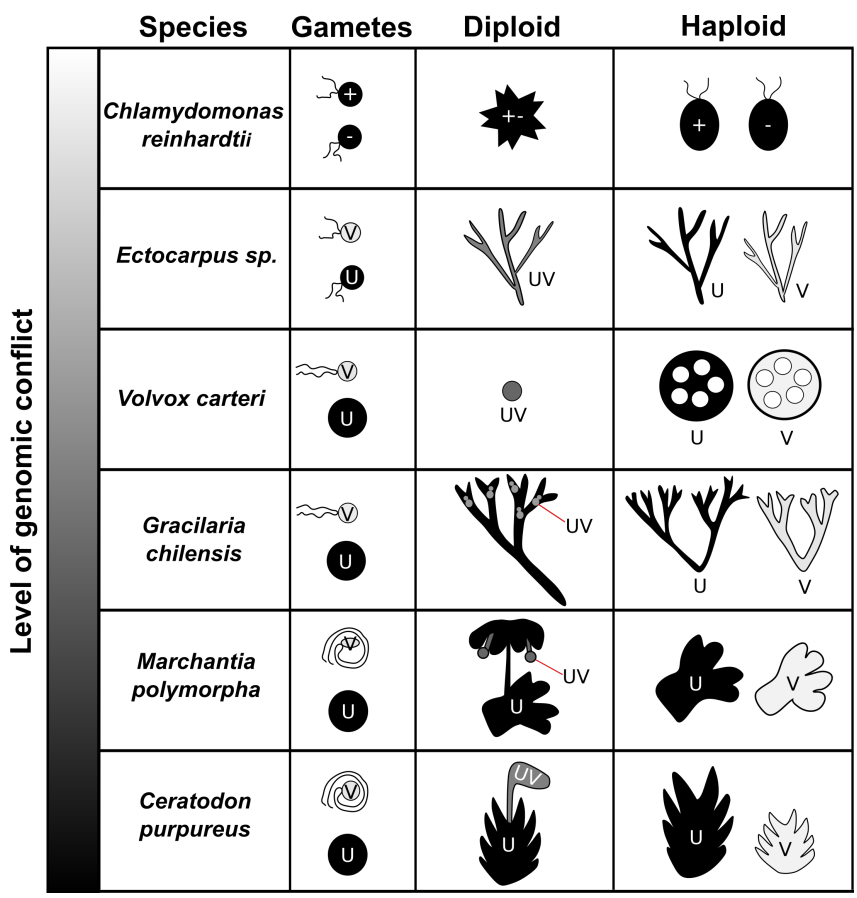


Figure 3. Predicted intensity of genomic conflict among UV systems. The level of genomic conflict is lower in mating-type loci because they do not have separate sexes, and therefore lack sexual dimorphism and parent-offspring conflict, although the mating type loci could experience transmission-ratio distortion. Across UV systems, the level of genomic conflict increases as the number or intensity of conflicts increases. Sexual dimorphism tends to be limited in algaes and is but may be obvious in bryophytes. Parent-offspring conflict requires a nutritional dependence between the embryo and the maternal plant, which is absent in some algae but lengthy in some mosses. Transmission ratio distortion is possible in all systems, although distorter systems may be more frequent in outbreeding species. We should note that this graphic is simply meant to illustrate a hypothesis, and that each of these groups is likely to be highly heterogeneous for the forms of conflict that we have depicted.

References

Ahmed S., Cock J.M., Pessia E., Luthringer R., Cormier A., Robuchon M., Sterck L., Peters A.F., Dittami S.M., Corre E., Valero M., Aury J.-M., Roze D., Van de Peer Y., Bothwell J., Marais G.A.B. & Coelho S.M. 2014. — A haploid system of sex determination in the brown alga Ectocarpus sp. *Current biology: CB* 24 (17): 1945–1957

Akagi T., Henry I.M., Tao R. & Comai L. 2014. — A Y-chromosome–encoded small RNA acts as a sex determinant in persimmons. *Science* 346 (6209): 646–650

Allen C.E. 1917. — A CHROMOSOME DIFFERENCE CORRELATED WITH SEX DIFFERENCES IN SPHAeROCARPOS. *Science* 46 (1193): 466–467

Allen C.E. 1945. — The genetics of bryophytes. II. *The Botanical review; interpreting botanical progress* 11 (5): 260–287

Anderson L.E. 2000. — Charles E. Allen and Sex Chromosomes. *The Bryologist* 103 (3): 442–448

Arnqvist G. & Rowe L. 2005. — *Sexual Conflict*.Princeton University Press. 330 p.

Avia K., Lipinska A.P., Mignerot L., Montecinos A.E., Jamy M., Ahmed S., Valero M., Peters A.F., Cock J.M., Roze D. & Coelho S.M. 2018. — Genetic Diversity in the UV Sex Chromosomes of the Brown Alga Ectocarpus. *Genes* 9 (6). https://doi.org/10.3390/genes9060286

Bachtrog D., Kirkpatrick M., Mank J.E., McDaniel S.F., Pires J.C., Rice W. & Valenzuela N. 2011. — Are all sex chromosomes created equal? *Trends in genetics: TIG* 27 (9): 350–357

Bateman A.J. 1948. — Intra-sexual selection in Drosophila. *Heredity* 2 (Pt. 3): 349–368

Baughman J.T., Payton A.C., Paasch A.E., Fisher K.M. & McDaniel S.F. 2017. — Multiple factors influence population sex ratios in the Mojave Desert moss Syntrichia caninervis. *American journal of botany* 104 (5): 733–742

Bazzicalupo A.L., Carpentier F., Otto S.P. & Giraud T. 2019. — Little Evidence of Antagonistic Selection in the Evolutionary Strata of Fungal Mating-Type Chromosomes (Microbotryum lychnidis-dioicae). *G3* 9 (6): 1987–1998

Begun D.J. & Aquadro C.F. 1992. — Levels of naturally occurring DNA polymorphism correlate with recombination rates in D. melanogaster. *Nature* 356 (6369): 519–520

Bengtsson B.O. & Cronberg N. 2009. — The effective size of bryophyte populations. *Journal of theoretical biology* 258 (1): 121–126

Berger F. 2019. — Emil Heitz, a true epigenetics pioneer. *Nature reviews. Molecular cell biology* 20 (10): 572

Bonduriansky R., Maklakov A., Zajitschek F. & Brooks R. 2008. — Sexual Selection, Sexual Conflict and the Evolution of Ageing and Life Span. *Functional ecology* 22 (3): 443–453

Bopp M. 1957. — Entwicklungsphysiologische Untersuchungen an Moosmutanten. *Zeitschrift fur induktive Abstammungs- und Vererbungslehre* 88 (4): 600–607

Bowman J.L., Kohchi T., Yamato K.T., Jenkins J., Shu S., Ishizaki K., Yamaoka S., Nishihama R., Nakamura Y., Berger F., Adam C., Aki S.S., Althoff F., Araki T., Arteaga-Vazquez M.A., Balasubrmanian S., Barry K., Bauer D., Boehm C.R., Briginshaw L., Caballero-Perez J., Catarino B., Chen F., Chiyoda S., Chovatia M., Davies K.M., Delmans M., Demura T., Dierschke T., Dolan L., Dorantes-Acosta A.E., Eklund D.M., Florent S.N., Flores-Sandoval E., Fujiyama A., Fukuzawa H., Galik B., Grimanelli D., Grimwood J., Grossniklaus U., Hamada T., Haseloff J., Hetherington A.J., Higo A., Hirakawa Y., Hundley H.N., Ikeda Y., Inoue K., Inoue S.-I., Ishida S., Jia Q., Kakita M., Kanazawa T., Kawai Y., Kawashima T., Kennedy M., Kinose K., Kinoshita T., Kohara Y., Koide E., Komatsu K., Kopischke S., Kubo M., Kyozuka J., Lagercrantz U., Lin S.-S., Lindquist E., Lipzen A.M., Lu C.-W., De Luna E., Martienssen R.A., Minamino N., Mizutani M., Mizutani M., Mochizuki N., Monte I., Mosher R., Nagasaki H., Nakagami H., Naramoto S., Nishitani K., Ohtani M., Okamoto T., Okumura M., Phillips J., Pollak B., Reinders A., Rövekamp M., Sano R., Sawa S., Schmid M.W., Shirakawa M., Solano R., Spunde A., Suetsugu N., Sugano S., Sugiyama A., Sun R., Suzuki Y., Takenaka M., Takezawa D., Tomogane H., Tsuzuki M., Ueda T., Umeda M., Ward J.M., Watanabe Y., Yazaki K., Yokoyama R., Yoshitake Y., Yotsui I., Zachgo S. & Schmutz J. 2017. — Insights into Land Plant Evolution Garnered from the Marchantia polymorpha Genome. *Cell* 171 (2): 287–304.e15

Branco S., Badouin H., Rodríguez de la Vega R.C., Gouzy J., Carpentier F., Aguileta G., Siguenza S., Brandenburg J.-T., Coelho M.A., Hood M.E. & Giraud T. 2017. — Evolutionary strata on young mating-type chromosomes despite the lack of sexual antagonism. *Proceedings of the National Academy of Sciences of the United States of America* 114 (27): 7067–7072

Bravo Núñez M.A., Nuckolls N.L. & Zanders S.E. 2018. — Genetic Villains: Killer Meiotic Drivers. *Trends in genetics: TIG* 34 (6): 424–433

Budke J.M. 2019. — The moss calyptra: A maternal structure influencing offspring development. *The Bryologist* 122 (3): 471–491

Bull J.J. 1983. — *Evolution of sex determining mechanisms*.The Benjamin/Cummings Publishing Company, Inc.

Carey S.B., Jenkins J., Payton A.C., Shu S., Lovell J.T., Maumus F., Sreedasyam A., Tiley G.P., Fernandez-Pozo N., Barry K., Chen C., Wang M., Lipzen A., Daum C., Saski C.A., McBreen J.C., Conrad R.E., Kollar L.M., Olsson S., Huttunen S., Landis J.B., Gordon Burleigh J., Wickett N.J., Johnson M.G., Rensing S.A., Grimwood J., Schmutz J. & McDaniel S.F. 2020. — Chromosome fusions shape an ancient UV sex chromosome system. *bioRxiv* https://doi.org/10.1101/2020.07.03.163634

Chapman T. 2006. — Evolutionary conflicts of interest between males and females. *Current biology: CB* 16 (17): R744–54

Charlesworth B. & Charlesworth D. 1978. — A model for the evolution of dioecy and gynodioecy. *The American naturalist*

Charlesworth B. 2009. — Effective population size and patterns of molecular evolution and variation. *Nature reviews. Genetics* 10 (3): 195–205

Charlesworth B. & Charlesworth D. 2000. — The degeneration of Y chromosomes. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 355 (1403): 1563–1572

610 Coelho S.M., Gueno J., Lipinska A.P., Cock J.M. & Umen J.G. 2018. — UV Chromosomes and
611 Haploid Sexual Systems. *Trends in plant science* 23 (9): 794–807

612 Comeron J.M., Williford A. & Kliman R.M. 2008. — The Hill–Robertson effect: evolutionary
613 consequences of weak selection and linkage in finite populations. *Heredity* 100 (1): 19–31

614 Crespi B. & Nosil P. 2013. — Conflictual speciation: species formation via genomic conflict.
615 *Trends in ecology & evolution* 28 (1): 48–57

616 Cronberg N., Natcheva R. & Hedlund K. 2006. — Microarthropods mediate sperm transfer in
617 mosses. *Science* 313 (5791): 1255

618 De Clerck O., Kao S.-M., Bogaert K.A., Blomme J., Foflonker F., Kwantes M., Vancaester E.,
619 Vanderstraeten L., Aydogdu E., Boesger J., Califano G., Charrier B., Clewes R., Del Cortona A.,
620 D'Hondt S., Fernandez-Pozo N., Gachon C.M., Hanikenne M., Lattermann L., Leliaert F., Liu X.,
621 Maggs C.A., Popper Z.A., Raven J.A., Van Bel M., Wilhelmsson P.K.I., Bhattacharya D., Coates
622 J.C., Rensing S.A., Van Der Straeten D., Vardi A., Sterck L., Vandepoele K., Van de Peer Y.,
623 Wichard T. & Bothwell J.H. 2018. — Insights into the Evolution of Multicellularity from the Sea
624 Lettuce Genome. *Current biology: CB* 28 (18): 2921–2933.e5

625 Dufaÿ M., Champelovier P., Käfer J., Henry J.-P., Mousset S. & Marais G.A.B. 2014. — An
626 angiosperm-wide analysis of the gynodioecy--dioecy pathway. *Annals of botany* 114 (3): 539–
627 548

628 Ellegren H. & Parsch J. 2007. — The evolution of sex-biased genes and sex-biased gene
629 expression. *Nature reviews. Genetics* 8 (9): 689–698

630 Eppley S.M., Taylor P.J. & Jesson L.K. 2007. — Self-fertilization in mosses: a comparison of
631 heterozygote deficiency between species with combined versus separate sexes. *Heredity* 98
632 (1): 38–44

633 Ferris P., Olson B.J.S.C., De Hoff P.L., Douglass S., Casero D., Prochnik S., Geng S., Rai R.,
634 Grimwood J., Schmutz J., Nishii I., Hamaji T., Nozaki H., Pellegrini M. & Umen J.G. 2010. —
635 Evolution of an expanded sex-determining locus in Volvox. *Science* 328 (5976): 351–354

636 Furman B.L.S., Metzger D.C.H., Darolti I., Wright A.E., Sandkam B.A., Almeida P., Shu J.J. &
637 Mank J.E. 2020. — Sex Chromosome Evolution: So Many Exceptions to the Rules. *Genome*
638 *biology and evolution* 12 (6): 750–763

639 Fuselier L. 2008. — Variation in life history characteristics between asexual and sexual
640 populations of marchantia inflexa. *The Bryologist* 111 (2): 248–259

641 Guillemin M.-L., Huanel O.R. & Martínez E.A. 2012. — CHARACTERIZATION OF GENETIC
642 MARKERS LINKED TO SEX DETERMINATION IN THE HAPLOID-DIPLOID RED ALGA
643 GRACILARIA CHILENSIS 1. *Journal of phycology* 48 (2): 365–372

644 Haig D. 2013. — Filial mistletoes: the functional morphology of moss sporophytes. *Annals of*
645 *botany* 111 (3): 337–345

646 Haig D. 2010. — Games in tetrads: segregation, recombination, and meiotic drive. *The*
647 *American naturalist* 176 (4): 404–413

648 Haig D. & Wilczek A. 2006. — Sexual conflict and the alternation of haploid and diploid

649 generations. *Philosophical transactions of the Royal Society of London. Series B, Biological*
650 *sciences* 361 (1466): 335–343

651 Hall D.W. 2004. — Meiotic drive and sex chromosome cycling. *Evolution; international journal of*
652 *organic evolution* 58 (5): 925–931

653 Hamaji T., Mogi Y., Ferris P.J., Mori T., Miyagishima S., Kabeya Y., Nishimura Y., Toyoda A.,
654 Noguchi H., Fujiyama A., Olson B.J.S.C., Marriage T.N., Nishii I., Umen J.G. & Nozaki H. 2016.
655 — Sequence of the Gonium pectorale Mating Locus Reveals a Complex and Dynamic History of
656 Changes in Volvocine Algal Mating Haplotypes. *G3* 6 (5): 1179–1189

657 Hammond T.M., Rehard D.G., Xiao H. & Shiu P.K.T. 2012. — Molecular dissection of
658 Neurospora Spore killer meiotic drive elements. *Proceedings of the National Academy of*
659 *Sciences of the United States of America* 109 (30): 12093–12098

660 Hedenäs L. & Bisang I. 2011. — The overlooked dwarf males in mosses—Unique among green
661 land plants. *Perspectives in plant ecology, evolution and systematics* 13 (2): 121–135

662 Heitz E. 1928. — *Das heterochromatin der moose*.Bornträger.

663 Herron M.D., Hackett J.D., Aylward F.O. & Michod R.E. 2009. — Triassic origin and early
664 radiation of multicellular volvocine algae. *Proceedings of the National Academy of Sciences of*
665 *the United States of America* 106 (9): 3254–3258

666 Hough J., Wang W., Barrett S.C.H. & Wright S.I. 2017. — Hill-Robertson Interference Reduces
667 Genetic Diversity on a Young Plant Y-Chromosome. *Genetics* 207 (2): 685–695

668 Immler S. & Otto S.P. 2015. — The evolution of sex chromosomes in organisms with separate
669 haploid sexes. *Evolution; international journal of organic evolution* 69 (3): 694–708

670 Ironside J.E. 2010. — No amicable divorce? Challenging the notion that sexual antagonism
671 drives sex chromosome evolution. *BioEssays: news and reviews in molecular, cellular and*
672 *developmental biology* 32 (8): 718–726

673 Korpelainen H., Bisang I., Hedenäs L. & Kolehmainen J. 2008. — The first sex-specific
674 molecular marker discovered in the moss Pseudocalliergon trifarium. *The Journal of heredity* 99
675 (6): 581–587

676 Laenen B., Machac A., Gradstein S.R., Shaw B., Patiño J., Désamoré A., Goffinet B., Cox C.J.,
677 Shaw A.J. & Vanderpoorten A. 2016. — Increased diversification rates follow shifts to
678 bisexuality in liverworts. *The New phytologist* 210 (3): 1121–1129

679 Lessells C.M. & Parker G.A. 1999. — Parent—offspring conflict: the full–sib—half–sib fallacy.
680 *Proceedings of the Royal Society of London. Series B: Biological Sciences* 266 (1429): 1637–
681 1643

682 Lipinska A., Cormier A., Luthringer R., Peters A.F., Corre E., Gachon C.M.M., Cock J.M. &
683 Coelho S.M. 2015. — Sexual dimorphism and the evolution of sex-biased gene expression in
684 the brown alga ectocarpus. *Molecular biology and evolution* 32 (6): 1581–1597

685 Lipinska A.P., Toda N.R.T., Heesch S., Peters A.F., Cock J.M. & Coelho S.M. 2017. — Multiple
686 gene movements into and out of haploid sex chromosomes. *Genome biology* 18 (1): 104

687 Luthringer R., Cormier A., Ahmed S., Peters A.F., Cock J.M. & Coelho S.M. 2014. — Sexual
688 dimorphism in the brown algae. *Perspect. Phycol* 1 (1): 11–25

689 Luthringer R., Lipinska A.P., Roze D., Cormier A., Macaisne N., Peters A.F., Cock J.M. &
690 Coelho S.M. 2015. — The Pseudoautosomal Regions of the U/V Sex Chromosomes of the
691 Brown Alga Ectocarpus Exhibit Unusual Features. *Molecular biology and evolution* 32 (11):
692 2973–2985

693 Lyttle T.W. 1993. — Cheaters sometimes prosper: distortion of mendelian segregation by
694 meiotic drive. *Trends in genetics: TIG* 9 (6): 205–210

695 Mank J.E. 2013. — Sex chromosome dosage compensation: definitely not for everyone. *Trends*
696 *in genetics: TIG* 29 (12): 677–683

697 McDaniel S.F. 2009. — The Genetic Basis of Natural Variation in Bryophyte Model Systems, *in*
698 Roberts J.A. (ed.), *Annual Plant Reviews online*Vol. 46. Chichester, UK, John Wiley & Sons,
699 Ltd. p. 16–41.

700 McDaniel S.F., Atwood J. & Burleigh J.G. 2013. — Recurrent evolution of dioecy in bryophytes.
701 *Evolution; international journal of organic evolution* 67 (2): 567–572

702 McDaniel S.F., Neubig K.M., Payton A.C., Quatrano R.S. & Cove D.J. 2013. — RECENT
703 GENE-CAPTURE ON THE UV SEX CHROMOSOMES OF THE MOSS CERATODON
704 PURPUREUS : SEX CHROMOSOME EVOLUTION IN C. PURPUREUS. *Evolution;*
705 *international journal of organic evolution* 110. https://doi.org/10.1111/evo.12165

706 McDaniel S.F. & Perroud P.-F. 2012. — Invited perspective: bryophytes as models for
707 understanding the evolution of sexual systems. *The Bryologist* 115 (1): 1–11

708 McDaniel S.F., Willis J.H. & Shaw A.J. 2007. — A linkage map reveals a complex basis for
709 segregation distortion in an interpopulation cross in the moss Ceratodon purpureus. *Genetics*
710 176 (4): 2489–2500

711 McLetchie D.N. & Puterbaugh M.N. 2000. — Population sex ratios, sex-specific clonal traits and
712 tradeoffs among these traits in the liverwort Marchantia inflexa. *Oikos* 90 (2): 227–237

713 Montgomery S.A., Tanizawa Y., Galik B., Wang N., Ito T., Mochizuki T., Akimcheva S., Bowman
714 J.L., Cognat V., Maréchal-Drouard L., Ekker H., Hong S.-F., Kohchi T., Lin S.-S., Liu L.-Y.D.,
715 Nakamura Y., Valeeva L.R., Shakirov E.V., Shippen D.E., Wei W.-L., Yagura M., Yamaoka S.,
716 Yamato K.T., Liu C. & Berger F. 2020. — Chromatin Organization in Early Land Plants Reveals
717 an Ancestral Association between H3K27me3, Transposons, and Constitutive Heterochromatin.
718 *Current biology: CB* 30 (4): 573–588.e7

719 Muller H.J. 1932. — Some Genetic Aspects of Sex. *The American naturalist* 66 (703): 118–138

720 Müller N.A., Kersten B., Leite Montalvão A.P., Mähler N., Bernhardsson C., Bräutigam K.,
721 Carracedo Lorenzo Z., Hoenicka H., Kumar V., Mader M., Pakull B., Robinson K.M., Sabatti M.,
722 Vettori C., Ingvarsson P.K., Cronk Q., Street N.R. & Fladung M. 2020. — A single gene
723 underlies the dynamic evolution of poplar sex determination. *Nature plants* 6 (6): 630–637

724 Nauta M.J. & Hoekstra R.F. 1993. — Evolutionary dynamics of spore killers. *Genetics* 135 (3):
725 923–930

Norrell T.E., Jones K.S., Payton A.C. & McDaniel S.F. 2014. — Meiotic sex ratio variation in natural populations of Ceratodon purpureus (Ditrichaceae). *American journal of botany* 101 (9): 1572–1576

Okada S., Sone T., Fujisawa M., Nakayama S., Takenaka M., Ishizaki K., Kono K., Shimizu-Ueda Y., Hanajiri T., Yamato K.T., Fukuzawa H., Brennicke A. & Ohyama K. 2001. — The Y chromosome in the liverwort Marchantia polymorpha has accumulated unique repeat sequences harboring a male-specific gene. *Proceedings of the National Academy of Sciences of the United States of America* 98 (16): 9454–9459

Otto S.P., Pannell J.R., Peichel C.L., Ashman T.-L., Charlesworth D., Chippindale A.K., Delph L.F., Guerrero R.F., Scarpino S.V. & McAllister B.F. 2011. — About PAR: the distinct evolutionary dynamics of the pseudoautosomal region. *Trends in genetics: TIG* 27 (9): 358–367

Parker G.A. & Others 1979. — Sexual selection and sexual conflict. *Sexual selection and reproductive competition in insects* 123: 166

Price T.A.R., Hurst G.D.D. & Wedell N. 2010. — Polyandry prevents extinction. *Current biology: CB* 20 (5): 471–475

Reik W. & Walter J. 2001. — Genomic imprinting: parental influence on the genome. *Nature reviews. Genetics* 2 (1): 21–32

Renner S.S. 2014. — The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. *American journal of botany* 101 (10): 1588–1596

Renner S.S., Heinrichs J. & Sousa A. 2017. — The sex chromosomes of bryophytes: Recent insights, open questions, and reinvestigations of Frullania dilatata and Plagiochila asplenioides. *Journal of Systematics and Evolution* 55 (4): 333–339

Reski R. 1998. — Development, Genetics and Molecular Biology of Mosses. *Botanica acta: Berichte der Deutschen Botanischen Gesellschaft = journal of the German Botanical Society* 111 (1): 1–15

Rice W.R. 1987. — THE ACCUMULATION OF SEXUALLY ANTAGONISTIC GENES AS A SELECTIVE AGENT PROMOTING THE EVOLUTION OF REDUCED RECOMBINATION BETWEEN PRIMITIVE SEX CHROMOSOMES. *Evolution; international journal of organic evolution* 41 (4): 911–914

Robert T. 1972. — Parental investment and sexual selection. *Sexual Selection & the Descent of Man, Aldine de Gruyter, New York*: 136–179

Rosengren F. & Cronberg N. 2014. — The adaptive background of nannandry: dwarf male distribution and fertilization in the moss Homalothecium lutescens. *Biological journal of the Linnean Society. Linnean Society of London* 113 (1): 74–84

Rosenstiel T.N., Shortlidge E.E., Melnychenko A.N., Pankow J.F. & Eppley S.M. 2012. — Sex-specific volatile compounds influence microarthropod-mediated fertilization of moss. *Nature* 489 (7416): 431–433

Sayres M.A.W. 2018. — Genetic diversity on the sex chromosomes. *Genome biology and*

765 *evolution* 10 (4): 1064

766 Shaw A.J. & Gaughan J.F. 1993. — CONTROL OF SEX RATIOS IN HAPLOID POPULATIONS
767 OF THE MOSS, CERATODON PURPUREUS. *American journal of botany* 80 (5): 584–591

768 Shortlidge E.E., Payton A.C., Carey S.B., Mc Daniel S.F., Rosenstiel T.N. & Eppley S.M. 2020.
769 — Microarthropod contributions to fitness variation in the common moss Ceratodon purpureus.
770 *bioRxiv* https://doi.org/10.1101/2020.12.02.408872

771 Stark L.R., Brinda J.C. & McLetchie D.N. 2009. — An experimental demonstration of the cost of
772 sex and a potential resource limitation on reproduction in the moss Pterygoneurum (Pottiaceae).
773 *American journal of botany* 96 (9): 1712–1721

774 Szövényi P., Perroud P.-F., Symeonidi A., Stevenson S., Quatrano R.S., Rensing S.A., Cuming
775 A.C. & McDaniel S.F. 2015. — De novo assembly and comparative analysis of the Ceratodon
776 purpureus transcriptome. *Molecular ecology resources* 15 (1): 203–215

777 Tao Y., Araripe L., Kingan S.B., Ke Y., Xiao H. & Hartl D.L. 2007. — A sex-ratio meiotic drive
778 system in Drosophila simulans. II: an X-linked distorter. *PLoS biology* 5 (11): e293

779 Tree of Sex Consortium 2014. — Tree of Sex: A database of sexual systems. *Scientific Data* 1

780 Vicoso B. 2019. — Molecular and evolutionary dynamics of animal sex-chromosome turnover.
781 *Nature ecology & evolution* 3 (12): 1632–1641

782 Vicoso B. & Charlesworth B. 2006. — Evolution on the X chromosome: unusual patterns and
783 processes. *Nature reviews. Genetics* 7 (8): 645–653

784 Villarreal J.C. & Renner S.S. 2013. — Correlates of monoicy and dioicy in hornworts, the
785 apparent sister group to vascular plants. *BMC evolutionary biology* 13: 239

786 Voglmayr H. 2000. — Nuclear DNA Amounts in Mosses (Musci). *Annals of botany* 85 (4): 531–
787 546

788 Werren J.H. 2011. — Selfish genetic elements, genetic conflict, and evolutionary innovation.
789 *Proceedings of the National Academy of Sciences of the United States of America* 108 Suppl 2:
790 10863–10870

791 Werren J.H. & Beukeboom L.W. 1998. — Sex determination, sex ratios, and genetic conflict.
792 *Annual review of ecology and systematics* 29 (1): 233–261

793 Westergaard M. 1958. — The mechanism of sex determination in dioecious flowering plants.
794 *Advances in genetics* 9: 217–281

795 Yamato K.T., Ishizaki K., Fujisawa M., Okada S., Nakayama S., Fujishita M., Bando H., Yodoya
796 K., Hayashi K., Bando T. & Others 2007. — Gene organization of the liverwort Y chromosome
797 reveals distinct sex chromosome evolution in a haploid system. *Proceedings of the National*
798 *Academy of Sciences* 104 (15): 6472–6477

799 Yamazaki T., Ichihara K., Suzuki R., Oshima K., Miyamura S., Kuwano K., Toyoda A., Suzuki
800 Y., Sugano S., Hattori M. & Kawano S. 2017. — Genomic structure and evolution of the mating
801 type locus in the green seaweed Ulva partita. *Scientific reports* 7 (1): 11679

802 Zeh D.W. & Zeh J.A. 2000. — Reproductive mode and speciation: the viviparity-driven conflict
803 hypothesis. *BioEssays: news and reviews in molecular, cellular and developmental biology* 22
804 (10): 938–946