doi:10.1111/evo.12602

The evolution of sex chromosomes in organisms with separate haploid sexes

Simone Immler[1,2] and Sarah Perin Otto[3]

[1]*Department of Ecology and Genetics, Evolutionary Biology, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden*

[2]*E-mail: simone.immler@ebc.uu.se*

[3]*Department of Zoology & Biodiversity Research Centre, 6270 University Blvd., University of British Columbia, Vancouver, BC V6T 1Z4, Canada*

Received September 24, 2014
Accepted December 11, 2014

The evolution of dimorphic sex chromosomes is driven largely by the evolution of reduced recombination and the subsequent accumulation of deleterious mutations. Although these processes are increasingly well understood in diploid organisms, the evolution of dimorphic sex chromosomes in haploid organisms (U/V) has been virtually unstudied theoretically. We analyze a model to investigate the evolution of linkage between fitness loci and the sex-determining region in U/V species. In a second step, we test how prone nonrecombining regions are to degeneration due to accumulation of deleterious mutations. Our modeling predicts that the decay of recombination on the sex chromosomes and the addition of strata via fusions will be just as much a part of the evolution of haploid sex chromosomes as in diploid sex chromosome systems. Reduced recombination is broadly favored, as long as there is some fitness difference between haploid males and females. The degeneration of the sex-determining region due to the accumulation of deleterious mutations is expected to be slower in haploid organisms because of the absence of masking. Nevertheless, balancing selection often drives greater differentiation between the U/V sex chromosomes than in X/Y and Z/W systems. We summarize empirical evidence for haploid sex chromosome evolution and discuss our predictions in light of these findings.

KEY WORDS: **Muller's ratchet, mutation load, recombination, sexual conflict, UV sex chromosomes.**

The evolution of genetically dimorphic sex chromosomes from autosomes has occurred independently in numerous taxa (Bull 1983; Charlesworth 1990, 1996; Rice 1996). The most commonly known are the XY and ZW systems and transitions to, from and among them have repeatedly occurred in diploid organisms such as mammals, birds, reptiles, amphibians, fish, and plants (see Charlesworth and Mank 2010; Bachtrog et al. 2011 for review). Factors such as the rate of mutation and recombination as well as intragenomic conflict play a pivotal role in the evolution of sex-determining regions (SDRs) and hence sex chromosomes. Genes subject to differential selection between the sexes favor tighter linkage to the sex-determining locus to keep alleles more often in the sex in which they are favored (Rice 1984; Charlesworth et al. 1987; Vicoso and Charlesworth 2006). Once recombination between the sex chromosomes has ceased, the nonrecombining region gradually decays due to the accumulation of deleterious mutations (e.g., Gordo and Charlesworth 2001; Bergero and Charlesworth 2009). These processes have been extensively studied in theory (e.g., Charlesworth 1978; Rice 1987b), as well as empirically for diploid organisms with XY systems as in mammals or ZW systems as in birds (e.g., Lawson-Handley et al. 2004; Matsubara et al. 2006; Bergero and Charlesworth 2009; see Bachtrog 2013 for review).

However, a large number of taxa, particularly protists, fungi, algae, and plants, spend some if not most of their life as haploids (Mable and Otto 1998). In species with a free-living haploid phase, it is often the haploid phase (the "gametophyte") that exhibits male and female sexual organs. By contrast, the diploid phase (the "sporophyte") lacks sexual differentiation and reproduces via meiotically produced spores. Whether there are

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

separate male and female haploids (dioicy) or both sexes can be found in the same haploid individual (hermaphroditism) is highly dynamic over evolutionary time, with 133 transitions between hermaphroditism and dioicy in mosses alone (McDaniel et al. 2013a). Similarly, haploid dioicy has evolved repeatedly and the haploid sex determination system is thought to have first evolved before X/Y or Z/W systems (Luthringer et al. 2014). Interestingly, genetic sex determination underlying dioicy is widespread in the haploid phase of many nonvascular plants (e.g., 50% of mosses, 40% liverworts, and 80% hornworts; Jesson and Garnock-Jones 2012).

The exact nature of the sex-determining system varies among organisms (Bachtrog et al. 2014). Here, we focus on the evolution of dimorphic SDRs and dimorphic sex chromosomes within haploid organisms. In many haploids, there are not sexes, per se, but mating types that govern compatibility (Casselton 2002). Nevertheless, our model also describes the evolution of regions surrounding mating-type loci, particularly those with two mating types. Historically, haploid sex chromosomes have been termed X and Y (e.g., Vitt 1968), but we use the more recent notation of U (for the female) and V (for the male; Bachtrog et al. 2011) to distinguish between diploid X/Y and haploid U/V systems.

It is currently not clear how well the theory developed for X/Y and Z/W systems applies to the U/V system. For one, the evolution of recombination per se and the effect of events such as fusions and inversions on the linkage between sex-specific genes and the sex-determining locus in haploid sex chromosomes has not previously been explored. Furthermore, as a consequence of haploid sex determination and hence a heterozygous diploid phase, any genes on either the U or V that are expressed during the diploid sporophyte phase will be sheltered and hence both chromosomes may potentially degenerate (Lewis and Benson-Evans 1960; Lewis 1961; Lewis and John 1968). However, genes are only sheltered in the diploids, whereas expression in the haploids results in efficient purging due to selection against deleterious mutations (Haldane 1933; Bull 1978). Hence, the accumulation of deleterious mutations and the resulting degeneration of haploid sex chromosomes (U/V) are expected to be much slower than for diploid sex chromosomes (X/Y or Z/W). Interestingly though, dimorphic U/V sex chromosomes have been repeatedly described in several groups of bryophytes (Allen 1945; Vitt 1968) and also in several algae (Martinez et al. 1999; Liu et al. 2009; Umen 2011). This phenomenon is somewhat puzzling given the fact that purifying selection in the haploid phase should efficiently eliminate deleterious mutations (Bull 1978). One possible reason for this discrepancy is that degeneration occurs at loci that are primarily expressed in the diploid phase or in the other sex. We explore the impact of expression differences in the haploid and diploid phase on the rate of mutation accumulation on haploid sex chromosomes, applying theory examining the speed of Muller's ratchet in haploid asexuals (Charlesworth and Charlesworth 1997; Gordo and Charlesworth 2001).

We begin by investigating how the rate of recombination and linkage of fitness loci with sex-determining loci may evolve in species with haploid sexes. In a second step, we test how prone nonrecombining regions are to degeneration due to the accumulation of deleterious mutations. We show that lower recombination rates are expected to evolve between haploid U and V sex chromosomes whenever haploid males and females experience different selection pressures. Furthermore, although deleterious alleles that are fully exposed to selection during the haploid phase rarely go to fixation on either the U or the V chromosome, loci subject to balancing selection—overdominance, sexually antagonistic selection, and/or ploidally antagonistic selection—become fixed for alternate alleles in nonrecombining regions of the U and V. That is, for loci tightly linked to the SDR, no polymorphism is expected on either the U or the V chromosome, only fixed differences between them. Thus, we expect U/V systems to be more highly differentiated at loci subject to balancing selection than diploid sex determination systems where polymorphism is often maintained on the X or the Z chromosome (Clark 1987; Otto 2014). We then discuss our model in light of recent genomic data testing for recombination and differentiation of SDRs that act in the haploid phase.

The Evolution of Recombination Rate

In this section, we examine how recombination evolves under sexually antagonistic selection in a haploid dioicious organism. Studies on the evolution of sex linkage in diploid dioecious organisms have shown that reduced recombination between a selected locus and the SDR is typically favored, as long as selection acts differently on alleles in males and females (Nei 1969; Charlesworth and Charlesworth 1980; Lenormand 2003; Otto 2014). Interestingly, selection can act differently on alleles in males and females even when the two sexes have the same fitness for each genotype, as long as genotypic frequencies differ between the sexes; while a seemingly odd requirement, Clark (1987) showed that genotypic frequencies generally do differ between males and females at sex-linked loci, causing alleles to experience different genotypic contexts in the two sexes in a manner that can favor reduced recombination (Otto 2014). Furthermore, it is possible for looser linkage to evolve in some cases with X/Y sex determination if diploid males are subject to heterozygous advantage (Otto 2014). Here, we show that this scenario does not arise in U/V systems where males and females exist only in the haploid phase, so that modifiers of recombination are favored only if they tighten linkage between selected loci and the SDR.

Table 1. Summary of notation used.

(A) Fitness of haploid males (V_i) and females (U_i) and diploid sporophytes (S_i).			
Haploid genotype:	A	a	
Female fitness:	U_A	U_a	
Male fitness:	V_A	V_a	
Diploid genotype:	AA	Aa	Aa
Diploid fitness:	S_{AA}	S_{Aa}	S_{aa}

(B) Additional terms	
o_i:	The frequency of genotype i among female haploid spores ($i = MA, Ma, mA, ma$).
p_i:	The frequency of genotype i among male haploid spores ($i = MA, Ma, mA, ma$).
r_{kl}:	Recombination rate between **A** and the sex-determining locus in diploids of modifier genotype kl.
R:	Recombination rate between **A** and **M** loci.
χ:	Rate of double recombination.
$U_{i\bullet}[V_{i\bullet}]$:	The marginal fitness of a U [V] chromosome carrying allele i considered over the life cycle (see eqs. (3), (4)).
δ_i:	Difference in frequency of allele i between the sexes (see eqs. (9a), (9b).
D_k:	Linkage disequilibrium in sex k (see eqs. (9c), (9d)).
ρ_i:	Average frequency of allele i across both sexes (see eq. 8).
$\bar{\delta}_r$:	Average effect on recombination of replacing an M allele with an m allele (see eq. (10b)).
u:	Mutation rate per chromosome
s_e:	Effective strength of selection across the life cycle against allele a on the U chromosome, $s_e = 1 - S_{Aa}U_a = 1 - (1 - h\,s)^d(1 - f\,s)^{1-d}$.
d:	Proportion of the sexual life cycle spent in the diploid phase.
f:	Importance of a gene to selection in the haploid phase relative to the diploid phase.

Specifically, we develop a model that allows the rate of recombination to evolve between a selected locus and the U/V SDR. Selection acts in both the haploid and diploid phases and can be sex specific in haploids. For ease of interpretation, we refer to fitness in male haploids, female haploids, and diploid sporophytes using the letters U_k, V_k, and S_{kl}, respectively, depending on the genotype at the selected locus **A** with alleles $k,l = A$ or a (Table 1A). During meiosis, the rate of recombination between the **A** locus and the U/V sex-determining locus occurs at a rate r_{kl} that depends on the genotype at a modifier locus **M** with alleles $k,l = M$ or m. The frequency of recombination between the modifier **M** and selected locus **A** is R, and double recombination between **M**, **A**, and the SDR occurs with probability χ (see Appendix for more details). Chromosome frequencies are censused at the beginning of the gametophytic stage (before selection), with o_i measuring the frequency of genotype i among female haploid spores bearing the U chromosome, and p_i measuring the frequency among male haploid spores bearing the V chromosome, where $i = MA, Ma, mA,$ or ma. These haploids then experience selection and produce gametes, with one female and one male gamete uniting at random. The resulting diploid sporophyte experiences selection followed by meiosis to produce haploid male and female spores. The full recursions are given in the Appendix, and a summary of parameters is provided in Table 1.

We explore the conditions under which linkage evolves between the selected locus **A** and the sex-determining locus. To do this, we consider three specific cases: where the **A** locus is initially unlinked to the SDR, where it is initially tightly linked, and where selection is weak relative to recombination. These cases are analytically tractable and provide complementary pictures of the evolution of recombination in haploid dioicious organisms. We then numerically evaluate how linkage evolves across the parameter space to determine whether these results are robust.

SELECTED LOCUS UNLINKED TO SDR

We first assume that the **A** locus is initially unlinked to the SDR and comes, by way of a fusion with either the U or the V chromosome, to be tightly linked to the SDR. This case parallels that considered by Charlesworth and Charlesworth (1980), who explored the fate of a fusion that brings a selected locus into linkage with the X/Y SDR in a diploid organism.

Initially, the **A** locus is autosomal and has one polymorphic equilibrium:

$$O_{MA} = p_{MA} = \frac{S_{Aa}U_AV_a + S_{Aa}U_aV_A - 2S_{aa}U_aV_a}{2\,(S_{Aa}U_AV_a + S_{Aa}U_aV_A - S_{AA}U_AV_A - S_{aa}U_aV_a)}, \quad (1)$$

which is valid (lies between 0 and 1) and stable if:

$$2 < \min\left[\frac{S_{Aa}}{S_{aa}}\left(\frac{U_A}{U_a} + \frac{V_A}{V_a}\right), \frac{S_{Aa}}{S_{AA}}\left(\frac{U_a}{U_A} + \frac{V_a}{V_A}\right)\right]. \quad (2)$$

Condition (2) can be met under a variety of circumstances, including heterozygous advantage in diploids, sexual antagonism

in the haploid phase, ploidy antagonism between selection in the haploid and diploid phases, or a mixture of the above.

If the selected locus then fuses with the U chromosome, causing tight linkage between the selected and sex-determining locus, the fusion spreads at a rate that depends on the larger of two eigenvalues, λ, of the local stability matrix describing the dynamics of the fusion while rare. These eigenvalues equal $U_{A\bullet}/\bar{W}$ and $U_{a\bullet}/\bar{W}$, where $U_{i\bullet}$ is the marginal fitness of a U chromosome carrying allele i considered over the life cycle and averaged over the two sperm types with which it might pair:

$$U_{i\bullet} = U_i\,(V_a p_{Ma} S_{ia} + V_A p_{MA} S_{iA})\,, \tag{3}$$

and where $\bar{W}$ represents the mean fitness over the life cycle, which equals $\bar{W} = o_{MA}U_{A\bullet} + o_{Ma}U_{a\bullet}$. As long as the marginal fitness of the alleles differs, however, one will be higher than the mean and one will be lower, so there will always be one eigenvalue greater than one. Furthermore, it can be shown that the marginal allele fitness of alleles A and a differs at equilibrium only if there are selective differences between haploid males and females (i.e., if $U_A/U_a \neq V_A/V_a$), otherwise the fusion is neutral (with a leading eigenvalue of one). Assuming that there are sex-specific differences in selection in the haploid phase, a fusion with the U chromosome will be favored as long as it captures the allele that causes female haploids to have a higher lifetime fitness. This is true even when polymorphism is maintained by ploidally antagonistic selection (e.g., even when selection acts in the same direction in males and females, but with $U_A/U_a \neq V_A/V_a$). In this case, the fixation of the fittest allele on the U chromosome ensures that female haploids have high fitness, as does the diploid, which are mostly heterozygotes after the spread of the fusion (condition 2 requires that the heterozygote have high fitness in diploids relative to the average homozygous fitness).

Equivalent results apply to a fusion with the V chromosome. In this case, the two eigenvalues are $V_{A\bullet}/\bar{W}$ and $V_{a\bullet}/\bar{W}$, where $V_{i\bullet}$ is the marginal fitness of a V chromosome carrying allele i averaged over the egg types with which it might pair:

$$V_{i\bullet} = V_i\,(U_a o_{Ma} S_{ia} + U_A o_{MA} S_{iA})\,. \tag{4}$$

Again, as long as male haploids differ in their marginal fitness, one of the eigenvalues will be greater than one. This in turn requires that there be selective differences between haploid males and females (otherwise, $V_{A\bullet} = V_{a\bullet}$ if $U_A/U_a = V_A/V_a$). Consequently, a fusion with the V chromosome will always be favored as long as it captures the allele that causes male haploids to have a higher lifetime fitness.

Charlesworth and Charlesworth (1980) found that the spread of a fusion to the Y chromosome in a diploid X/Y system is similarly described by the larger of two eigenvalues, which are equivalent to equation (4) in describing the marginal fitness across the life cycle of sperm bearing allele A ($Y_{A\bullet}/\bar{W}$) and those bearing allele a ($Y_{a\bullet}/\bar{W}$) (their eq. A1). Because of masking by the X chromosome during the diploid phase, the difference between the marginal fitness across the life cycle of sperm bearing allele A and those bearing allele a may often be milder than in the dioicious case where fitness differences in the haploid phase also contribute to the eigenvalues. Although the results of Charlesworth and Charlesworth (1980) for a fusion to the X are slightly more complicated, the net result is multiplied by 1/3, relative to the case of a Y-fusion (compare their eqs. A7–A1). These eigenvalues are approximately 2/3 $X^{\female}_{i\bullet}/\bar{W}^{\female}$+1/3 $X^{\male}_{i\bullet}/\bar{W}^{\male}$ for the two alleles ($i = A$ or a), where $X^{k}_{i\bullet}/\bar{W}^{k}$ is the marginal fitness of allele i in sex k relative to the mean fitness (assuming only a small difference in allele frequency between the sexes, see Supporting Information). With sexually antagonistic selection, any benefit gained by a fusion the 2/3 of the time when the X is in a female is undone by the 1/3 of the time it is in a male. This counterselection, along with masking in diploids, makes it plausible that the leading eigenvalue for X-autosome fusions will tend to be smaller—and the spread of the fusion slower—than in the dioicious case. Assuming that fusion rates and population sizes are similar and that the strength of selection in haploids is similar to the strength of selection between diploid homozygotes (i.e., assuming "all else is equal"), we thus expect that reduced recombination would be more strongly favored in haploid U/V systems than in diploid X/Y systems. Whether all else is equal, however, depends on the organisms in question and particularly on the strength of selection across ploidy levels.

In the case of X/Y sex determination, Charlesworth and Charlesworth (1980) interpreted their results by stating that the spread of fusions depends on the existence of a difference in allele frequency between eggs and sperm. In our model, for a locus unlinked to the SDR, there can be no initial difference in allele frequencies between male and female haploid spores (see eq. (4)). Nevertheless, there will be a difference in the frequency of A-bearing males and females after haploid selection (and hence among the sperm and eggs that they produce) as long as selection acts differently in male and female haploids (i.e., $U_A/U_a \neq V_A/V_a$), a condition that we have shown is required for fusions to spread.

In summary, fusions are generally favored as long as they better couple female-beneficial alleles with the U chromosome and male-beneficial alleles with the V chromosome. If, however, selection does not differ between males and females in the haploid phase, the A and a alleles are equally fit at equilibrium in each sex, on average, and the fusion behaves neutrally.

SELECTED LOCUS TIGHTLY LINKED TO SDR

The next case investigates the opposite scenario, where the selected **A** locus has evolved into tight linkage with the SDR and hence r_{MM} and χ are both small (of the order of a small term ξ). Assuming the modifier locus is fixed for allele *M*, the system has three polymorphic equilibria, which are (to leading order in ξ) near:

$$\begin{aligned}
&\text{(A)} \quad o_{MA} = 1,\ p_{MA} = 0 \\
&\text{(B)} \quad o_{MA} = 0,\ p_{MA} = 1 \\
&\text{(C)} \quad o_{MA} = \frac{(V_a S_{aa} - V_A S_{Aa}) U_a}{U_a S_{aa} V_a - U_A S_{Aa} V_a - U_a S_{Aa} V_A + U_A S_{AA} V_A} \\
&\qquad\quad p_{MA} = \frac{(U_a S_{aa} - U_A S_{Aa}) V_a}{U_a S_{aa} V_a - U_A S_{Aa} V_a - U_a S_{Aa} V_A + U_A S_{AA} V_A}.
\end{aligned} \tag{5}$$

Recombination slightly alters the position of these equilibria by an amount proportional to ξ, bringing the frequencies of allele *A* in male and female haploids (p_{MA} and o_{MA}) slightly closer together.

It can be shown that equilibrium (C) is never stable, whereas equilibria (A) and (B) are stable when the fractions *x* and *y* are both less than one, where for equilibrium:

$$\begin{aligned}
&\text{(A)} \quad x = \frac{U_a S_{aa}}{U_A S_{Aa}} \text{ and } y = \frac{V_A S_{AA}}{V_a S_{Aa}} \\
&\text{(B)} \quad x = \frac{U_A S_{AA}}{U_a S_{Aa}} \text{ and } y = \frac{V_a S_{aa}}{V_A S_{Aa}}.
\end{aligned} \tag{6}$$

If selection is absent during the diploid phase ($S_{kl} = 1$), the stability conditions for equilibria (A) and (B) require antagonistic selection between the sexes, where one of the two alleles is favored in females and the other in males. If selection is absent in the haploid phase, *x* and *y* will be less than one only if there is heterozygote advantage in the diploid phase. With selection in both phases, the geometric mean fitness must be higher for one allele when averaged across the female haploid stage and the diploid stage, whereas the other allele must be fitter when averaged over the male haploid stage and the diploid stage.

Equilibria (A) and (B) are examples of "high-complementary equilibria," where linkage disequilibrium is maximized when linkage is tight (e.g., Feldman et al. 1974). At these two equilibria, allele *A* is fixed on the female chromosome U (equilibrium A) or on the male chromosome V (equilibrium B), and all diploids are *Aa* heterozygotes (or nearly so if there is some recombination). The fact that we expect fixed differences between the U and V chromosomes holds regardless of the fitness regime maintaining a polymorphism: heterozygote advantage, sexually antagonistic selection, and/or ploidally antagonistic selection.

We now introduce a weak modifier allele *m*, which slightly alters the recombination rate between U and V so that both ($r_{Mm} - r_{MM}$) and ($r_{mm} - r_{MM}$) are small (of order ξ). Again, we assume that recombination rates involving the region between locus **A** and the SDR (r_{kl} and χ) are small (of order ξ), although the modifier may be anywhere in the genome. We also adjust equilibria A and B in equation (5) by including the smaller order terms (of order ξ) to ensure that the rate of spread of the modifier is calculated correctly to leading order (see Supporting Information). These assumptions allow us to approximate the leading eigenvalue, λ, describing the dynamics of the system while the modifier is rare (setting $\lambda = 1 + \lambda_0 \xi + \lambda_1 \xi^2 + \cdots$ and solving for the largest terms). The modifier spreads whenever $\lambda > 1$, which occurs when:

$$-(r_{Mm} - r_{MM}) \times \frac{2(1-x)(1-y) + R(x(1-y) + y(1-x))}{2(1 - x(1-R))(1 - y(1-R))} > 0, \tag{7}$$

using the appropriate values of *x* and *y* for equilibrium (A) and (B) from equation (6). Because *x* and *y* must be positive and less than one for the equilibrium to be stable before the new modifier is introduced, the fraction in (7) must be positive. Hence, genetic modifiers of recombination will spread only if they tighten linkage between the selected locus and the SDR ($r_{Mm} < r_{MM}$). Interestingly, this does not require that there be sexually antagonistic selection; any form of selection that maintains a polymorphism will do. In particular, selection favors tighter linkage with the SDR even for loci that are subject solely to heterozygote advantage in the diploid phase. In this case, it is not sexual antagonism that is driving selection for sex linkage, but rather the fact that tighter sex linkage causes male and female gametes to differ more in allele frequency, making carriers of a modifier that reduces recombination more likely to produce the most fit heterozygous diploids.

WEAK SELECTION APPROXIMATION

To examine the evolution of recombination without assuming initially tight or loose linkage between the selected locus **A** and the SDR, we performed a quasi-linkage equilibrium (QLE) approximation, which assumes that the genetic associations among the loci rapidly reach their steady-state values and then follow slower changes in allele frequencies. As detailed in the Supporting Information *Mathematica* file, this separation of time scales is appropriate when selection is weak relative to the recombination rates among the loci. Unlike the previous cases, the QLE approximation is valid even when the allele frequencies are not at equilibrium and so applies with directional selection.

In this approximation, we replace the haploid chromosome frequencies, o_i and p_i, with two allele frequencies and four genetic association measures, which provide a dynamically equivalent description of the system. Specifically, we track the average frequency of allele *A* and allele *M* across the sexes as:

$$\rho_A = (o_{MA} + o_{mA})/2 + (p_{MA} + p_{mA})/2 \tag{8a}$$

$$\rho_M = (o_{AM} + o_{aM})/2 + (p_{AM} + p_{aM})/2. \quad (8b)$$

For genetic associations, we track the difference in allele frequencies between the sexes:

$$\delta_A = (o_{MA} + o_{mA}) - (p_{MA} + p_{mA}) \quad (9a)$$

$$\delta_M = (o_{AM} + o_{aM}) - (p_{AM} + p_{aM}) \quad (9b)$$

as well as the linkage disequilibrium between loci **A** and **M** in males and females:

$$D_f = o_{AM} o_{am} - o_{Am} o_{aM} \quad (9c)$$

$$D_m = p_{AM} p_{am} - p_{Am} p_{aM}. \quad (9d)$$

We then replace the steady-state values for the associations in (9) into equation (8) for the allele frequency change to determine the change in allele frequencies over one generation (as detailed in Supporting Information *Mathematica* file). Assuming selection is weak and that the modifier has only a small effect on recombination, the modifier allele, m, changes across a generation by:

$$\Delta\rho_m \approx -\bar{\delta}_r \times \frac{(1-\rho_A)\rho_A(1-\rho_M)\rho_M\,(U_A - U_a - V_A + V_a)^2}{4r_{MM}(R + 2(r_{MM} - \chi))}, \quad (10a)$$

where $\bar{\delta}_r$ is the average effect on the recombination rate of replacing an M allele with an m allele (assumed small):

$$\bar{\delta}_r = ((1-\rho_M)\,r_{mm} + \rho_M r_{Mm}) - ((1-\rho_M)\,r_{Mm} + \rho_M r_{MM})\,. \quad (10b)$$

Again, because the fraction in (10a) is generally positive, modifiers rise in frequency only if they reduce the recombination rate between the selected locus and the SDR ($\bar{\delta}_r < 0$). Selection is, however, required in the haploid phase and must not be exactly equivalent in males and females (i.e., $U_A - U_a$ must not equal $V_A - V_a$). Notice, however, that selection can act in the same direction in male and female haploids—sexual antagonism is not needed. This parallels the result of Lenormand (2003), who used a similar QLE approximation to show that the evolution of tighter linkage to the SDR in XY (or ZW) species requires only that male and female diploids differ in fitness. Here, as long as selection differs between male and female haploids, then associations will build between allele A and one of the two sexes; this association is stronger if recombination is tighter, generating linkage disequilibrium that favors the evolution of reduced recombination.

TRANSITION BETWEEN LOOSE AND TIGHT LINKAGE REGIMES

From the above analyses, if linkage is initially loose (unlinked case or QLE), selection must differ between male and female haploids for decreased recombination to be favored ($U_a/U_A \neq V_a/V_A$, which is equivalent to $U_A - U_a \neq V_A - V_a$ for weak selection). By contrast, with initially tight linkage, all parameters that maintain a polymorphism favor the evolution of tighter linkage, even if there is no selection in the haploid phase. To determine the transition between these two regimes, we eliminated differences in selection between male and female haploids (setting $U_a/U_A = V_a/V_A = c$) and determined when the sex-symmetric equilibrium (1) with $o_{MA} = p_{MA}$ is stable, finding that it is stable only if $r_{MM} > r^*$, where:

$$r^* = \frac{\left(c\,S_{Aa} - c^2\,S_{aa}\right)\left(c\,S_{Aa} - S_{AA}\right)}{2\,c\,S_{Aa}\left(2\,c\,S_{Aa} - c^2\,S_{aa} - S_{AA}\right)}. \quad (11)$$

When this equilibrium is stable, an invasion analysis demonstrates that changes in recombination are indeed neutral (see Supporting Information), as expected from the unlinked case considered above with $U_a/U_A = V_a/V_A$. With tighter linkage ($r_{MM} < r^*$), a pair of asymmetric equilibria become stable where the allele frequencies differ between the sexes ($o_{MA} \neq p_{MA}$). We conjecture that decreased recombination is always selected when starting from these asymmetric equilibria, which is consistent with the numerical analysis conducted next.

NUMERICAL ANALYSIS

The above analyses suggest that increased recombination is never favored between any locus that remains polymorphic due to selection and the SDR. These analyses required specific assumptions, however: an initially unlinked locus in the first case, tight linkage in the second case, and weak selection in the third case. We thus set out to confirm these results by randomly drawing fitness and recombination parameters, calculating all stable equilibria, and determining if a modifier that increases recombination would spread at any of these equilibria. An exploration of 70,000 random parameter sets failed to identify any cases of increased recombination. If, however, we assumed no difference in selection within male and female haploids ($U_A/U_a = V_A/V_a$), then tighter linkage was favored only when recombination was sufficiently low initially. If the initial level of recombination was too high ($r_{MM} > r^*$), allele frequencies were the same in males and females at equilibrium, and a modifier of recombination behaved neutrally, neither spreading nor disappearing. With tight enough linkage between the selected locus and the SDR ($r_{MM} < r^*$), however, the system approached an equilibrium with sex differences in allele frequency, and reduced recombination was then always favored.

Degeneration in Haploid Sex Chromosomes

The above models indicate that the U and V chromosomes should both, over evolutionary time, become nonrecombining as loci with

differences in selection between the sexes are brought into tighter linkage with the SDR. Of course, the need for proper segregation of the sex chromosomes during meiosis in diploids may place constraints against the complete loss of recombination for U/V as well as X/Y and Z/W systems (Otto et al. 2011). Once reduced recombination around the SDR has evolved, an important subsequent process in the evolution of diploid sex chromosomes is the degeneration of Y or W chromosomes through the accumulation of deleterious mutations, which are largely masked by the X or Z, respectively. This process is well described for the evolution of diploid sex chromosomes and has found ample empirical evidence (Charlesworth et al. 2005; Bachtrog 2006). Bull (1978) argued that degeneration in haploid sex chromosomes should not be expected, however, due to the efficacy of purifying selection in haploids. This assumes that the alleles are expressed and subject to selection in both haploid males and females. Here, we estimate the time it takes for a haploid sex chromosome to lose the least-loaded class (one click of Muller's ratchet) as a function of the degree of selection acting on a gene in the haploid stage relative to the diploid stage.

Briefly, we use the approximation developed by Charlesworth and Charlesworth (1997) to estimate the time to loss of the least-loaded class. The method is based on a one-dimensional diffusion process, assuming a haploid model with multiplicative selection among loci, which is expected to generate a Poisson distribution of number of mutations per chromosome. The per chromosome mutation rate is assumed to be u, with effective selection against each deleterious mutation of s_e. The diffusion then tracks the frequency x of the least-loaded class as it varies around the deterministic mutation–selection balance of $x_0 = exp\,(-u/s_e)$. The expected time until loss is then given by:

$$T = 2\left(\int_0^{x_0} \frac{\int_0^x \psi(y)\mathrm{d}y}{b(x)\psi(x)}\mathrm{d}x\right) + 2\left(\int_{x_0}^1 \frac{\int_0^{x_0} \psi(y)\mathrm{d}y}{b(x)\psi(x)}\mathrm{d}x\right), \quad (12)$$

where

$$\psi(y) = \exp\left(-2\int_0^y \frac{a(z)}{b(z)}\mathrm{d}z\right) = \exp\left(2N_e e^u y\left(\frac{y}{2} - x_0\right)\right), \quad (13)$$

using the drift and diffusion coefficients, $a(x) \approx -x(x - x_0)e^u$ and $b(x) \approx x/N_e$, respectively, in a population of effective size N_e (Charlesworth and Charlesworth 1997).

We can directly apply the above model to a population of U chromosomes, with effective population size N_e, where we census the population in the haploid phase (V chromosomes may be treated similarly.) We assume complete linkage between the selected locus and the SDR and assume that sites that accumulate deleterious mutations on the U do not do so on the V. Assuming A-bearing haploids and AA-bearing diploids have fitness of one, the effective strength of selection across the life cycle against a on the U chromosome is $s_e = 1 - S_{Aa}U_a$ (note that the only haploid selection relevant to the U is in females). If selection acted only in the diploid phase, we would set $S_{Aa} = 1 - h\,s$, where s is the strength of selection and h the dominance coefficient. If selection acted only in the haploid phase, we would set $U_a = 1 - f\,s$, where f measures the relative importance of a particular gene to selection in the haploid phase relative to the diploid phase. For example, if a gene is not expressed in the haploid phase (or is expressed in males but not females), then f would be 0, whereas if it is more strongly selected in female haploids, then $f > 1$. Finally, we assume that the proportion of the sexual life cycle spent in the diploid phase is d and that selection in the diploid and haploid phase scales with the proportion of time in that phase (as in Otto 1994). The above assumptions allow us to explore the impact of the relative lengths of the haploid and diploid phases (through d) and differences in the strength of haploid versus diploid selection for particular genes (through f), via the effective selection coefficient, $s_e = 1 - (1 - h\,s)^d\,(1 - f\,s)^{1-d}$.

As illustrated in Figure 1, Muller's ratchet rarely clicks in organisms that are primarily haploid (d low) for genes that are expressed and subject to selection in the haploid phase (f high). Nevertheless, Muller's ratchet will click for genes on the U (or V) chromosome either when the haploid phase experiences relatively little selection (d high) or when a gene has only a weak effect on the survival of female (or male) haploids (f low). Indeed, Muller's ratchet can click faster per sexual cycle on the U sex chromosome than on a Y sex chromosome for the subset of genes that are less important during the haploid phase than the diploid phase, such that $f < h$ (comparing red and black curves to the blue dashed line). Furthermore, if male haploids and female haploids differ in effective population size, N_e (e.g., due to differences in variance in reproductive success), Muller's ratchet would click faster on the sex chromosome (U or V) associated with the lower N_e. Similarly, Muller's ratchet would click faster on whichever sex chromosome is subject to weaker selection. If both N_e and selection differ between the sexes, the relative rate of U and V degeneration can be predicted from equation (12). Thus, although Bull's (1978) insight was largely correct in predicting that the U and V chromosomes would not decay, there are some important exceptions, including in species with limited haploid phases and at genes with diploid-limited or sex-limited selection.

Importantly, even if Muller's ratchet clicks too slowly for U and V sex chromosomes to degenerate, this does not necessarily mean that sex chromosomes will differentiate slowly. According to equation (5), the U and V chromosomes are expected to fix alternate alleles at any locus linked to the SDR that is maintained polymorphic through a combination of heterozygous advantage,

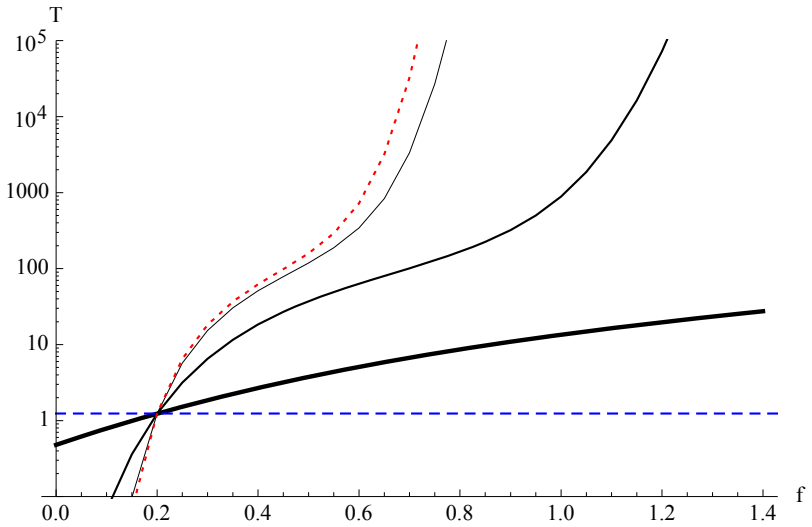

Figure 1. **Waiting time for a click of Muller's ratchet on the U (or V) chromosome. Figure shows the waiting time in generations until the loss of the least-loaded class, based on the approximation of Charlesworth and Charlesworth (1997). The selective importance per unit time of a gene in haploids relative to diploids, *f*, is varied along the horizontal axis. The life cycle is varied from being exclusively haploid ($d = 0$; red dotted curve) to exclusively diploid ($d = 1$; blue dashed line), with intermediate life cycles in black curves (thin: $d = 1/10$, medium: 1/2, thick: 9/10). The blue line also describes the speed of the ratchet on a Y chromosome in exclusively diploid organisms (assuming the same N_e for the U and Y). Parameters: $u = 0.2$, $h = 0.2$, $s = 0.1$, $N_e = 2000$.**

sexually antagonistic selection, and/or ploidally antagonistic selection. This contrasts with selection in X/Y (or Z/W) systems, where alleles that are fixed on the Y (or W) often remain polymorphic on the X (or Z) (Clark 1987; Otto 2014). Furthermore, as alternate alleles rise to fixation on the U and V (e.g., following a new fusion and/or the onset of balancing selection at a sex-linked locus), the spreading alleles might drag along with them linked deleterious alleles. The net result is that we might expect substantial differentiation between U and V chromosomes, even without degeneration.

Discussion

Our modeling predicts that the decay of recombination on the sex chromosomes and the addition of strata via fusions will be just as much a part of the evolution of haploid sex chromosomes (U/V) as diploid sex chromosome systems (XX/XY or ZW/ZZ). Indeed, reduced recombination is broadly favored, with or without sexually antagonistic selection, as long as there is some fitness difference between haploid males and females (i.e., $V_A/V_a \neq U_A/U_a$). Furthermore, even if selection acts only in the diploid phase, tighter linkage is favored if the selected locus is initially close enough to the SDR, such that the allele frequencies differ between males and females at equilibrium. In other words, the accumulation of sex-specific alleles (i.e., alleles slightly favored in one sex over the other) is expected to be commonplace around the SDR for selectively maintained polymorphisms and hence may well be detectable even in young haploid sex chromosomes. Genetic divergence between U and V chromosomes should be slow until recombination rate r approaches zero, at which point all of the stable polymorphisms involve fixed differences between U and V sex chromosomes (eq. (5)).

Despite the similarities between the evolution of sex chromosomes with haploid versus diploid sex determination, some fundamental differences exist, as highlighted by our findings. For one, in systems with haploid sex determination, recombination is absent in the individuals exhibiting the sexes—this is not true for diploid systems. In particular, there cannot be sex differences in recombination with haploid sex determination. Furthermore, with U and V chromosomes being equally exposed to haploid selection, there is no asymmetry allowing one chromosome to maintain polymorphism (as on the X) more readily than the other chromosome (as on the Y). Indeed, we find that the only selectively maintained polymorphism at loci completely linked to the SDR involves fixed differences, with a U-specific allele and a V-specific allele, regardless of the nature of balancing selection (assumed to be frequency independent). Consequently, we predict much lower genetic variance in fitness among U's or among V's once recombination is reduced, in contrast to the high amount of genetic variance on the X thought to be maintained by sexually antagonistic selection (e.g., Gibson et al. 2002).

A further important difference between diploid and haploid sex determination is the speed with which deleterious mutations accumulate: masking of deleterious mutations does not occur in

haploid males and females and hence the degeneration of the SDR is expected to be slower with haploid sex determination than with diploid sex determination. Indeed, the accumulation of deleterious mutations is essentially halted by selection for loci that are highly expressed in the haploid phase (unless selection is very weak). Our numerical evaluations of the speed of Muller's ratchet, using Charlesworth and Charlesworth (1997) approximation, support Bull's (1978) argument that degeneration on haploid sex chromosomes should be minimal, at least for genes highly expressed in the haploid phase. This is not true, however, for loci subject primarily to selection in the other sex or in the diploid phase. In the latter case, masking allows the accumulation of different deleterious mutations on the U and V chromosomes, without much reduction in fitness in the diploid phase. In addition, we predict nearly fixed differences between the U and V at any locus tightly linked to the SDR that is maintained polymorphic by selection, whether through overdominance, sexually antagonistic selection, and/or ploidally antagonistic selection. As a consequence, we expect differentiation to arise more readily than degeneration on U/V sex chromosomes relative to X/Y or Z/W systems, with degeneration observed primarily at loci with limited haploid expression.

In this study, we have focused on only two aspects of sex chromosome evolution in haploids: the evolution of reduced recombination and degeneration due to the accumulation of deleterious mutations. Other aspects of sex chromosome evolution also merit investigation in haploid U/V systems. For example, the spread of deleterious alleles by hitchhiking with beneficial alleles will differ for nonrecombining U (and V) sex chromosomes, because alleles (both beneficial and deleterious) are fully exposed to haploid selection compared to alleles on the Y, which are masked in diploids (Rice 1987a). Furthermore, although nonrecombining and masked Y chromosomes are adaptively crippled and expected to lag increasingly far behind the X in fitness (Orr and Kim 1998), the U and V should adapt more similarly and readily given that alleles are fully exposed to haploid selection. Consequently, U and V sex chromosomes may consist of a larger fraction of long-standing functional genes, in contrast to Y chromosomes where, at least in humans, many functional genes are recently transposed or are maintained by gene conversion (Skaletsky et al. 2003). Nevertheless, the lack of recombination would continue to hamper adaptation on nonrecombining U and V chromosomes, relative to autosomes, potentially favoring the appearance and spread of functional gene copies on the autosomes, followed by their loss on the sex chromosomes. Another question of interest is whether the relative importance of hitchhiking versus Muller's ratchet differs for U/V and X/Y or Z/W systems, and how this changes over the course of sex chromosome divergence (Bachtrog 2008). Finally, because we expect selection to drive differences in allele frequencies between U and V sex chromosomes, divergence between the sex chromosomes may well be faster initially, but taper off, relative to the process of degeneration differentiating the Y (W) from the X (Z).

Empirical data on the genomic landscape and structure of SDRs in haploid organisms is still scarce, despite the fact that Allen (1917) described the likely existence of sex chromosomes in bryophytes nearly a century ago. We performed a literature review and collected information from studies examining the evolution of sex determination regions in haploid organisms ranging from brown, red, and green algae to bryophytes and fungi (Table 2). Only a few of the included studies provide detailed descriptions of the SDRs based on sequencing data. However, in all cases, signs of reduced recombination had been reported, confirming our predictions about the efficacy of selection to reduce recombination. Differentiation is also commonly observed. In the liverwort *Marchantia polymorpha*, the long established V chromosome carries genes of which only half have putative homologues on the U chromosome and those that do have homologs show signs of 6–30% divergence (Yamato et al. 2007). Similarly, in the moss *Ceratodon purpureus*, the U/V chromosomes exhibit recent and repeated gene capture and low polymorphism (McDaniel et al. 2013b), in combination with high numbers of repetitive elements and asymmetric gene loss between homologs on the U and V (S. F. McDaniel, unpubl. data). Finally, in the brown alga *Ectocarpus*, the SDRs in males and females are extremely divergent with only small regions showing sequence similarity between the two (Ahmed et al. 2014).

Signs of degeneration have been putatively reported for a few species (Table 2), although most examples cannot distinguish between degeneration due to the accumulation of deleterious mutations and differentiation due to selection favoring different alleles on the U and V chromosomes. Furthermore, some of the genetic changes (e.g., repeat differences) could be neutral and differ simply because of the lack of recombination to equalize them between the two sex chromosomes. In the rhodophyte *Gracilaria chilensis*, for example, male- and female-specific SCAR markers (sequence characterized amplified regions) were shown to differ between the sexes at 8–9% of sites, although the authors noted that this might represent either degeneration or differentiation (Guillemin and Huanel 2012). Furthermore, in the alga *Volvox carteri*, the sex-linked region appears to have accumulated repeats, exhibits low gene density, and has accumulated sex-limited genes present in one sex chromosome but not the other (Ferris et al. 2010; Umen and Olson 2012). Again, however, the authors note that these patterns may reflect either degeneration or divergent natural selection. Indeed, one gene (*MAT3*) exhibited signs of sex-specific selection driving the alleles apart, with parts of the gene more strongly conserved in females and others in males. Similarly, a genomic analysis of the V chromosome in the liverwort *M. polymorpha* found that it had accumulated several repeats, is relatively

Table 2. Overview of taxa with evidence of sex chromosomes or differentiated mating-type regions in the haploid phase.

Taxonomic group	Species	System	Size of nonrecombining region	Age	Recombination	Degeneration	Source
Bryophyta	*Ceratodon purpureus*	UV	80 Mb (size of sex chromosome)	>1.3–3.5 mya	Rich in sex-linked genes (reduced recombination) and loss of homologs	Accumulation of deleterious mutations uncertain	McDaniel et al. (2007), McDaniel et al. (2013b)
Bryophyta	*Marchantia polymorpha*	UV*	10 Mb (V chromosome)		Suppressed for a long time between U and V	Repeats accumulated on V (U needs to be investigated)	Yamato et al. (2007)
Bryophyta	*Sphaerocarpos donnellii*	UV	Unknown	Unknown	Sex-specific markers suggesting nonrecombining regions and sex-linked loci	Unknown	Allen (1917)
Bryophyta	*Pseudocalliergon trifarium* (also known as *Drepanocladus trifarius*)	Sex-specific markers	Unknown	Unknown	Sex-specific markers suggesting nonrecombining regions and sex-linked loci	Unknown	Korpelainen et al. (2008)
Rhodophyta	*Gracilaria chilensis*	UV	Unknown	Unknown	Nonrecombining regions	Potential degeneration or diverged	Guillemin and Huanel (2012)
Rhodophyta	*Gracilaria gracilis*	Male and female mating types (mt^m, mt^f—needs verification)	Average chromosome size = 0.8 Mb (size of coding DNA)	Unknown	Sex-specific markers suggesting nonrecombining regions and sex-linked loci	Unknown	Martinez et al. (1999)
Phaeophyta	*Saccharina japonica*	Sex-specific marker	Unknown (very small chromosomes)	Unknown	Sex-specific markers suggesting nonrecombining regions and sex-linked loci	Unknown	Liu et al. (2009)
Phaeophyta	*Undaria pinnatifida*	Sex (female) specific marker	Unknown (very small chromosomes)	Unknown	Sex-specific markers suggesting nonrecombining regions and sex-linked loci	Unknown	Shan and Pang (2010)
Phaeophyta	*Ectocarpus* spp.	UV (chromosomes with SDR region)	~1 Mb, containing about 20 genes	>70 mya	Suppressed recombination	Signs of mild degeneration, repeat rich and gene poor	Ahmed et al. (2014)

(*Continued*)

Table 2. Continued.

Taxonomic group	Species	System	Size of nonrecombining region	Age	Recombination	Degeneration	Source
Volvocales	*Chlamydomonas reinhardtii*	MT+, MT−	200–300 kb	Unknown	Suppressed recombination, sequence rearrangement (inversions and transpositions), emergent asymmetry	Unknown	De Hoff et al. (2013)
Volvocales	*Volvox carteri*	MTF, MTM	>1 Mb	Unknown	Suppressed recombination due to rearrangements	Repeat rich, low gene density	Ferris et al. (2010), Umen and Olson (2012)
Volvocales	*Gonium pectorale*	MT+, MT−	Unknown	Unknown	Suppressed recombination due to rearrangements	Low GC content	Hamaji et al. (2008)
Volvocales	*Pleodorina starrii*	MTF, MTM	Unknown	Unknown	Sex-specific markers suggesting nonrecombining regions and sex-linked loci	Unknown	Nozaki et al. (2007)
Ascomycetes	*Neurospora tetrasperma*	mat *A*, mat *a*	>6.6 Mb	~1 mya	Nonrecombining region with two evolutionary strata, flanked by pseudo-autosomal regions, accumulation of sex-specific genes	Signs of degeneration, such as lower usage of preferred codon	Menkis et al. (2008), Whittle et al. (2011), Samils et al. (2013), Corcoran et al. (2014)
Microbotryomycetes, Basidiomycetes	*Microbotryum violaceum*	UV*	2.8–3.1 Mb and 3.4–4.2 Mb	Unknown	Suppressed recombination, possible evolutionary strata (at least three not confirmed), divergence	Accumulation of transposable elements	Hood (2002), Hood et al. (2004), Votintseva and Filatov (2009), Hood et al. (2013)
Microbotryomycetes, Basidiomycetes	*Microbotryum lychnidis-dioicae*	UV	3.3 Mb and 4.0 Mb	370 mya	Suppressed recombination	Strong evidence for degenration with accumulation of transposable elements, weak gene expression and gene loss	Fontanillas et al. (2015)
Tremellomycetes, Basidiomycetes	*Cryptococcus neoformans*	Mata, Matα	MAT locus: >100 kb with >20 genes		Suppressed recombination and; substantial rearrangement, four primary groupings (strata?) identified based on phylogeny, nucleotide identity, and synonymous substitution rates, diverging sequences within MAT	Unknown	Fraser et al. (2004)

*UV refers to morphologically distinct sex chromosomes (U in females and V in males) and may differ from the nomenclature used in the original publication.

gene poor, and also has several male-specific genes (Yamato et al. 2007), but other genes had divergent male and female homologs expressed in sexual organs, consistent with differentiation rather than degeneration. On the other hand, Ahmed et al. (2014) concluded that degeneration was surprisingly mild in the *Ectocarpus* UV system, despite the relatively old age of the sex chromosomes (Ahmed et al. 2014). It remains an open empirical challenge to determine the nature of selection at loci that differ between U and V chromosomes in a way that distinguishes among degeneration versus selectively favored differentiation versus neutral divergence. For example, the lack of a homolog in one sex could represent any one of these possibilities, depending on whether gene loss from one of the sex chromosomes reduced fitness slightly, increased fitness, or had no effect on fitness.

It is worth highlighting one taxonomic group in our table that has been excluded from previous discussions on the evolution of sex chromosomes—the fungi. The reason for avoiding the fungi was based on the fact that anisogamy in most fungi is limited or absent, and hence the scope for sexual dimorphism and sexually antagonistic selection appears to be rather limited. This also applies to some of the volvocine algae including *Chlamydomonas reinhardtii* and *Gonium pectorale* (Hamaji et al. 2008; De Hoff et al. 2013). Nevertheless, isogamy in these species does not exclude the fact that genes may be differentially expressed among the two types of gametes that need to fuse, where one is sometimes referred to as the donor and the other the receptor (Nieuwenhuis and Aanen 2012). Therefore, given that any differences in allele frequencies due to even slight differential selection in the two mating types may lead to the reduction of recombination, we believe that our models apply to these taxonomic groups as well. In fact, for all three fungal species listed in our table and for both volvocine algae, suppressed recombination and signs of genetic divergence have been reported. Perhaps the best evidence to date of degeneration per se comes from regions linked to the mating-type locus of *Neurospora tetrasperma* (Whittle et al. 2011). These authors showed an elevated level of transitions from preferred to nonpreferred codons in the nonrecombining region near the *mat* locus. Such degeneration may occur despite the efficacy of haploid selection because selection against nonpreferred codons is weak and more readily overcome by drift and Muller's ratchet (eq. (12)) or by hitchhiking with positively selected changes within nonrecombining regions. Interestingly, sex-specific genes have started to accumulate around the mating-type locus in *N. tetrasperma*, with *mat A* becoming associated with sexual/female traits (protoperithecia) and *mat a* with asexual/male traits (conidia), blurring the distinction between a mating-type locus and a sex-determining locus (Samils et al. 2013). These represent some of the first detailed studies investigating the genomic landscape around mating-type loci in fungi. More examples of the accumulation of sex-specific genes around mating-type loci likely remain to be discovered.

In addition to suppressed recombination and sex chromosome differentiation, chromosomal rearrangements such as fusions, transpositions, and inversions certainly play a crucial role in the evolution of dimorphic sex chromosomes in haploid organisms as well. As demonstrated by our model, a fusion that brings a selected locus into the immediate vicinity of the SDR will spread through the population as long as selection differs between male and female haploids. Furthermore, the modifier locus **M** that we have modeled can also describe inversions and transpositions that lead to tighter linkage of selected loci with the SDR. Chromosomal rearrangements due to inversions and translocations have been reported in three of the four species of volvocine algae listed in our table. Chromosomal fusion and subsequent inversions appear to have played an important role also in the evolution of the mating-type locus in the fungal pathogen *Cryptococcus neoformans* (Fraser et al. 2004). With the increasing emergence of next-generation sequencing data from nonmodel organisms, more information will soon become available to investigate the processes involved in the evolution of sex chromosomes in organisms where it is the haploid phase that determines sex.

ACKNOWLEDGMENTS

We are grateful to S. Coelho and S. McDaniel for sharing their unpublished results. We thank S. McDaniel, S. Coelho, H. Johannesson, B. Nieuwenhuis, and two anonymous reviewers for valuable comments on earlier drafts of the manuscript. This study was supported by grants from the Nilsson-Ehle Foundation, the Swedish Research Council, and the European Research Council to SI and by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to SPO.

DATA ARCHIVING

The doi for our data is 10.5061/dryad.n4v3b.

LITERATURE CITED

Ahmed, S., J. M. Cock, E. Pessia, R. Luthringer, A. Cormier, M. Robuchon, L. Sterck, A. F. Peters, S. M. Dittami, E. Corre, et al. 2014. A haploid system of sex determination in the brown alga *Ectocarpus* sp. Curr. Biol. 24:1945–1957.

Allen, C. 1917. A chromosome difference correlated with sex differences in *Sphaerocarpos*. Science 46:466–467.

Allen, C. E. 1945. The genetics of bryophytes. II. Bot. Rev. 5:260–287.

Bachtrog, D. 2006. A dynamical view of sex chromosome evolution. Curr. Opin. Genet. Dev. 16:578–585.

———. 2008. Positive selection at the binding sites of the male-specific lethal complex involved in dosage compensation in *Drosophila*. Genetics 180:1123–1129.

———. 2013. Y-chromosome evolution: emerging insights into the processes of Y-chromosome degeneration. Nat. Rev. Genet. 14:113–124.

Bachtrog, D., M. Kirkpatrick, J. E. Mank, S. F. McDaniel, J. C. Pires, W. R. Rice, and N. Valenzuela. 2011. Are all sex chromosomes created equal? TREE 27:350–357.

Bachtrog, D., J. Mank, C. Peichel, S. Otto, M. Kirkpatrick, T. Ashman, M. Hahn, J. Kitano, I. Mayrose, R. Ming, et al. 2014. Sex determination: why so many ways of doing it? PLoS Biol. 12:001899.

Bergero, R., and D. Charlesworth. 2009. The evolution of restricted recombination in sex chromosomes. TREE 24:94–102.

Bull, J. 1978. Sex chromosomes in haploid dioecy: a unique contrast to Muller's theory for diploid dioecy. Am. Nat. 112:245–250.

———. 1983. Evolution of sex determining mechanisms. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

Casselton, L. 2002. Mate recognition in fungi. Heredity 88:142–147.

Charlesworth, B. 1978. Model for evolution of Y-chromosomes and dosage compensation. PNAS 75:5618–5622.

———. 1990. The evolution of sex chromosomes. Science 251:1030–1033.

———. 1990. The evolution of chromosomal sex determination and dosage compensation. Curr. Biol. 6:149–162.

Charlesworth, B., and D. Charlesworth. 1997. Rapid fixation of deleterious alleles can be caused by Muller's ratchet. Genet. Res. 70:63–73.

Charlesworth, D., and B. Charlesworth. 1980. Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes. Genet. Res. 35:205–214.

Charlesworth, D., and J. E. Mank. 2010. The birds and the bees and the flowers and the trees: lessons from genetic mapping of sex determination in plants and animals. Genetics 186:9–31.

Charlesworth, B., J. Coyne, and N. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130:113–146.

Charlesworth, D., B. Charlesworth, and G. Marais. 2005. Steps in the evolution of heteromorphic sex chromosomes. Heredity 95:118–128.

Clark, A. G. 1987. Natural selection and Y-linked polymorphism. Genetics 115:569–577.

Corcoran, P., J. R. Dettman, Y. Sun, E. M. Luque, L. M. Corrochano, J. W. Taylor, M. Lascoux, and H. Johannesson. 2014. A global multilocus analysis of the model fungus Neurospora reveals a single recent origin of a novel genetic system. Mol. Phylogen. Evol. 78:136–147.

De Hoff, P., P. Ferris, B. Olson, A. Mjyagi, S. Geng, and J. Umen. 2013. Species and population level molecular profiling reveals cryptic recombination and emergent asymmetry in the dimorphic mating locus of *C. reinhardtii*. PLoS Genet. 9:e1003724.

Feldman, M. W., I. Franklin, and G. J. Thomson. 1974. Selection in complex genetic systems. I. The symmetric equilibria of the three-locus symmetric viability model. Genetics 76:135–162.

Ferris, P., B. Olson, P. De Hoff, S. Douglass, D. Casero, S. Prochnik, S. Geng, R. Rai, J. Grimwood, J. Schmutz, et al. 2010. Evolution of an expanded sex-determining locus in *Volvox*. Science 328:351–354.

Fontanillas, E., M. E. Hood, H. Badouin, V. Barbe, J. Gouzy, D. M. de Vienne, G. Aguileta, J. Poulain, P. Wincker, Z. Chen, et al. 2015. Degeneration of the nonrecombining regios in the mating-type chromosomes of the anther-smut fungi. Mol. Biol. Evol. doi: 10.1093/molbev/msu396.

Fraser, J., S. Diezmann, R. Subaran, A. Allen, K. Lengeler, F. Dietrich, and J. Heitman. 2004. Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. PLoS Biol. 2:e384.

Gibson, J. R., A. K. Chippindale, and W. R. Rice. 2002. The X chromosome is a hot spot for sexually antagonistic fitness variation. Proc. R Soc. Lond. B 269:499–505.

Gordo, I., and B. Charlesworth. 2001. The speed of Muller's ratchet with background selection, and the degeneration of Y chromosome. Genet. Res. 78:149–161.

Guillemin, M.-L., and O. Huanel. 2012. Characterization of genetic markers linked to sex determination in the haploid-diploid red alga *Gracilaria chilensis*. J. Phycol. 48:365–372.

Haldane, J. 1933. The part played by recurrent mutation in evolution. Am. Nat. 67:5–19.

Hamaji, T., P. Ferris, A. Coleman, S. Waffenschmidt, F. Takahashi, I. Nishii, and H. Nozaki. 2008. Identification of the minus-dominance gene ortholog in the mating-type locus of *Gonium pectorale*. Genetics 178:283–294.

Hood, M. 2002. Dimorphic mating-type chomosomes in the fungus *Mycrobotryum violaceum*. Genetics 160:457–461.

Hood, M., J. Antonovics, and B. Koskella. 2004. Shared forces of sex chromosome evolution in haploid-mating and diploid-mating organisms: *Microbotryum violaceum* and other model organisms. Genetics 168:141–146.

Hood, M. E., E. Petit, and T. Giraud. 2013. Extensive divergence between mating-type chromosomes of the anther-smut fungus. Genetics 193:309–315.

Jesson, L., and P. Garnock-Jones. 2012. Can classifications of functional gender be extended to all land plants? Perspectives in Plant Ecology, Evolution and Systematics 14:153–160.

Korpelainen, H., I. Bisang, L. Hedenäs, and J. Kolehmainen. 2008. The first sex-specific molecular marker discovered in the moss *Pseudocalliergon trifarium*. J. Hered. 99:581–587.

Lawson-Handley, L., H. Ceplitis, and H. Ellegren. 2004. Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution. Genetics 167:367–376.

Lenormand, T. 2003. The evolution of sex dimorphism in recombination. Genetics 163:811–822.

Lewis, K. R. 1961. The genetics of bryophytes. Trans. Brit. Bryol. Soc. 4:111–130.

Lewis, K. R., and K. Benson-Evans. 1960. The chromosomes of *Cryptothallbus mirabilis* (Hepaticae: Riccardiaceae). Phyton 14:21–35.

Lewis, K. R., and B. John. 1968. The chromosomal basis of sex determination. Int. Rev. Cyto. 17:277–379.

Liu, Y.-S., L.-H. Li, W.-K. Wu, and Z.-G. Zhou. 2009. A SCAR molecular marker specifically related to the female gametophytes of *Saccharina* (*Laminaria*) *japonica* (Phaeophyta). J. Phycol. 45:894–897.

Luthringer, R., A. Cormier, S. Ahmed, A. F. Peters, J. M. Cock, and S. M. Coelho. 2014. Sexual dimorphism in the brown algae. Perspect. Phycol. 1:11–25.

Mable, B., and S. Otto. 1998. The evolution of life cycles with haploid and diploid phases. BioEssays 20:453–462.

Martinez, E., C. Destombe, M. Quillet, and M. Valero. 1999. Identification of random amplified polymorphic DNA (RAPD) markers highly linked to sex determination in the red alga *Gracilaria gracilis*. Mol. Ecol. 8:1533–1538.

Matsubara, K., H. Tarui, M. Toriba, K. Yamada, C. Nishida-Umehara, K. Agata, and Y. Matsuda. 2006. Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. PNAS 103:18190–18195.

McDaniel, S. F., J. Willis, and A. Shaw. 2007. A linkage map reveals a complex basis for segregation distortion in an interpopulation cross in the moss *Ceratodon pupureus*. Genetics 176:2489–2500.

McDaniel, S. F., J. Atwood, and J. Burleigh. 2013a. Recurrent evolution of dioecy in bryophytes. Evolution 67:567–572.

McDaniel, S. F., K. Neubig, A. Payton, R. Quatrano, and D. Cove. 2013b. Recent gene-capture on the UV sex chromosomes of the moss *Ceratodon pupureus*. Evolution 67:2811–2822.

Menkis, A., D. J. Jacobson, T. Gustafsson, and H. Johannesson. 2008. The mating-type chromosome in the filamentous ascomycete *Neuropora tetrasperma* represents a model for early evolution of sex chromosomes. PLoS Genet. 4:e1000030.

Nei, M. 1969. Linkage modification and sex difference in recombination. Genetics 63:681–669.

Nieuwenhuis, B. P. S., and D. K. Aanen. 2012. Sexual selection in fungi. J. Evol. Biol. 25:2397–2411.

Nozaki, H., T. Mori, O. Misumi, S. Matsunanga, and T. Kuroiwa. 2007. Males evolved from the dominant isogametic mating type. Curr. Biol. 16:R1018–R1020.

Orr, H. A., and Y. Kim. 1998. An adaptive hypothesis for the evolution of the Y chromosome. Genetics 150:1693–1698.

Otto, S. P. 1994. The role of deleterious and beneficial mutations in the evolution of ploidy levels. Pp. 69–98 *in* M. Kirkpatrick, ed. The evolution of haploid-diploid life cycles. American Mathematical Society, Snowbird, UT.

———. 2014. Selective maintenance of recombination between the sex chromosomes. J. Evol. Biol. 27:1431–1442.

Otto, S. P., J. R. Pannell, C. L. Peichel, T. L. Ashman, D. Charlesworth, A. K. Chippindale, L. F. Delph, R. F. Guerrero, S. V. Scarpino, and B. F. McAllister. 2011. About PAR: the distinct evolutionary dynamics of the pseudoautosomal region. Trends Genet. 27:358–367.

Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.

———. 1987a. The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex-chromosomes. Evolution 41:911–914.

———. 1987b. Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. Genetics 116:161–167.

———. 1996. Evolution of the Y sex chromosome in animals. BioScience 46:331–343.

Samils, N., A. Gioti, M. Karlsson, Y. Sun, T. Kasuga, E. Bastiaans, Z. Wang, N. Li, J. Townsend, and H. Johannesson. 2013. Sex-linked transcriptional divergence in the hermaphrodite fungus *Neurospora tetrasperma*. Proc. R. Soc. B Biol. Sci. 280:20130862.

Shan, T., and S. Pang. 2010. Sex-linked microsatellite marker detected in the female gametophytes of *Undaria pinnatifida* (Phaeophyta). Phycol. Res. 58:171–176.

Skaletsky, H., T. Kuroda-Kawaguchi, P. J. Minx, H. S. Cordum, L. W. Hillier, L. G. Brown, S. Repping, T. Pyntikova, J. Ali, T. Bieri, et al. 2003. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 423:825–837.

Umen, J. G. 2011. Evolution of sex and mating loci: an expanded view from Volvocine algae. Curr. Opin. Microbiol. 14:634–641.

Umen, J. G., and B. J., Olson. 2012. Genomics of Volvocine Algae. Adv. Bot. Res. 64:185–243.

Vicoso, B., and B. Charlesworth. 2006. Evolution on the X chromosome: unusual patterns and processes. Nat. Rev. Genet. 7:645–653.

Vitt, D. H. 1968. Sex determination in mosses. Mich. Bot. 7:195–203.

Votintseva, A. A., and D. A. Filatov. 2009. Evolutionary strata in a small mating-type-specific region of the smut fungus *Microbotryum violaceum*. Genetics 182:1391–1396.

Whittle, C., Y. Sun, and H. Johannesson. 2011. Degeneration in codon usage within the region of suppressed recombination in the mating-type chromosomes of *Neurospora tetrasperma*. Eukaryot. Cell 10:594–603.

Yamato, K. T., K. Ishizaki, M. Fujisawa, S. Okada, S. Nakayama, M. Fujishita, H. Bando, K. Yodoya, K. Hayashi, T. Bando, et al. 2007. Gene organization of the liverwort Y chromosome reveals distinct sex chromosome evolution in a haploid system. PNAS 104:6472–6477.

Associate Editor: D. Roze
Handling Editor: R. Shaw

Appendix

RECURSIONS

We follow chromosomes, starting at the beginning of the haploid phase, with female U-bearing chromosome frequencies given by o_i and male V-bearing chromosome frequencies given by p_i. Here, i is an index that numbers the chromosomes, with $1 = MA$, $2 = Ma$, $3 = mA$, and $4 = ma$.

Selection first acts in the haploid phase, depending on the allele, k, that the haploid bears at the **A** locus (e.g., $k = A$ for haploids of genotype $i = 1$ or 3). Selection thus changes the chromosome frequencies in haploid females to $o_i^s = U_k\, o_i/\bar{U}$, where $\bar{U} = \sum_{i=1}^{4} U_k\, o_i$ is the mean fitness of haploid females. Similarly, selection changes the chromosome frequencies in haploid males to $p_i^s = V_k\, p_i/\bar{V}$, where $\bar{V} = \sum_{i=1}^{4} V_k\, p_i$.

At the next stage, haploid females produce eggs and haploid males produce sperm, which unite at random to produce a diploid sporophyte of genotype ij with probability $x_{ij} = o_i^s\, p_j^s$, where i refers to the haplotype bearing the U chromosome and j the haplotype bearing the V chromosome. After selection in the diploid phase, these genotype frequencies become $x_{ij}^s = S_k\, x_{ij}/\bar{S}$, where $\bar{S} = \sum_{i=1}^{4}\sum_{j=1}^{4} S_k\, x_{ij}$ is the mean sporophytic fitness and k now describes the diploid genotype at locus **A** (e.g., $k = Aa$ for individuals where $i = 1$ and $j = 2$).

Finally, the diploid sporophytes undergo meiosis with recombination to produce the next generation of haploid U-bearing females:

$$\begin{aligned} o'_{MA} = {} & \left(\sum_{j=1}^{4} x_{1j}^s\right) - r_{MM}\left(x_{12}^s - x_{21}^s\right) \\ & - (R + r_{Mm} - 2\chi)\left(x_{13}^s - x_{31}^s\right) - (R + r_{Mm} - \chi)\, x_{14}^s \\ & + (r_{Mm} - \chi)\, x_{41}^s + \chi\, x_{23}^s + (R - \chi)\, x_{32}^s \end{aligned} \tag{A1a}$$

$$\begin{aligned} o'_{Ma} = {} & \left(\sum_{j=1}^{4} x_{2j}^s\right) - r_{MM}\left(x_{21}^s - x_{12}^s\right) \\ & - (R + r_{Mm} - 2\chi)\left(x_{24}^s - x_{42}^s\right) - (R + r_{Mm} - \chi)\, x_{23}^s \\ & + (r_{Mm} - \chi)\, x_{32}^s + \chi\, x_{14}^s + (R - \chi)\, x_{41}^s \end{aligned} \tag{A1b}$$

$$\begin{aligned} o'_{mA} = {} & \left(\sum_{j=1}^{4} x_{3j}^s\right) - r_{mm}\left(x_{34}^s - x_{43}^s\right) \\ & - (R + r_{Mm} - 2\chi)\left(x_{31}^s - x_{13}^s\right) - (R + r_{Mm} - \chi)\, x_{32}^s \\ & + (r_{Mm} - \chi)\, x_{23}^s + \chi\, x_{41}^s + (R - \chi)\, x_{14}^s \end{aligned} \tag{A1c}$$

$$o'_{ma} = \left(\sum_{j=1}^{4} x^s_{4j}\right) - r_{mm}\left(x^s_{43} - x^s_{34}\right) - (R + r_{Mm} - 2\chi)\left(x^s_{42} - x^s_{24}\right) - (R + r_{Mm} - \chi)\, x^s_{41} + (r_{Mm} - \chi)\, x^s_{14} + \chi\, x^s_{32} + (R - \chi)\, x^s_{23} \quad \text{(A1d)}$$

$$p'_{mA} = \left(\sum_{j=1}^{4} x^s_{j3}\right) - r_{mm}\left(x^s_{43} - x^s_{34}\right) - (R + r_{Mm} - 2\chi)\left(x^s_{13} - x^s_{31}\right) - (R + r_{Mm} - \chi)\, x^s_{23} + (r_{Mm} - \chi)\, x^s_{32} + \chi\, x^s_{14} + (R - \chi)\, x^s_{41} \quad \text{(A2c)}$$

and haploid V-bearing males:

$$p'_{MA} = \left(\sum_{j=1}^{4} x^s_{j1}\right) - r_{MM}\left(x^s_{21} - x^s_{12}\right) - (R + r_{Mm} - 2\chi)\left(x^s_{31} - x^s_{13}\right) - (R + r_{Mm} - \chi)\, x^s_{41} + (r_{Mm} - \chi)\, x^s_{14} + \chi\, x^s_{32} + (R - \chi)\, x^s_{23} \quad \text{(A2a)}$$

$$p'_{ma} = \left(\sum_{j=1}^{4} x^s_{j4}\right) - r_{mm}\left(x^s_{34} - x^s_{43}\right) - (R + r_{Mm} - 2\chi)\left(x^s_{24} - x^s_{42}\right) - (R + r_{Mm} - \chi)\, x^s_{14}. + (r_{Mm} - \chi)\, x^s_{41} + \chi\, x^s_{23} + (R - \chi)\, x^s_{32} \quad \text{(A2d)}$$

$$p'_{Ma} = \left(\sum_{j=1}^{4} x^s_{j2}\right) - r_{MM}\left(x^s_{12} - x^s_{21}\right) - (R + r_{Mm} - 2\chi)\left(x^s_{42} - x^s_{24}\right) - (R + r_{Mm} - \chi)\, x^s_{32} + (r_{Mm} - \chi)\, x^s_{23} + \chi\, x^s_{41} + (R - \chi)\, x^s_{14} \quad \text{(A2b)}$$

If the gene order is **M**—**A**—U/V SDR and there is no genetic interference, then $\chi = R\, r_{Mm}$. If the gene order is **A**—**M**—U/V SDR (as with a fusion) and there is no genetic interference, then $\chi = R\,(1 - R - r_{Mm})\,(1 - 2R)$. If a fusion brings the selected locus into complete linkage with the SDR, then $r_{Mm} = R = \chi = 0$.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Immler & Otto 2015 Supplementary Matematica file.