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EVOLUTIONARILY STABLE SEX RATIOS AND MUTATION LOAD

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Frequency-dependent selection should drive dioecious populations toward a 1:1 sex ratio, but biased sex ratios are widespread, especially among plants with sex chromosomes. Here, we develop population genetic models to investigate the relationships between evolutionarily stable sex ratios, haploid selection, and deleterious mutation load. We confirm that when haploid selection acts only on the relative fitness of X- and Y-bearing pollen and the sex ratio is controlled by the maternal genotype, seed sex ratios evolve toward 1:1. When we also consider haploid selection acting on deleterious mutations, however, we find that biased sex ratios can be stably maintained, reflecting a balance between the advantages of purging deleterious mutations via haploid selection, and the disadvantages of haploid selection on the sex ratio. Our results provide a plausible evolutionary explanation for biased sex ratios in dioecious plants, given the extensive gene expression that occurs across plant genomes at the haploid stage.

KEY WORDS: **Evolutionarily stable strategy, gametophytic selection, heteromorphic sex chromosomes, mutation load, plant life cycles, sex-ratio evolution.**

The evolution of the sex ratio is a frequency-dependent process in which the least frequent sex obtains fitness benefits proportional to its rarity in the population (Maynard Smith 1974; Charnov 1982). This is referred to as negative frequency-dependent selection, and its effects on sex-ratio evolution were discussed in early work by Darwin (1871) and formalized mathematically by Düsing (1884). Fisher (1930) showed that sex ratios evolve toward 1:1 when parents invest equally in the two sexes (see Trivers 1972). Selection for an even sex ratio is referred to here as Fisherian sex-ratio selection and is described in explicit genetic terms by Shaw and Mohler (1953) and reviewed in Karlin and Lessard (1986).

Populations of dioecious plants commonly exhibit deviations from the 1:1 sex ratio, and these biases can involve an excess of females or males (Barrett et al. 2010; Sinclair et al. 2012). A recent survey of sex ratios in 243 dioecious angiosperm species, including 123 genera and 61 families, found significantly biased sex ratios in 49.8% of species (Field et al. 2012a). Of these, 76 exhibited male-biased sex ratios and 45 were female-biased, with a median male percentage of 63% and 36%, respectively. The frequent occurrence of sex-ratio bias in plants raises questions about the proximate and ultimate causes of this phenomenon, especially when it involves biased primary (seed) sex ratios. Several mechanisms have been proposed to explain bias in seed sex ratios, including competition between male- versus female-determining gametophytes (pollen tubes), a process known as "certation" (Correns 1922), X-linked meiotic drive (Taylor and Ingvarsson 2003), and local mate competition (de Jong and Klinkhamer 2005). Other mechanisms that could conceivably affect primary sex ratios include selective abortion of ovules (e.g., Stephenson and Winsor 1986; Casper 1988) and maternally induced selection among pollen tubes (Bachelier and Friedman 2011).

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The possibility that competition among male gametophytes is a mechanism causing biased sex ratios is suggested by the observation that the amount of pollen deposited on stigmas (pollination intensity) affects seed sex ratios. Correns (1922, 1928) demonstrated experimentally that increasing pollen loads in *Silene* and *Rumex* species, two groups with sex chromosomes, was associated with more female-biased sex ratios, whereas sparse pollination resulted in sex ratios closer to unity. This pattern has been subsequently confirmed in several additional *Rumex* species (Rychlewski and Zarxycki 1975; Conn and Blum 1981; Stehlik and Barrett 2006; Field et al. 2012b). These observations are consistent with the certation hypothesis involving poor performance of Y-bearing pollen, which in turn may be due to the degeneration of the Y chromosome caused by suppressed X-Y recombination (Smith 1963; Lloyd 1974; Charlesworth and Charlesworth 2000). In *S. latifolia*, the Y chromosome is known to have partially degenerated and ∼20% of the genes on this chromosome have either impaired function or severely reduced expression (Bergero and Charlesworth 2011; Chibalina and Filatov 2011). In *R. acetosa* and *R. hastatulus*, sex chromosomes are heteromorphic, and Y chromosomes have accumulated repetitive sequences (Mariotti et al. 2006; Ester et al. 2011). Such degeneration could contribute to mutation load and therefore to fitness differences between female- and male-determining pollen. In the absence of dosage compensation, this accumulating load could also affect the fitness of heterogametic (XY) males in the diploid sporophytic stage because of the strong overlap in gene expression between the haploid and diploid phases of the life cycle in plants (Mascarenhas 1990; Borg et al. 2009). Indeed, a higher mutation load among male diploids has been suggested in *R. nivalis*, in which the sex ratio becomes progressively more female biased from the seed to the flowering stage (Stehlik et al. 2007).

Several studies including those mentioned above have emphasized mechanisms that operate during the progamic phase—from pollination to fertilization—to account for biased seed sex ratios in plants. These are proximate explanations however, and ultimately we want to know why plants do not evolve sex ratios closer to unity in the face of Fisherian sex-ratio selection, for example, by weakening selection during the haploid stage. This issue is the main focus of the present study.

The role that gametophytic selection may play in purging the deleterious mutation load and its effects on sex-ratio evolution have not been previously considered. Mutation load refers to the reduction in individual fitness (relative to a mutation-free genotype) caused by segregating deleterious alleles, and its effects on fitness can be substantial (Agrawal and Whitlock 2012). With thousands of genes expressed during the haploid stage of plant life cycles (Borg et al. 2009), strong selection against deleterious mutations during this stage could have pervasive genome-wide effects on the mutation load of plants (Klekowski 1984; Walbot and Evans 2003). In animals, haploid expression is much less extensive, but some genes, particularly those involved in spermatogenesis, also show potential for strong haploid selection (Joseph and Kirkpatrick 2004).

Here, we develop population genetic models to investigate sex-ratio evolution in a dioecious plant with sex chromosomes. We assume that males are heterogametic (XY), both because this form of sex determination is predominant among known plant sex chromosome systems (Ming et al. 2011), and because selection among pollen does not affect the sex ratio in ZW species (because all pollen is Z-bearing). We consider genes that act in the maternal plant either to modify the sex ratio directly, or to modify the strength of selection among X- and Y-bearing pollen, and ask how these genes evolve in the face of Fisherian sex-ratio selection. We then add recurrent deleterious mutations throughout the genome to assess their effects on modifiers that alter the strength of haploid selection. Finally, we determine the evolutionarily stable sex ratio (ESS) and the level of mutation load that result in the presence of these conflicting selective pressures. We discuss the implications of our theoretical results for explaining empirical observations of sex-ratio bias in dioecious plants and for the evolution of life cycles with extensive haploid gene expression.

The Models

In the presence of Fisherian sex-ratio selection, we consider the evolution of modifier alleles that affect the sex ratio in a dioecious plant population in which the haploid gametophytic phase is contained within female sporophytes. We evaluate the conditions under which such modifiers can spread and determine the ESS under three scenarios that differ with regard to the stage at which female sporophytes influence the pool of pollen used at fertilization (Fig. 1): (1) an early-acting sex-ratio modifier that influences the frequency (c_{ij}) of Y-bearing pollen that germinates on the stigma before gametophytic selection, (2) a late-acting sex-ratio modifier that influences the frequency (c_{ij}) of Y-bearing pollen tubes entering ovules after gametophytic selection, and (3) a modifier that alters the strength of selection in females (c_{ij}) among haploid male gametophytes, without directly selecting among them.

In each case, we assume that c_{ij} depends on the genotype at the modifier locus, which bears two alleles (M and m) that have no direct fitness effects. Because sex chromosomes may not segregate randomly during meiosis in males, we assume that males produce a fraction, α, of Y-bearing pollen, and a fraction, $1-\alpha$, of X-bearing pollen such that in the absence of sex ratio or gametophytic selection, the male to female ratio among seeds would be $\alpha : 1-\alpha$. Selection among pollen tubes during their growth in the style causes the frequency of Y-bearing gametophytes to change by an amount proportional to $1-\gamma$ relative to X-bearing gametophytes. See Table 1 for model notation.

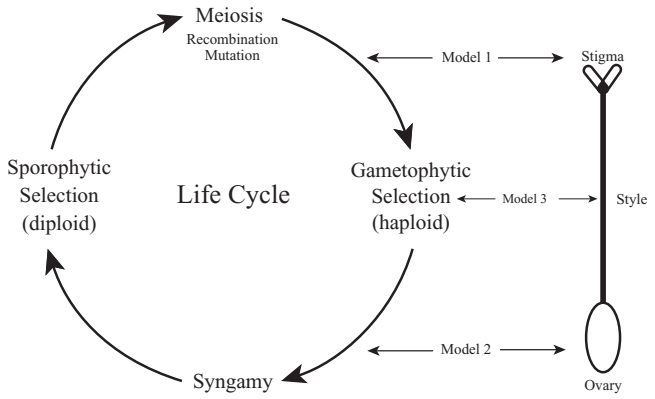

Figure 1. Modifier evolution at different stages of the plant life cycle. Our models track the evolution of modifier alleles that affect either the sex ratio or the strength of gametophytic selection at different life cycle stages: (1) sex-ratio regulation at the stage of pollen receipt before gametophytic selection (Model 1), (2) sex-ratio regulation after gametophytic selection (Model 2), and (3) regulation of the strength of gametophytic selection (Model 3). We census maternal genotypes after meiosis.

MODEL 1: EARLY-ACTING SEX-RATIO MODIFIER

In the first model (Fig. 1: Model 1), females exert control over the sex ratio at the stigma, during the stage at which pollen-tube growth is initiated (see recursions in Appendix A). Depending on the female genotype *ij* at the modifier locus, a fraction c_{ij} of Y-bearing pollen tubes and $1 - c_{ij}$ of X-bearing pollen tubes enter the style on average. The c_{ij} values that are possible depend on the genetic variation that could arise to alter the ratio of pollen tube types entering the style. For example, if all of the pollen produced is Y-bearing ($\alpha = 0$), then c_{ij} must be zero. Although modifiers may be more abundant for certain c_{ij} values (e.g., for c_{ij} nearer α), we assume for now that the ratio of X to Y carrying pollen could be modified to any level as long as $0 < \alpha < 1$.

We first calculate the sex ratio when the modifier allele *M* is fixed in the population (i.e., before allowing evolution to adjust the sex ratio). Because sex-ratio control occurs before gametophytic selection, the frequency of male seeds, ψ, reflects both female sex-ratio control (c_{MM}) and the relative fitness of Y-bearing pollen grains (described by γ):

$$\psi = \frac{(1-\gamma)c_{MM}}{1-\gamma c_{MM}}. \quad (1)$$

A new modifier allele, *m*, that alters the ratio of X- to Y-bearing pollen tubes entering the stigma is predicted to spread when the leading eigenvalue, λ, is greater than one, where λ is calculated from the local stability matrix describing the dynamics when *m* is rare, as derived from the recursions in Appendix A. We show in our supplementary *Mathematica* file (doi:10.5061/dryad.74tg7) that:

$$\lambda \approx 1 + \frac{(c_{Mm} - c_{MM})(1 - 2c_{MM} + \gamma c_{MM})}{4c_{MM}(1 - c_{MM})(1 - \gamma c_{MM})}, \quad (2)$$

where we assume that the effect of the new modifier is small (c_{Mm} near c_{MM}) to simplify the solution (qualitatively, the results are similar for large-effect modifiers; see supplementary material (doi:10.5061/dryad.74tg7)). Thus, if $c_{MM} < 1/(2-\gamma)$, modifier alleles will invade if they increase the fraction of germinating pollen that is Y-bearing ($c_{Mm} > c_{MM}$). With small modifier effects, the system thus evolves toward:

$$c^* = \frac{1}{2-\gamma}, \quad (3)$$

meaning that c^* is a convergence stable strategy (Eshel et al. 1997). According to (2), c^* also cannot be invaded by any other strategy (implying that c^* is also an ESS). Note that c^* is the sex ratio before gametophytic selection, and inserting equation (3) into (1) indicates that the ESS sex ratio among seeds after gametophytic selection (ψ^*) is 1:1. Thus, when females exert control over the initial growth of male- versus female-determining pollen tubes, they evolve to do so in a manner that counterbalances gametophytic selection.

MODEL 2: SEED PRODUCTION WITH LATE-ACTING SEX-RATIO MODIFIER

Similar results are obtained if females exert control over the sex ratio by discriminating among pollen tubes that have survived

Table 1. Notation used in the models.

M	Modifier locus, with alleles *M* (resident) and *m* (rare)
A	Selected locus, with alleles *A* (wild type) and *a* (deleterious)
c_{ij}	Maternal control of the sex ratio in a female carrying modifier alleles *i* and *j*.
α	The frequency of Y-bearing pollen produced by males
γ	Selection against Y-bearing pollen at the haploid stage (after receipt on the stigma)
ψ	The frequency of male seeds
ψ^*	The ESS frequency of male seeds
s^k	Selection against the mutant *a* allele in diploid individuals of sex *k*
h^k	Dominance of the mutant *a* allele in diploid individuals of sex *k* (fitness of heterozygotes being $1 - h^k s^k$)
t^k	Selection against the mutant *a* allele in haploids of sex *k*

gametophytic selection and have reached the ovary (Fig. 1: Model 2), with c_{ij} now describing the fraction of Y-bearing pollen tubes involved in ovule fertilization (recursions in Appendix A). Here, sex-ratio control is assumed to occur immediately before fertilization with no other selective events following, and the seed sex ratio is thus given by $\psi = c_{MM}$, when the modifier allele, M, is fixed. The invasion of a new modifier is now determined by:

$$\lambda \approx 1 + \frac{(c_{Mm} - c_{MM})(1 - 2c_{MM})}{4c_{MM}(1 - c_{MM})}, \quad (4)$$

where again we have assumed that the modifier is weak. Neither biased production of X and Y pollen (described by α) nor gametophytic selection (described by γ) affect the dynamics of rare sex-ratio modifiers, because these forces are neutralized when females can manipulate whether female- or male-determining pollen tubes are allowed to enter ovules. Instead, modifiers invade ($c_{Mm} > c_{MM}$) whenever they increase the fraction of the rarer sex in the population. With small modifier effects, the system therefore evolves toward the ESS $c^* = 1/2$ such that the sex ratio among seeds is again 1:1.

MODEL 3: SEED PRODUCTION WITH MODIFIER OF GAMETOPHYTIC SELECTION

The preceding models assume that females can detect X- or Y-bearing pollen tubes and manipulate their growth, but this may be mechanistically unrealistic. An alternative possibility is that females alter the strength of selection experienced by pollen tubes (Fig. 1: Model 3). Females could, for example, modify the length, shape, or structure of the style in a manner that indirectly influences the intensity of pollen-tube competition (Lankinen and Skogsmyr 2001), or females could alter the amount or type of resource provisioning for growing pollen tubes, which could conceivably accentuate or mute fitness differences among the pollen.

Regardless of the exact mechanism, we assume that there is genetic variation for the strength of gametophytic selection in females, with this strength given by c_{ij} for a female of modifier genotype *ij*. Specifically, Y-bearing pollen now has fitness $1 - \gamma c_{ij}$ relative to X-bearing pollen (recursions in Appendix A). Here, we define γ as the maximal strength of gametophytic selection given other constraints (e.g., constraints on style length or resources supporting pollen-tube growth); we thus consider c_{ij} values between 0 and 1. With modifier allele M fixed, the frequency of male seeds becomes:

$$\psi = \frac{\alpha(1 - \gamma c_{MM})}{1 - \alpha\gamma c_{MM}}. \quad (5)$$

A new modifier allele, m, that alters the strength of gametophytic selection can then spread when $\lambda > 1$, where:

$$\lambda \approx 1 + \frac{(c_{Mm} - c_{MM})\gamma(2\alpha - 1 - \alpha\gamma c_{MM})}{4(1 - \gamma c_{MM})(1 - \alpha\gamma c_{MM})}. \quad (6)$$

Thus, when Y-bearing pollen is less fit ($\gamma > 0$) modifier alleles increasing the strength of gametophytic selection ($c_{Mm} > c_{MM}$) invade if Y-bearing pollen is produced in excess, with $\alpha > 1/(2 - \gamma c_{MM})$, and otherwise weaker gametophytic selection evolves. With small modifier effects, the strength of gametophytic selection evolves toward the ESS:

$$c^* = \frac{2\alpha - 1}{\alpha\gamma}. \quad (7)$$

Thus, when females receive equal proportions of X- and Y-bearing pollen ($\alpha = 1/2$), they evolve to minimize selection among gametophytes ($c^* = 0$), keeping the sex ratio even. More generally, if we insert equation (7) into (5), the sex ratio among seeds at this ESS is $\psi^* = 1/2$, so that again the system evolves toward a 1:1 sex ratio among seeds. These calculations assume, however, that sex-ratio selection is the only factor influencing the evolution of gametophytic selection, an assumption relaxed in the next section.

INCORPORATING DELETERIOUS MUTATIONS INTO THE MODIFIER MODEL OF GAMETOPHYTIC SELECTION

The above results imply that females evolve to neutralize any process that perturbs the sex ratio among seeds from 1:1, a result consistent with Fisherian sex-ratio theory. This assumes, however, that there are no costs to doing so. In particular, altering the strength of gametophytic selection is likely to have major consequences for purging deleterious alleles from the genome, assuming that pollen with a high mutation load has low gametophytic fitness (Charlesworth and Charlesworth 1992). We thus seek to determine how sex-ratio selection and the benefits of purging together affect the evolution of modifiers controlling the strength of gametophytic selection.

We assume that all selected loci are loosely linked, autosomal, and nonepistatic so that we can ignore genetic associations among selected loci and between each selected locus and the sex-determining region. In this case, the strength of indirect selection acting on a modifier of weak effect can be approximated as the sum of indirect selective forces arising in models with the modifier locus plus each other locus, considered in turn. Specifically, invasion of a rare modifier depends on:

$$\lambda_{net} = 1 + \sum_l (\lambda_l - 1), \quad (8)$$

where $\lambda_l - 1$ measures the asymptotic strength of indirect selection acting on a rare modifier allele, m, due to interactions with locus l, once the system has approached the eigenvector associated with the leading eigenvalue (e.g., see Appendix in Otto and Bourguet 1999). In the previous section, we obtained λ_l, as given by equation 6, when locus l is the sex-determining gene. Here, we calculate λ_l for a selected locus, **A**, subject to recurrent

deleterious mutations, with mutation occurring from allele A to a at rate μ. From these calculations we obtain the net evolutionary force acting on a modifier, λ_{net}, and use this to predict the sex ratio and mutation load when the strength of gametophytic selection has reached the ESS.

Again, the modifier genotype of a female determines the strength of gametophytic selection, c_{ij} (see Table 1), with stronger selection reducing the frequency of the mutant allele among seeds (see recursions in Appendix B). Assuming that selection coefficients acting on allele a are small (but large relative to the inverse of the population size) and that the mutation rate is even smaller, the equilibrium frequency of allele a averaged across the sexes at the lth selected locus is:

$$\bar{q}_l = \frac{\mu}{(h^{\varphi} s^{\varphi} + h^{\sigma} s^{\sigma})/2 + (c_{MM} t^{\sigma})/2} \tag{9}$$

(to simplify the notation, we have suppressed the locus-specific subscript, l, on the selection coefficients, which are defined in Table 1). The difference in allele frequency between the sexes is of lower order and does not appreciably influence the spread of the modifier. Observe that equation (9) reduces to the classic mutation-selection balance, $\bar{q}_l = \mu/(hs)$, when gametophytic selection is absent ($t^{\sigma} = 0$) and selection is the same in both sexes ($s^{\sigma} = s^{\varphi}$). Recurrent deleterious mutations thus reduce the mean fitness in diploids of sex k by an amount $\approx 2h^k s^k \bar{q}_l$ (the "mutation load").

A new modifier allele, m, that alters the strength of selection among haploid pollen can then spread when $\lambda > 1$, where:

$$\lambda \approx 1 + \left\{ \frac{(c_{Mm} - c_{MM})\mu t^{\sigma} (2h^{\varphi} s^{\varphi} + 2h^{\sigma} s^{\sigma} + c_{MM} t^{\sigma})}{2(h^{\varphi} s^{\varphi} + h^{\sigma} s^{\sigma} + c_{MM} t^{\sigma})} \right\}. \tag{10}$$

As expected, a rare modifier experiences no indirect selection if there is no selection in the haploid phase ($t^{\sigma} = 0$), or no genetic variation at the **A** locus ($\mu = 0$), or no effect of the modifier ($c_{Mm} = c_{MM}$). Otherwise, when allele a is deleterious, modifier alleles invade if they increase the strength of gametophytic selection ($c_{Mm} > c_{MM}$), thereby purging mutations more efficiently from the male gametes involved in fertilization.

We now combine the indirect selection on a modifier that alters the strength of gametophytic selection arising from sex-ratio selection (eq. 6), and from each of L loci at mutation-selection balance (eq. 9). Overall, the leading eigenvalue describing the spread of a modifier is:

$$\lambda_{net} \approx 1 + (c_{Mm} - c_{MM}) \left\{ \frac{\gamma(2\alpha - 1 - \alpha\gamma c_{MM})}{4(1 - \gamma c_{MM})(1 - \alpha\gamma c_{MM})} + \frac{U t^{\sigma} (2h^{\varphi} s^{\varphi} + 2h^{\sigma} s^{\sigma} + c_{MM} t^{\sigma})}{2(h^{\varphi} s^{\varphi} + h^{\sigma} s^{\sigma} + c_{MM} t^{\sigma})} \right\}, \tag{11}$$

where $U = L\mu$ is the rate of deleterious mutations per haploid genome given L loci subject to selection in the haploid phase. Technically, the last fraction is calculated per locus and averaged over loci, but we simplify the presentation by assuming equal selection coefficients across loci. Modifiers increasing the strength of gametophytic selection ($c_{Mm} > c_{MM}$) spread when the term in braces is positive. The ESS level of gametophytic selection is thus obtained by setting this term to zero and solving for $c^* = c_{MM}$. As this is cubic in c^*, the solution is not presented but is instead manipulated numerically.

In Figure 2, we plot the ESS frequency of male seeds, ψ^* (obtained by inserting c^*into eq. 5), and the mutation load experienced by a diploid sporophyte (obtained by inserting c^* into eq. 9 and then $\bar{q}_l$ into the load). To calculate the genome-wide mutation load, we assume that the fitness effects of each locus are similar and independent, so that they multiply together to give an overall diploid fitness of:

$$\bar{W}^k_{(diploid)} = \prod_{l=1}^{L} (1 - 2h^k s^k \bar{q}_l) \approx e^{-4h^k s^k U/(h^{\varphi} s^{\varphi} + h^{\sigma} s^{\sigma} + c_{MM} t^{\sigma})} \tag{12}$$

for individuals of sex k. When the mutation rate U is high, the advantages of strengthening gametophytic selection through purging can be much greater than the disadvantages arising from skewed sex ratios, especially when the relative fitness of Y-bearing pollen is high (γ near 0). The ESS value of c^* predicted by equation 11 can then reach or even exceed its maximal value (recall that $c^* = 1$ is the maximal strength of gametophytic selection that can evolve, given other constraints on floral structure). In such cases, we set $c^* = 1$, assuming that these constraints are sufficiently strong to prevent higher levels of gametophytic selection from evolving (dashed curves in Fig. 2).

The equilibrium sex ratios in Figure 2 reflect a balance between selective pressures favoring the removal of deleterious mutations through gametophytic selection and the countervailing pressures of Fisherian sex-ratio selection. As expected, when either U or t^{σ} are zero, the sex ratio at equilibrium evolves toward 1:1, and this occurs regardless of the value of α or γ. Increasing the genome-wide deleterious mutation rate (U) and the strength of gametophytic selection (t^{σ}) cause an increase in the advantages of purging, leading to a more biased ESS sex ratio (Fig. 2, panel A) but a lower mutation load in the diploid phase compared to the load expected in the absence of gametophytic selection (Fig. 2, panel B). On the other hand, greater differences in the relative fitness of X- and Y-bearing sperm (γ) favor weaker gametophytic selection because of stronger Fisherian sex-ratio selection, which leads to a heavier burden of mutations among the diploid offspring (Fig. 2, panel B).

Discussion

Considering haploid selection on the sex chromosomes, we find that when sex-ratio adjustment is controlled by maternal genotype

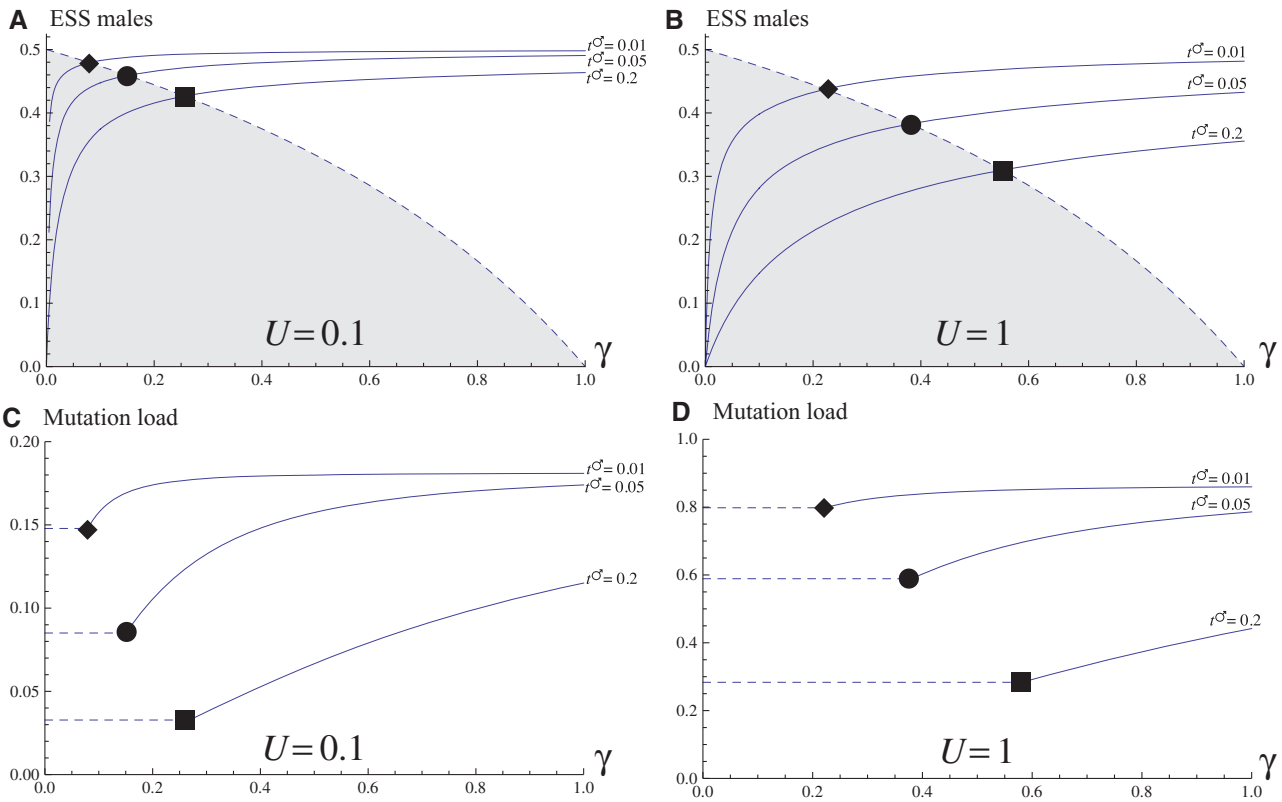

Figure 2. Evolutionarily stable sex ratios and mutation load. Panels A ($U = 0.1$) and B ($U = 1$) illustrate the evolutionarily stable sex ratio in the face of conflicting selection pressures, with Fisherian sex-ratio selection favoring no gametophytic selection and purging of deleterious mutations favoring the expansion of the gametophytic phase. Where the solid curves enter the shaded regions (at diamonds for $t^{\sigma} = 0.01$, circles for $t^{\sigma} = 0.05$, and squares for $t^{\sigma} = 0.2$), the ESS gametophytic selection becomes as strong as possible given other possible constraints ($c_{ij} = 1$), and the frequency of males is then constrained to the dashed curve. Panels C ($U = 0.1$) and D ($U = 1$) represent the mutation load in the sporophytic phase (one minus the mean fitness in diploids), assuming multiplicative selection and independent loci, with the dashed curves representing the load once gametophytic selection is maximized. Gametophytic selection can lead to substantial reductions in the mutation load, which would be 0.18 (with $U = 0.1$) and 0.86 (with $U = 1$) in the absence of gametophytic selection. On the other hand, as γ rises, the mutation load rises because sex-ratio selection becomes stronger and favors reduced gametophytic selection. Other parameters: $h^{\sigma} = h^{\varphi} = 0.1$, $s^{\sigma} = s^{\varphi} = 0.2$, $\alpha = 0.5$.

(Fig. 1), sex ratios at the end of parental care in plants (i.e., among seeds) should ultimately evolve toward 1:1, as predicted from Fisherian sex-ratio theory. Thus, the several proposed hypotheses for biased sex ratios in plant populations, such as the certation hypothesis or meiotic drive, represent proximate explanations and assume that the sex ratio either is not under maternal control or has not had time to reach an evolutionarily stable frequency. By contrast, when we also consider selection against deleterious mutations, we find that a biased sex ratio can be maintained at an evolutionarily stable equilibrium. This striking result arises because females are under conflicting evolutionary pressures: to reduce gametophytic selection within their styles to decrease the extent of sex-ratio bias in their offspring, but also to increase the intensity of gametophytic selection to purge deleterious mutations.

The extent of sex-ratio bias at the ESS depends on the strength of selection among male- and female-determining gametophytes and the rate at which deleterious mutations occur (Fig. 2). As expected, increasing the genome-wide mutation rate or the strength of selection resulted in a stronger sex-ratio bias at ESS due to the increased advantages of purging. Indeed, over much of the parameter space that we explored, gametophytic selection was so favorable because of purging that it evolved to its maximal possible strength ($c_{ij} = 1$; dashed curve in Fig. 2), despite the resulting skew in the offspring sex ratio (Fig. 2, panels A and B). Below we discuss the implications of these findings for understanding observed patterns of mutation load and sex-ratio bias in dioecious plants and, more generally, for the evolution of life cycles with extensive gene expression in both haploid and diploid phases.

MUTATION LOAD AND HAPLOID SELECTION IN PLANTS

Mutation load is known to have a large effect on fitness (Muller 1950; Crow 1970; Charlesworth and Charlesworth 1998; Agrawal

and Whitlock 2012) and has consequently been included in evolutionary explanations for a variety of phenomena, including ploidy level (Otto and Goldstein 1992), recombination and sex (Keightley and Otto 2006), mating-system evolution (Lande and Schemske 1985; Barrett and Charlesworth 1991; Charlesworth and Charlesworth 1999), and sexual selection (Whitlock and Agrawal 2009). Our models demonstrate that the benefits of reducing genome-wide deleterious mutation load through haploid selection can also influence the evolution of sex ratios for organisms with extensive overlap in gene expression between haploid and diploid phases of the life cycle.

Our models confirm that the effects of purging on the mutation load through haploid selection may be particularly important in plants, where widespread gene expression in the haploid stage has been demonstrated (e.g., up to 60% of expression overlap with the diploid stage, according to some studies; Mascarenhas 1990; Borg et al. 2009) and haploid selection appears to be widespread (e.g., Searcy and Mulcahy 1985; Sari-Gorla et al. 1989; Chibalina and Filatov 2011). In particular, our finding that gametophytic selection can evolve to be maximal in the presence of recurrent deleterious mutations, despite the fitness cost associated with biasing the sex ratio, suggests that purging may be an important factor contributing to the maintenance of the haploid phase in plants.

It is thought that the diploid sporophytic phase in plants has expanded over evolutionary time because diploids, which carry two copies of every gene, are able to mask deleterious recessive mutations, giving them an advantage over haploids (Valero et al. 1992; Orr 1995). However, an advantage to haploidy is that it enables purging of deleterious mutations (Otto and Marks 1996; reviewed in Mable and Otto 1998). To the extent that there is overlap in gene expression between haploid and diploid life cycle phases, the haploid phase may therefore act to screen against poorly functioning genomes, allowing only the most metabolically vigorous gametophytes to contribute genes to future generations (Mulcahy 1979). This is consistent with the finding that gametophytic selection can increase progeny fitness (e.g., Marshall et al. 2007). Thus, while flowering plant life cycles are physically and temporally dominated by the diploid phase, viewed from the perspective of selection, the haploid phase is also of fundamental importance, potentially more so than the diploid phase for some fraction of the genome.

IMPLICATIONS FOR UNDERSTANDING OBSERVED PATTERNS OF SEX-RATIO BIAS

Our results suggest that the benefits of selection against deleterious mutations during the haploid phase can also contribute to the maintenance of sex-ratio bias in dioecious plants, at least among species with male heterogamety whose X- and Y-bearing pollen differ in fitness. This finding is particularly relevant for species in which an association between pollination intensity and the degree of sex-ratio bias has been established, as this suggests that gametophytic selection may be involved in causing the bias (Correns 1928; Conn and Blum 1981; Stehlik and Barrett 2006; Field et al. 2012b). Previous suggestions that gametophytic selection can account for observed sex-ratio biases have not considered, however, that such bias would generate strong sex-ratio selection in females to equalize the representation of X- and Y-bearing gametophytes during fertilization. Our results confirm that sex ratios will tend toward 1:1 in the absence of opposing forces acting to maintain selection in the haploid phase. However, with recurrent deleterious mutations, our analysis (Model 3) indicates that strong gametophytic selection can be maintained, preventing the population from evolving a 1:1 sex ratio. Indeed, for realistic genome-wide mutation rates and gametophytic selection coefficients (e.g., $U = 0.1$, $t^{\sigma} = 0.2$), our analysis illustrates that the trade-off between gametophytic and sex-ratio selection results in patterns of bias similar to those observed in dioecious plant populations (Barrett et al. 2010; Field et al. 2012a).

A mechanism that may cause increased gametophytic selection and sex-ratio bias involves the suppression of recombination between sex-determining loci, which can lead to the accumulation of rearrangements, transposable elements, and deleterious mutations on Y chromosomes and hence to sex chromosome heteromorphism (Charlesworth et al. 2005). There is evidence that this has occurred in dioecious plants to varying degrees (Charlesworth 2012), and a recent comparative analysis reports an association between the possession of heteromorphic sex chromosomes and female-biased sex ratios in angiosperm species (Field et al. 2012a, and see Lloyd 1974). Indeed, to the extent that Y chromosome degeneration reduces the fitness of Y-bearing pollen relative to X-bearing pollen, then our model of gametophytic selection against deleterious mutations predicts this pattern. Testing the predictions of our model quantitatively should become increasingly possible as genomic studies provide markers to distinguish X- and Y-bearing pollen and improve our understanding of sex chromosome evolution in plants (Bergero and Charlesworth 2011; Chibalina and Filatov 2011).

In many species, however, sex ratios are male biased. Indeed, in the survey by Field et al. (2012a), 63% of the cases with sex ratios significantly different from 1:1 exhibited male-biased sex ratios. In some cases, male-biased sex ratios could result from Y-bearing pollen being positively selected in the gametophytic phase. This is expected early in the evolution of dioecy, before degeneration, because alleles that are favorable in the pollen of males, but disadvantageous to females or at other stages, can preferentially accumulate on the Y chromosome. That is, with sexually antagonistic and/or ploidally antagonistic selection, genes linked to the sex-determining region on the Y experience proportionately more selection in the male gametophytic stage and can thus accumulate alleles enhancing pollen fitness (Immler et al. 2011). Once

sex-linked markers become readily available in plants, future empirical studies comparing the growth rates of pollen bearing different sex chromosomes could confirm whether species with male-biased sex ratios have high Y-bearing pollen fitness.

Several caveats should be considered when comparing our models to empirical data on plant sex ratios. First, our models only consider the sex ratios of seeds, and very few studies have estimated these in natural populations (but see Taylor 1999; Stehlik and Barrett 2005). Instead, the vast majority of empirical work has focused on the sex ratios of reproductively mature plants, which are considerably easier to measure (Field et al. 2012a). Second, we have assumed that a large pool of pollen is available to each female, and we have not taken into account stochasticity in pollen dispersal and the consequences of pollen limitation for the parameters in our model. Finally, our models do not consider the possibility that males are under countervailing selection pressures to mask the deleterious mutations in the pollen they produce. Such masking occurs in animals, where gene expression in the sperm largely reflects the paternal diploid genome, with both homologous chromosomes contributing gene products to the haploid sperm (Joseph and Kirkpatrick 2004). It may, however, be that continuous protein synthesis for pollen-tube growth during the haploid phase constrains the extent to which male plants can evolve mechanisms to mask deleterious mutations in their haploid gametes.

Although many questions remain to be addressed, our study has demonstrated that incorporating selection against deleterious mutations in the haploid gametophyte phase provides a plausible evolutionary explanation for biased sex ratios in dioecious plants when X- and Y-bearing pollen differ in fitness and deleterious mutations are widespread. Future empirical studies aimed at estimating the strength and direction of gametophytic selection on sex chromosomes and the ways in which females might manipulate this selection would help strengthen our understanding of the proximate mechanisms causing sex-ratio bias and, perhaps more importantly, the ultimate causes of this variation.

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Appendix A

The following recursions describe the per generation change in the frequency of each modifier genotype i (i being MM, Mm, or mm) among female plants, F_i, and the frequency of each modifier allele j (j being M or m) among X- and Y-bearing pollen grains deposited on stigmas (p_{Xj} and p_{Yj}, respectively, where $p_{XM} + p_{Xm} = 1$ and $p_{YM} + p_{Ym} = 1$). We assume throughout that ample pollen is received on each stigma and ignore pollen limitation and stochastic sampling.

We first derive the frequency among all seeds (including both males and females) that inherited haplotype Xk from the ovule and Xj or Yj from the pollen (freq($XkXj$) or freq($XkYj$), respectively), where k and j represent the allele at the modifier locus (M or m). To account for transmission from a maternal parent of diploid genotype i at the modifier locus to an ovule of genotype k, we define $T_{i\to k}$, where, for example, $T_{MM\to M} = 1$, $T_{Mm\to M} = 0.5$, and $T_{mm\to M} = 0$.

MODEL 1: SEED PRODUCTION WITH EARLY-ACTING SEX-RATIO MODIFIER

Females adjust the pollen received so that a fraction m_i is Y-bearing. Gametophytic selection then occurs, followed by syngamy (Fig. 1). Immediately after fertilization, the frequency of each seed genotype is given by:

$$freq(XkXj) = \sum_{i\in\{MM,Mm,mm\}} F_i \left((1-c_i)\frac{(1-\alpha)p_{Xj}}{(1-\alpha)(p_{XM}+p_{Xm})}\right)\frac{1}{N_i}T_{i\to k}$$

$$freq(XkYj) = \sum_{i\in\{MM,Mm,mm\}} F_i \left(c_i \frac{\alpha p_{Yj}}{\alpha(p_{YM} + p_{Ym})} \right) \frac{(1-\gamma)}{N_i} T_{i\to k},$$

where

$$N_i = (1 - c_i) + (c_i)(1-\gamma)$$

is a normalization factor ensuring that the frequencies of pollen available for fertilization (after gametophytic selection) sum to one. Observe that α cancels out because females choose pollen bearing a particular sex chromosome.

MODEL 2: SEED PRODUCTION WITH LATE-ACTING SEX-RATIO MODIFIER

Females adjust the pollen received after gametophytic selection, choosing among the Y-bearing sperm that survive. Immediately after fertilization, the frequency of each seed genotype is:

$$freq(XkXj) = \sum_{i\in\{MM,Mm,mm\}} F_i(1-c_i)\frac{(1-\alpha)p_{Xj}}{N_{Xi}} T_{i\to k}$$

$$freq(XkYj) = \sum_{i\in\{MM,Mm,mm\}} F_i c_i \frac{\alpha(1-\gamma)p_{Yj}}{N_{Yi}} T_{i\to k},$$

where

$$N_{Xi} = (1-\alpha)(p_{XM} + p_{Xm})$$

$$N_{Yi} = \alpha(1-\gamma)(p_{YM} + p_{Ym})$$

are normalization constants that ensure that the frequency of pollen bearing an X or Y, respectively, each sum to one after gametophytic selection. Observe that α and γ both cancel out upon normalization, because females choose pollen bearing a particular sex chromosome after gametophytic selection.

MODEL 3: SEED PRODUCTION WITH MODIFIER OF GAMETOPHYTIC SELECTION

In this model, females alter the strength of gametophytic selection but do not directly choose the type of pollen grain used for fertilization. Immediately after fertilization, the frequency of each seed genotype is given by:

$$freq(XkXj) = \sum_{i\in\{MM,Mm,mm\}} F_i \frac{(1-\alpha)p_{Xj}}{N_i} T_{i\to k}$$

$$freq(XkYj) = \sum_{i\in\{MM,Mm,mm\}} F_i \frac{\alpha(1-\gamma c_i)p_{Yj}}{N_i} T_{i\to k},$$

where

$$N_i = (1-\alpha)(p_{XM} + p_{Xm}) + \alpha(1-\gamma c_i)(p_{YM} + p_{Ym})$$

normalizes the frequencies of pollen surviving gametophytic selection.

ALL MODELS: SPOROPHYTE AND POLLEN PRODUCTION

Assuming that the modifier does not directly affect survival, the frequency of each genotype among the adult females in the next generation becomes:

$$F'_{MM} = freq(XMXM)/(1-\psi)$$
$$F'_{Mm} = (freq(XMXm) + freq(XmXM))/(1-\psi),$$
$$F'_{mm} = freq(XmXm)/(1-\psi)$$

where $1-\psi$ equals the frequency of females among the seeds. To determine the frequency of the pollen haplotypes produced by fathers, we must account for recombination between the modifier and the hemizygous sex-determining locus:

$$\begin{aligned} p'_{XM} &= (freq(XMYM) + freq(XMYm)(1-r) \\ &\quad + freq(XmYM)r)/\psi \\ p'_{Xm} &= (freq(XmYm) + freq(XMYm)r \\ &\quad + freq(XmYM)(1-r))/\psi \\ p'_{YM} &= (freq(XMYM) + freq(XMYm)r \\ &\quad + freq(XmYM)(1-r))/\psi \\ p'_{Ym} &= (freq(XmYm) + freq(XMYm)(1-r) \\ &\quad + freq(XmYM)r)/\psi, \end{aligned}$$

where ψ equals the frequency of males among the seeds. Different survival rates for female and male sporophytes have not been explicitly included, but they would not affect the dynamics because each female or male seed would be multiplied by a sex-specific fitness, which would then cancel out when dividing by the total female frequency or the total male frequency after sporophytic selection.

Appendix B

The following recursions describe the change across a generation in the frequency of a modifier of the strength of gametophytic selection (like Model 3 above), but where the second locus is not the sex-determining region but a locus **A** at mutation-selection balance.

MODEL 3: INCORPORATING DELETERIOUS MUTATIONS IN A MODIFIER MODEL OF GAMETOPHYTIC SELECTION

In this model, we keep track of ovule and pollen haplotypes, o_{ij} and p_{kl}, respectively, where i and k denote the modifier allele whereas j and l denote the selected allele. Because the loci are now assumed autosomal, we do not separately track X- and Y-bearing pollen. In the recursions, we keep track of the maternal

genotype for each ovule because her genotype determines the strength of gametophytic selection experienced by the pollen. We do so by specifying the "type" of ovule, which denotes whether the ovule is carried by a homozygous (type = *hom*) or heterozygous (type = *het*) mother at the modifier locus. For example, $o_{MA,hom}$ represents the frequency of ovules with haplotype *MA* that are carried by homozygous mothers (which must be *MM*).

Females alter the strength of gametophytic selection but do not directly choose the type of pollen grain used for fertilization. After fertilization, sporophytic selection, and meiosis, the frequency of each haplotype, *g h* (*g* being *M* or *m*, *h* being *A* or *a*) among the ovules ($freq(gh)_{type} = o_{gh,type}$ produced by sporophytes of sex = *f*) or among the pollen grains ($freq(gh)_{hom\,or\,het} = p_{gh}$ produced by sporophytes of sex = *m*) is given by:

$$freq(gh)_{type} = \sum_{\substack{i,k\in\{M,m\}\\ j,l\in\{A,a\}}} \left(o_{ij,\ hom} \frac{(1-\delta_l t^{\male} c_{ii}) p_{kl}}{N_{ii}} + o_{ij,\ het} \frac{(1-\delta_l t^{\male} c_{Mm}) p_{kl}}{N_{Mm}} \right) \frac{W^{sex}_{ij,kl}}{\bar{W}^{sex}_{ij,kl}} T_{ij,kl\to gh,type},$$

where

$$N_{xy} = p_{MA} + p_{mA} + (1 - t^{\male} c_{xy})(p_{Ma} + p_{ma})$$

normalizes the pollen pool after gametophytic selection (*xy* is *MM* or *mm* in "hom" mothers when *i* = *M* or *m*, respectively, but always *Mm* in "het" mothers), δ_l is one if the pollen carries the *a* allele and zero otherwise, $W^{sex}_{ij,kl}$ represents the sporophytic fitness of the resulting diploid of a particular sex, and $\bar{W}^{sex}_{ij,kl}$ represents the mean diploid fitness of that sex. We assume that sex is determined elsewhere in the genome and track the frequencies of gametes within each sex. The transmission coefficient, $T_{ij,kl\to gh,type}$, now specifies the probability that a sporophyte produced from an ovule of haplotype *ij* and a pollen grain of haplotype *kl* produces a gamete of genotype *gh*, as well as the probability that the sporophyte was of the correct type (i.e., if type = *hom*, then *i* must equal *k* or else the transmission probability is zero). In addition to recombination at rate *R* between the **M** and **A** locus, the transmission coefficient also accounts for mutation at the **A** locus, with *A* mutating to *a* at rate μ (back mutation is assumed rare and is ignored). For example, $T_{MA,ma\to Ma,hom}$ is zero (the maternal sporophyte is *Mm* and not homozygous), whereas $T_{MA,ma\to Ma,het}$ is $(1-\mu)(R/2) + \mu/2$.