Reduced Nucleotide Diversity on a Plant Y Chromosome Following Recent Recombination Suppression

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ABSTRACT X and Y chromosomes differ in effective population size (N_e), rates of recombination, and exposure to natural selection, all of which can significantly affect levels of genetic variability within the genome. On Y chromosomes with suppressed recombination, selection can remove genetically-linked neutral varia tion, resulting in reduced N_e and the level of polymorphism on Y compared to X chromosomes or autosomes. However, non-selective factors including female biased sex ratios and high variance in male fitness can also reduce Y-linked N_e, making it difficult infer the causes of low Y-chromosome diversity. Here, we investigate the factors affecting X- and Y-linked polymorphism during the early stages of plant sex chromosome evolution in *Rumex hastatulus* (Polygonaceae). Strikingly, we find that nucleotide diversity on the Y chromosome is approximately 40 fold lower than on the X chromosome, suggesting an extensive and rapid loss of diversity. We demonstrate that the magnitude of this diversity loss is not consistent with a purely neutral model based on the occurrence of female biased sex ratios in this species, or by reduced male N_e caused by high variance in male fitness. Rather, using forward simulations, we show that observed Y diversity is consistent selection on deleterious mutations.

KEYWORDS Sex Chromosome Evolution; Nucleotide Diversity; Recombination; Deleterious Mutations

Introduction

Morphologically distinct sex chromosomes have evolved multiple times independently in both plant and animal kingdoms (Westergaard 1958; Ohno 1967; Bull 1983; Charlesworth 1991). Despite numerous biological differences between the kingdoms, X and Y chromosomes in both lineages have undergone similar genetic changes, suggesting that general evolutionary mechanisms might drive sex chromosome divergence (Charlesworth 1978, 1996b; Charlesworth and Charlesworth 2000). Investigating the shared features of plant and animal sex chromosomes, including the loss of recombination (Bergero and Charlesworth 2009), the degeneration of Y chromosomes (Hough *et al.* 2014; Bergero *et al.* 2015), and the evolution of dosage compensation (Muyle *et al.* 2012; Papadopulos *et al.* 2015), thus provides an informative context for investigating the evolutionary forces driving sex chromosome evolution.

One fundamental difference between sex chromosomes and autosomes is their respective copy number in a population; whereas autosomes in diploid species are present in two copies in each sex, the X chromosome is present in two copies in females and only one copy in males. As a consequence, the effective population size (N_e) of the X chromosome is predicted to be 3/4 that of autosomes, whereas the N_e of the male-limited Y chromosome is expected to equal 1/4 that of autosomes (assuming an equal number of reproducing females and males). Such differences in N_e are predicted to directly affect relative levels of neutral polymorphism maintained on these chromosomes, which is proportional to the product of N_e and the neutral mutation rate, μ (Kimura 1984). This variation in neutral polymorphism is in turn expected to have important consequences on patterns of DNA sequence evolution, including the effectiveness of both positive and negative selection (Charlesworth *et al.* 1987).

Several demographic factors can modulate or accentuate differences in polymorphism levels maintained on sex chromosomes (Ellegren 2009). These include population subdivision

and sex-biased dispersal, deviations from a 1:1 breeding sex ratio, and high variance in reproductive success (Caballero 1995; Charlesworth 2001; Laporte and Charlesworth 2002; Pool and Nielsen 2007). For example, studies comparing levels of variability on the X chromosome and autosomes in humans have found evidence that sex-biased dispersal has reduced levels of X-to-autosome diversity below the neutrally expected level of 0.75 (Keinan et al. 2009), though there is considerable variation in estimates of the X/A ratio in humans, and other demographic processes have also been suggested (Hammer et al. 2010; Bustamante and Ramachandran 2009). Theoretical work also indicates that population bottlenecks can lead to disproportionate decreases in sex-linked compared to autosomal variation (Pool and Nielsen 2007), a prediction that has been supported in a diverse array of taxa, including chimpanzees and orangutans (Kaessmann et al. 2001; Fischer et al. 2006), the house mouse *Mus musculus* (Baines and Harr 2007), Drosophila (Andolfatto 2001), and humans (Keinan et al. 2009).

In addition to demographic factors, evolutionary theory predicts that genetic variability on sex chromosomes should be affected by the evolution of suppressed X-Y recombination, which lowers the coalescent N_e for Y-linked genes and increases their vulnerability to the effects of selective sweeps (Maynard Smith and Haigh 1974; Aquadro et al. 1994) and background selection (Charlesworth 1996a, 1994). Moreover, both background selection and selective sweeps predict a reduction in N_e and, as a result, reduced levels of neutral polymorphism, selection efficacy, and rate of adaptation (Comeron et al. 2008). Although the such linked selective effects can occur throughout the genome (McVean and Charlesworth 2000), large genomic regions in which recombination is suppressed, such as the Y chromosome, are expected to experience the most severe reductions in neutral diversity due to chromosome-wide linkage (Charlesworth 1996b; Charlesworth and Charlesworth 2000; Bachtrog 2013). Consistent with this, studies examining Y chromosome variability in mammals (Hellborg and Ellegren 2004; Wilson Sayres et al. 2014) and Drosophila (McAllister and Charlesworth 1999; Bachtrog and Charlesworth 2000) have found that Y-linked diversity is considerably lower than predicted under models of neutral evolution, suggesting that the linked selection plays a key role determining patterns of nucleotide diversity during sex chromosome evolution across a broad range of animal taxa.

Despite widespread interest in determining the effects of recombination on patterns of genetic diversity (Ellegren 2011; Bachtrog 2013), we still know very little about the influence of linked selection on patterns nucleotide polymorphism in younger sex chromosome systems, where *de novo* recombination suppression evolved relatively recently (Charlesworth 2016). Recent studies of young plant sex chromosome systems have revealed that several canonical features of X and Y chromosome evolution are shared between plants and animals, including the genetic deterioration of the Y chromosome (Bergero et al. 2015; Hough et al. 2014), the occurrence of 'evolutionary strata' (Bergero and Charlesworth 2009), and the evolution of dosage compensation (Muyle et al. 2012; Papadopulos et al. 2015). Nonetheless, except for a small number of genes in *Silene latifolia* (Filatov et al. 2001; Qiu et al. 2010), the associated changes in genetic diversity that are predicted from evolutionary models of X- and Y-chromosome evolution have not been widely studied in plant sex chromosomes. Given that background selection and selective sweeps are predicted to have the strongest effects during the earliest stages of Y-chromosome evolution, before Y-chromosomes have lost the majority of their genes (Bachtrog 2008), studying recently evolved sex chromosomes provides an important test of the temporal dynamics of the linked selection.

To investigate the factors affecting nucleotide diversity in the early stages of sex chromosome evolution, we analyzed single nucleotide polymorphisms (SNPs) on sex chromosomes and autosomes in the plant *R. hastatulus* (Polygonaceae). This species is a dioecious annual with highly heteromorphic X and Y chromosomes that are estimated to have evolved approximately 15 MYA (Quesada del Bosque et al. 2011; Grabowska-Joachimiak et al. 2015; Navajas-Pérez et al. 2005), making sex chromosomes in this species over 100 million years younger than the well-studied mammalian sex chromosomes (Lahn and Page 1999; Ross et al. 2005). *Rumex hastatulus* has also received particular attention because of the unique occurrence of an intraspecific sex chromosome system, in which both XY and XY_1XY_2 males occur in geographically distinct 'chromosomal races' (Smith 1963). The XY_1XY_2 sex chromosome system in this species (the North Carolina race) is thought to have originated through an X-autosome fusion, with the XY system (the Texas race) maintaining the ancestral chromosome complement (Smith 1964). Despite the recent origin of sex chromosomes in both races, a recent study revealed that both the ancestral and neo-Y chromosomes have undergone significant gene loss and functional deterioration (Hough et al. 2014).

Of particular relevance to the present study, *R. hastatulus* populations have also been found to consistently exhibit female-biased sex ratios, with a mean sex ratio of $N_m/N_f = 0.6$ (Pickup and Barrett 2013). This is important because female-biased sex ratios are predicted to affect chromosomal N_e and therefore the level of neutral polymorphism maintained on sex chromosomes and autosomes (Ellegren 2009). Here, we test whether patterns of nucleotide diversity on sex chromosomes and autosomes in *R. hastatulus* are jointly consistent with neutral evolutionary processes - including the effects of ratio bias and variance in reproductive success - or whether the removal of linked neutral variation caused by positive and purifying selection has played a more significant role.

Materials and Methods

Population Samples and Sex-Linked Genes

We analyzed sex-linked and autosomal genes identified from Illumina RNA sequence data from 24 individuals (12 males and 12 females, with 1 male and 1 female from each of 12 populations; 6 populations of each sex chromosome race). Samples were collected in 2010 from throughout the native range of *R. hastatulus* (locations in Table S1), and plants were grown in the glasshouse from seeds collected from open-pollinated females. We extracted RNA from leaf tissue using Spectrum Plant Total RNA kits (Sigma-Aldrich). The isolation of mRNA and cDNA synthesis was conducted according to standard Illumina RNAseq procedures, with sequencing conducted on two Illumina HiSeq lanes with 150-bp end reads at the Genome Quebec Innovation Center. Reads from these 24 samples were mapped to the *R. hastatulus* reference transcriptome (Hough et al. 2014), and raw sequences are available from the Gen-Bank Short Read Archive under accession no. SRP041588. Reads were mapped using the Burrows–Wheeler Aligner (Li and Durbin 2010), followed by Stampy (Lunter and Goodson 2011). We used Picard tools (http://picard.sourceforge.net) to modify mapping output into the format required for the Genome Analysis Toolkit (McKenna et al. 2010) variant calling software, and subsequently

removed genes with low coverage (<10x) and Phred Quality Scores <20. The population samples analyzed here were previously reported in Hough et al. (2014), where they were used to validate the ascertainment of sex-linked genes identified through segregation analysis. Here, to consider sex linked genes that were identified in each of our sequenced population samples, we focused on the set of 460 X/Y genes for which the Y homolog was found in both the Texas and North Carolina races (i.e., X/Y genes where the Y copy was inferred to be on the Y_1 chromosome).

Autosomal Genes

In evaluating evidence for nucleotide diversity differences between X and Y chromosomes, it is important to distinguish between reduced Y-linked diversity, and the possibility that X-linked diversity is elevated above the level predicted from a neutral model. To do this, we normalized sex-linked diversity estimates by autosomal diversity, and compared empirical X/A and Y/A nucleotide diversity ratios to those predicted from neutral models and from simulations (described below). Because the criteria for identifying autosomal loci in Hough et al. (2014) were based on the occurrence of four segregating SNPs per locus, this set of genes is probably higher in diversity than the average autosome. Therefore, for the present analysis we incorporated the broader set of all non-sex linked (punitively autosomal) genes from our transcriptome data. We also filtered this set to remove genes that may have been sex-linked but were not identified as such by Hough et al.'s conservative ascertainment criteria. In particular, we removed: (i) any genes in which there was evidence for at least one SNP with a sex-linked segregation pattern, (ii) any genes with SNPs showing fixed heterozygosity in males and fixed homozygosity in females, (iii) any genes with less than 10X coverage or greater than 100X coverage from independently obtained genomic coverage data (to filter out duplicate genes or those with highly repetitive sequences), and (iv) any genes containing SNPs with large (>0.4) allele frequency differences between males and females. Finally, we removed genes with fewer than 100 synonymous sites following this filtering to avoid biasing our results toward genes that may have been particularly short due to assembly problems. This filtering resulted in a set of 12,356 and 11,350 autosomal genes in the Texas and North Carolina races, respectively.

Phasing X and Y alleles

To estimate polymorphism for the X and Y sequences separately, it is necessary to infer the phase of SNPs in sex-linked transcripts in males. In previous work, phasing alleles on *R. hastatulus* sex chromosomes was achieved using segregation analysis from a genetic cross. Here, to phase SNPs from population samples where such segregation data was unavailable, we used HAPCUT (Bansal and Bafna 2008), a maximum-cut based algorithm that reconstructs haplotypes using sequenced fragments (Illumina read data) from the two homologous chromosomes to output a list of phased haplotype blocks containing the SNP variants on each chromosome. Because the resulting haplotype blocks produced by HAPCUT contained SNPs that were phased relative to each other, but not designated to either the X or Y chromosome, we assigned individual variants to X or Y by independently identifying fixed X-Y differences with each haplotype block (i.e., sites in which all females were homozygous, and all males were heterozygous). Identifying such fixed differences within phased haplotype blocks enabled us to then infer the correct phase (X

or Y) of the polymorphisms from HAPCUT's output. This was done by matching the phase of fixed X-Y differences with their neighboring polymorphic sites: when a fixed X-Y difference occurred in the same phased haplotype block as a polymorphic site, the polymorphic variants in that block were assigned to either X or Y based on the known phase of the fixed difference with which they were matched. SNPs that were identified outside of phased blocks, or in blocks without fixed X-Y differences, were recorded as missing data. Finally, we filtered out SNPs with coverage > 60, QUAL score > 60, and those within a distance of 10bp or less from indels. This procedure was conducted using a combination of Perl and Bash scripts, and resulted in fasta-formatted alignments of X and Y sequences for 372 sex-linked genes from the 24 individuals in our study.

We further validated the results of HAPCUT's allele phasing by comparing the accuracy of this method with the phasing-by-segregation method that was conducted in Hough et al. (2014). To do this, we first phased the sequence data from parents and their progeny using HAPCUT's algorithm (using the same parameters as for the population data), and then identified cases where SNPs were inferred on the Y chromosome by HAPCUT, but where the 'true' level polymorphism was inferred to be zero by phasing-by-segregation. We identified 7 percent of sex-linked genes with phasing errors of this kind, or which were otherwise determined to have genotyping errors resulting in a false SNP calls. This corresponds to SNP error rate estimate of 1.7 x 10-4. Note that this rate is very low relative to population-based estimates of polymorphism on the X and autosomes (Table 1), and therefore should have minimal effects on our estimation of X/A polymorphism. However, because the rate is high relative to the expected level of true polymorphism on the Y-chromosome, we further filtered genes in which we found evidence for false-positive Y-polymorphisms arising from: (i) phasing errors caused by gene duplicates (more than two haplotypes), (ii) polymorphisms around indels, and (iii) genotyping errors caused by low Y-expression. This filtering was done by manually inspecting sequences in IGV (Robinson *et al.* 2011) and identifying each individual putative polymorphism on the Y chromosome.

Estimating nucleotide diversity on sex chromosomes and autosomes

For each locus in our analysis, we calculated Watterson's (1975) estimator of the population parameter $\theta = 4N_e\mu$, where N_e is the effective population size, and μ is the mutation rate (Watterson 1975), using a modified version of the Perl program Polymorphurama (Bachtrog and Andolfatto 2006). To compare sex-linked and autosomal loci, we calculated the average value of θ, weighted by the number of synonymous sites in each gene (**X** Figure 2; Table 1). We obtained 95 percent confidence intervals for our estimates of the X/A and Y/A diversity ratios by bootstrapping by gene using the BCa method (Efron and Tibshirani 1994) implemented in the Boot package in R (Canty and Ripley 2012), and calculating X/A and Y/A diversity on each iteration for 20000 replicates each. Bootstrapping was conducted on the final filtered set of 173 sex-linked, and 12355 autosomal genes from the Texas race, and separately for the 176 sex-linked and 11349 autosomal genes from the North Carolina race. Note that the lack of recombination on the Y chromosome implies that assumptions about independence across loci are likely violated, suggesting that the true uncertainty in our Y/A diversity estimate is likely an underestimate. To address this issue, we

used a maximum likelihood approach, implemented a modified version of the MLHKA software ([Wright and Charlesworth 2004](#)) to independently estimate a credibility interval for the Y/A ratio (Figure S1). We also tested whether estimates of diversity for the X chromosome calculated from phased sequences from females were consistent with estimates from phased sequences from males. As no significant difference was observed (**X** Figure? Table?), we report only results from females.

Finally, we tested for population substructure within and between the two sex chromosome races to control for the possibility that hidden substructure in our sampled populations could affect estimates of diversity. To do this, we conducted a phylogenetic analysis of sex-linked sequences from all populations in our study. The analysis was conducted on an alignment of the X- and Y-linked genes from *R. hastatulus*, with orthologous autosomal sequences from the non-dioecious but closely related outgroup species *R. bucephalophorus* used to root the tree (Figure S2). The phylogeny was inferred using the Neighbor-Joining method ([Saitou and Nei 1987](#)), with evolutionary distances computed using Maximum Composite Likelihood ([Tamura *et al.* 2011](#)). The inferred sequence phylogeny revealed strong support for Y-linked genes from the XY_1XY_2 ("North Carolina") race being paraphyletic (98% bootstrap support), with samples from two populations (hereafter the "SC sub-clade") forming a monophyletic group, and samples from Florida and Georgia (hereafter the "FL sub-clade") also forming a monophyletic group, that was more closely related to the XY ("Texas") race (Figure S2). In light of this evidence for significant population substructure, and to consider between population differences, we estimated X and Y diversity in each of the three sub-clades (SC, FL, TX) separately.

Neutral predictions and the effect of sex ratio bias on diversity
To test whether the levels of diversity we observed on X, Y and autosomal chromosomes could be explained by the occurrence of biased reproductive sex ratios or high variance in reproductive success, we compared our empirical diversity estimates to predictions from a neutral model that jointly considers these two effects. Here, following Nomura (2002), the neutrally-expected effective population size for autosomal loci is given by:

$$N_{eA} = \frac{4N_mN_f}{N_m(1 + C_f^2) + N_f(1 + C_m^2)} \quad (1)$$

where N_m and N_f are effective population sizes for males and females, respectively, and C_i^2 is the coefficient of variation in mating success for the *i*th sex, defined as the ratio of the standard deviation to the mean number of mates σ_i/μ_{di}. Similarly, for X and Y linked loci,

$$N_{eX} = \frac{9N_mN_f}{4N_m(1 + C_f^2) + 2N_f(1 + C_m^2)} \quad (2)$$

and

$$N_{eY} = \frac{N_m(1 + C_f^2)}{2} \quad (3)$$

With with no variation in mating success in either sex ($C_m^2 = C_f^2 = 0$), equations 1-3 reduce to the familiar expressions from ([Wright 1931](#)):

$$N_{eA} = \frac{4N_mN_f}{N_m + N_f} \quad (4)$$

$$N_{eX} = \frac{9N_mN_f}{4N_m + 2N_f} \quad (5)$$

$$N_{eY} = \frac{N_m}{2} \quad (6)$$

To consider the joint effects of biased sex ratios and variance in male reproductive success, neutrally-expected levels of diversity were calculated using equations 1-3, assuming no variance in female mating (e.g., $C_f^2 = 0$), and we considered a range of values for σ_m: 0 (no variance), 1/2 (Poisson/2) 1 (Poisson distribution of offspring), 2 (Poisson*2), and 3 (Poisson*3). Figure 1 shows the predicted effective population size of autosomes and sex chromosomes as a function of the sex ratio under this model. Using these predictions, we calculated the expected X/A and Y/A ratios of diversity for the estimated *R. hastatulus* sex ratio $N_m/N_f = 0.6$ ([Pickup and Barrett 2013](#)), assuming that the level of neutral polymorphism is given by the product of the mutation rate and the effective population size, $\theta = 4N_e\mu$ ([Watterson 1975](#)). Note that we considered variance in male reproductive success here because this is most likely to occur in wind pollinated plant like *R. hastatulus*, where males produce copious amounts of pollen and compete to fertilize uniovulate females (**X** ref).
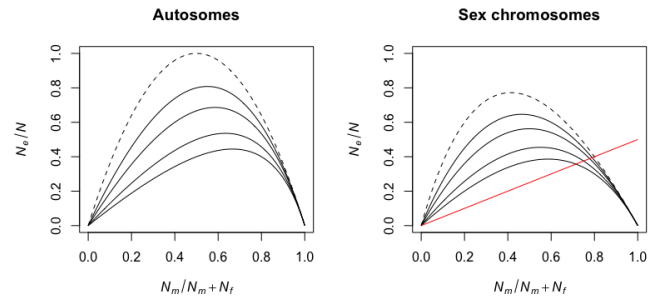

Figure 1 The relation between relative effective population size and sex ratio bias for genes on autosomes (**A**) and sex chromosomes (**B**) (Y chromosome in red). The sex ratio is shown as the proportion of males, $N_m/(N_f + N_m)$, where N_m and N_f are the effective number of breeding males and females, respectively, plotted against N_e/N, where $N = N_m + N_f$ and the N_e for sex chromosomes and autosomes is given in equations 4-6. Dotted curves show predictions in the standard neutral model, where both males and females produce Poisson-distributed offspring numbers and the chromosomal N_e's are given by equations 4-6 ([Wright 1931](#)). Solid curves correspond to increasing levels of variance in male reproductive success ([Nomura 2002](#)) (see Methods). Assuming $\theta = 4N_e\mu$ and equal neutral mutation rates among genes, the predicted N_e's are used to generate null predictions for X/A and Y/A ratios of diversity.

Simulations of positive and purifying selection
To test whether our observed level of Y-chromosome diversity could be explained by the effects of linked selection, we followed the approach used in ([Wilson Sayres *et al.* 2014](#)) and conducted forward-time simulations using the software SFSCODE ([Hernandez 2008](#)) and compared our empirical diversity estimates with those from simulations. This approach was preferred over using

Table 1 Estimates of neutral diversity by race on *R. hastatulus* sex chromosomes and autosomes.

chromosome	Texas θ	Texas θ/θ_A	South Carolina θ	South Carolina θ/θ_A	Florida θ	Florida θ/θ_A
A	0.006	1	0.006	1	0.005	1
X	0.0047	0.85	0.002	0.33	0.0047	0.37
Y	10^{-4}	0.002	10^{-4}	0.002	10^{-4}	0.002

analytical predictions primarily because the background selection model over-predicts the reduction in diversity when there are many linked sites under selection (Kaiser and Charlesworth 2009), as is expected to be the case for Y chromosomes that lack crossing over.

For purifying selection simulations, we assumed that selection coefficients followed a gamma distribution, and we considered a range of values for the mean selection coefficient against deleterious mutations, *s*, by varying the scale parameter from 0.0001 to 0.1 while keeping the distribution shape parameter constant (see Supporting Information for simulation commands). To make the simulation output comparable to our data, we initialized the simulations with our empirically estimated autosomal θ, adjusted for a sex ratio of $N_m/N_f = 0.4$, and we sampled 8 haploid chromosomes per simulation. Simulated sequences contained 500 kb of linked neutral sequence from which we calculated diversity.

For positive selection with recurrent hitchhiking, we used sfscoder to implement the method of (Uricchio and Hernandez 2014), and simulated a 500kb (**X**) neutral locus that was flanked by 100kb loci on either side that were subject to positive selection. We ran 1000 replicates for each model and calculated the average θ_{Ysim} values and the corresponding Y/A ratios for each model seperately. Finally, we tested the significance of the fit of the simulated diversity to our empirical Y/A ratio (**X** new figure) by calculating the proportion of simulation replicates for which θ_{Ysim} was not significantly different from $\theta_{Yobserved}$, using a two-sided P value. Commands for running the simulations are provided in the Supporting Information.

Results and Discussion

Y-chromosome diversity in R. hastatulus is very low

Our analysis revealed that diversity on the *R. hastatulus* Y chromosome is significantly lower than expected under neutrality, with estimates indicating Y/A=0.02, which is 12.5 fold lower than the standard neutral prediction of Y/A = 0.25 (P<0.0001), and 40-fold lower compared to mean diversity on the X chromosome (Table 1). Note that by normalizing X and Y diversity by autosomal diversity, our results indicate that the X-Y difference we observed was not due to an elevation of X chromosome diversity, but rather a Y-specific reduction. Conceivably, such low diversity on the Y could arise from a low mutation rate on the Y chromosome, or a lower mutation rate in males compared to females. However, these possibilities can be rejected because there is no evidence that the number of synonymous mutations in X and Y lineages, estimated by both parsimony and maximum likelihood, are significantly different (Hough *et al.* 2014).

Although our sampling of each *R. hastatulus* sub-clade is

limited, the discovery of three phylogenetically distinct mono-phyletic groups is interesting because it suggests the possibility that introgression occurred between the ancestral Texas (XY race) and the derived North Carolina (XY_1Y_2) race, leading to a derived XY_1Y_2 sub-clade. As the North Carolina and Texas races are known to be inter-fertile (Smith 1964), we suggest that the SC sub-clade inferred here likely originated through hybridization between a female from the FL clade harboring the X-autosome fusion, and a male from the XY Texas race. Notably, when we pooled the samples from each sub-clade together, the estimated level of Y diversity was significantly higher in the pooled data, highlighting the presence of strong substructure between these groups (Figure S2).

Our results also indicate a significant reduction in X/A diversity in the derived SC and FL sub-clades of the North Carolina race ($X/A_{FL} = 0.33$ and $X/A_{SC} = 0.37$) compared to the Texas race ($X/A_{TX} = 0.85$) (Figure 2). Although not expected, this reduction in diversity may be associated with the recent origin of the XY_1Y_2 sex chromosome system, which is thought to have originated through an X-autosome fusion involving the ancestral 3rd chromosome in the Texas race (Smith 1964). Evidence supporting this autosomal origin was recently obtained by (Grabowska-Joachimiak *et al.* 2015), who reported that the ancestral third chromosome in the Texas race carries the 5S rDNA locus, which is now found on both the neo-X and the Y_2 sex chromosomes in the derived North Carolina race. If recent positive selection was involved in driving the evolution of this X-A fusion, which theory suggests can driving the evolution of such fusions (Charlesworth and Charlesworth 1980), then the formerly autosomal segment on the X chromosome in the XY_1Y_2 sub-clades may have experienced a strong selective sweep, resulting in reduced X-linked diversity in the derived XY_1Y_2 sub-clades. It will be important for future work to investigate in more detail the factors driving the establishment of the X-autosome fusion in this species, and how they might impact patterns of X-linked neutral diversity.

In contrast to the X chromosome, however, our data indicate a strong and consistent diversity reduction on the Y chromosome: an approximately 40 fold reduction compared to the mean θ_X. We next consider several possible models - neutral and selective - that might explain this reduction.

Female biased sex ratio and high variance in male fitness

The occurrence of female-biased sex ratios in this species has been predicted to lower Y diversity due to its effects on reducing male N_e and therefore the neutrally expected N_e of the Y chromosome (**X** ref). This reduction N_e on the Y is expected to be further accented if there is high variance in male reproductive success (Figure 1), which is not unusual in annual plants such as *R. hastatulus* that commonly exhibit extensive phenotypic plasticity in plant size and flower production (**X** Harper 1977). Moreover, given that male plants in this wind-pollinated species produce large amounts of pollen, and that female flowers are uniovulate, we expected that there is strong competition among males to fertilize females.

In common with most flowering plants we do not have marker-based estimates of the variance in male reproductive success in *R. hastatulus*. However, by comparing our empirical estimates of diversity to predictions from models that jointly predict the effects on diversity of sex ratio bias and male reproductive variance, we evaluated whether these effects could explain the level of Y/A diversity that we observed (see Meth-

ods). Conditioning on estimates of sex ratio bias in *R. hastatulus* that have been estimated, ranging from $N_m/(N_m + N_f) = 0.4$ to $N_m/(N_m + N_f) = 0.35$ (Pickup and Barrett 2013), the predicted Y/A diversity ratio is approximately 0.2 (Table1, Figure 3). This is significantly lower than our estimated mean Y/A ratio of 0.02 ($P < 0.0001$), and remains significant even if we consider an upper bound estimate obtained from our likelihood based estimated of the confidence interval. This suggests that the sex ratio effect alone is insufficient to explain our data under the standard sex ratio model. (**X** I think some of this can be written better...)

Assuming that there is extensive variance in male reproductive success (**X**), the predicted ratios of Y/A diversity (**X** supp) were also significantly higher than our estimates for the empirically estimated sex ratio (**X** table) . We also find, however, that purely neutral models in which the sex ratio was highly female-biased (**X**), with level of variance in male reproductive success on the order of (**X**), predicted a Y/A ratio that could not be rejected (**X**). However, because a highly females biased sex ratio is expected to increase the X/A ratio as well, these models simultaneously predicted a range of X/A ratios that were significantly different from what we observed (**X** Figure). Thus, our results indicate that the combined effects of sex ratio bias and variance in reproductive success cannot jointly explain our observed levels of X, Y, and autosomal diversity.

Background Selection and Selective Sweeps

It is worth noting that our results only apply to the Y_1 chromosome, as estimates of diversity were calculated for sex-linked genes that were shared between the XX/XY and the XX/XY_1Y_2 sex chromosome systems. Previous work found that both the Y_1 and the more recently evolved "neo" Y_2 chromosomes exhibited signs of genetic degeneration, including gene loss, loss of expression, and an accumulation of amino acid-changing mutations (Hough *et al.* 2014), but we cannot say from the present study whether the neo-Y chromosome has also undergone a reduction in diversity. However, the extensive reduction in diversity estimated on the Y_1 chromosome occurs in each of the three *R. hastatulus* sub-clades (**X** Figure 2; Table 1; Figure S2), suggesting that this effect is not population-specific.

Conclusions

Acknowledgments

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