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, both positive and negative selection can remove genetically linked neutral variation, resulting in a reduction in N_e and the level of neutral polymorphism on Y compared to X chromosomes or autosomes. However, non-selective factors including 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, our analysis revealed that nucleotide diversity on the Y is approximately 12.5 fold lower than predicted from models of neutral evolution. We demonstrate that the magnitude of this reduction is unlikely to be explained by the observed 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 the diversity reduction on the Y is consistent with the predicted effects of background selection arising from suppressed recombination. Given the recent origin of *R. hastatulus* sex chromosomes, our results indicate that the low level of sequence diversity commonly observed on ancient Y chromosomes might evolve soon after sex chromosomes originate, with background selection playing an important role during the early stages of their evolution.

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 also 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). These processes can be viewed as examples of the Hill–Robertson (HR) effect, in which the effective population size for a given chromosomal region depends strongly on the rate of recombination, such that selection at a focal site is not independent of selection at nearby sites (Hill and Robertson 1966). 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 HR effect 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 HR effect 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 and the forces contributing to sex chromosome divergence (Ellegren 2011; Otto *et al.* 2011; Bachtrog 2013), we still know little about the influence of the HR effect on patterns nucleotide polymorphism in younger sex chromosome systems, where *de novo* recombination suppression evolved relatively recently (Charlesworth 2016). The timescales over which selection affects neutral polymor-phism on sex chromosomes is therefore not well understood.

Recent studies of young sex chromosome system in plants 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 young 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 plant sex chromosomes provides an important test of the temporal dynamics of the HR effect.

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). *R. hastatulus* has also received particular attention because of the unique occurrence of an intraspecific polymorphism for 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, there is evidence that both the ancestral and neo-Y chromosomes have undergone significant genetic degeneration (Hough *et al.* 2014).

Of particular relevance to the present study, *R. hastatulus* populations throughout their geographical range (southern USA) 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 genetic diversity on sex chromosomes and autosomes (Ellegren 2009). Here, we investigated the factors affecting nucleotide diversity on X, Y, and autosomal chromosomes. Our goal was to determine whether levels of diversity on these chromosomes were jointly consistent with predictions from models of sex ratio bias and variance in reproductive success, or whether linked selection has been the dominant factor affecting patterns of neutral polymorphism on sex chromosomes.

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 of

both sex chromosomal race 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 contigs identified through segregation analysis. From the list of sex-linked genes identified in Hough et al. (2014) that were shared between the Texas (XY) and North Carolina (XY1Y2) sex chromosome races, we identified a total of 460 sex-linked genes for analysis.

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 a neutral model (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. Here, we incorporated the broader set of all non-sex linked (punitively autosomal) genes from our transcriptome data. We filtered this set to remove those that may have been sex-linked but were not identified as such by the ascertainment criteria in Hough et al. (2014). 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 - i.e., 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 using data from a genetic cross 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). In particular, we looked for 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 that were determined to have genotyping errors resulting in a false SNP calls on the Y chromosome, with a SNP error rate estimate of 1.7 x 10-4. This rate is very low relative to the 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 this 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 polymorphism levels 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 of independent loci are likely violated such that the true uncertainty in our Y/A diversity estimate obtained by this method is likely underestimated. To address this issue, we used a maximum likelihood approach, implemented in the software (Wright and Charlesworth 2004), to independently estimate a credibility interval for the Y/A ratio (see 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 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 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 the Y-linked genes from the XY_1XY_2 ("North Carolina") race being paraphyletic (98 percent 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, but more closely related to the XY (Texas) race. In light of this evidence for substructure among our samples, we used the inferred phylogeny (Figure S2) as the basis for our estimates of X and Y diversity.

Neutral predictions and the effect of sex ratio bias on diversity

To test whether the levels of neutral 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 estimates of diversity to predictions from a neutral model that jointly considers these two effects. Here, following Nomura (2002), the effective population sizes for autosomal loci are given by:

$$N_{eA} = \frac{4N_m N_f}{N_m(1 + C_{df}^2) + N_f(1 + C_{dm}^2)} \qquad (1)$$

where N_m and N_f are effective population sizes for males and females, respectively, and C_{di}^2 is the coefficient of variation in mating success for the ith sex. Similarly, for X loci,

$$N_{eX} = \frac{9N_m N_f}{4N_m(1 + C_{df}^2) + 2N_f(1 + C_{dm}^2)} \qquad (2)$$

and

$$N_{eY} = \frac{N_m(1 + C_{df}^2)}{2} \qquad (3)$$

Such that with no variation in mating success for males or females ($C_{dm}^2 = C_{df}^2 = 0$), equations 2 and 3, respectively, reduce to the familiar expressions from (Wright 1931):

$$N_{eX} = \frac{9N_m N_f}{4N_m + 2N_f}, \qquad (4)$$

and

$$N_{eY} = \frac{N_m}{2} \qquad (5)$$

To consider the joint effects of biased sex ratios and variance in male reproductive success (which is more likely in a wind-pollinated annual), our neutrally-expected X/A and Y/A levels of diversity were calculated assuming no variance in female mating (e.g., $C_{df}^2 = 0$) and we considered values for C_{dm}^2: 0 (no variance), 1 (Poisson distribution of offspring), 2 (Poisson*2), and 3 (Poisson*3). With the level of neutral polymorphism in a population equalling the product of the mutation rate and the effective population size, $\theta = 4N_e\mu$ (Watterson 1975; **?**), Figure 1 shows the predicted X/A and Y/A diversity ratios as a function of the sex ratio and variance in male reproductive success (assuming equal mutation rates among genes).

Given a female effective population size N_f, and a male effective population size N_m, the predicted effective population size for X-linked genes, relative to autosomes, is given by $N_{e_X}/N_{e_A} = 9(N_f + N_m)/8(2N_f + N_m)$ (Wright 1931). Similarly, for the Y chromosome, the effective population size is given by 1/4 the total N_e or 1/2 the N_e of males, $N_m/2$. Thus, with equal sex ratios, $N_{e_X}/N_{e_A} = 0.75$, and $N_{e_Y}/N_{e_A} = 0.25$. For populations with female-biased sex ratios, however, N_m becomes small relative to N_f and this ratio can become larger than 1, approaching 1.125 at the limit (Caballero 1995). With the level of nucleotide polymorphism maintained in a population is proportional to the product of the mutation rate and the effective population size: $\theta = 4N_e\mu$ (Watterson 1975; **?**). Figure 1 shows the predicted effective population size ratios for autosomal, X-linked, and Y-linked genes (left) and the corresponding X/A and Y/A ratios of diversity (right) for a sex ratio bias ranging from $N_f/(N_f + N_m) = 0$ to $N_f/(N_f + N_m) = 1$.

Simulations of Background Selection and Selective Sweeps

To investigate whether our observed level of Y-chromosome diversity could be explained by background selection, we conducted forward time simulations.....We modeled both purifying and positive selection using the forward simulation software SFSCODE (Hernandez 2008). To make the simulation output more comparable to our data, we used estimates of θ from our polymorphism data as a simulation input parameter, and sampled 8 Y chromosomes per simulation from 24 populations to match out empirical data. To consider the effects of selection against deleterious alleles, we sampled selective coefficients from a gamma distribution with shape parameter 0.1, mean selection coefficient $s = -0.01$, neutral mutation rate $\mu = 0.005$, deleterious mutation rate $U = 0.01$ (diploid), and dominance term $h = 1$. We then
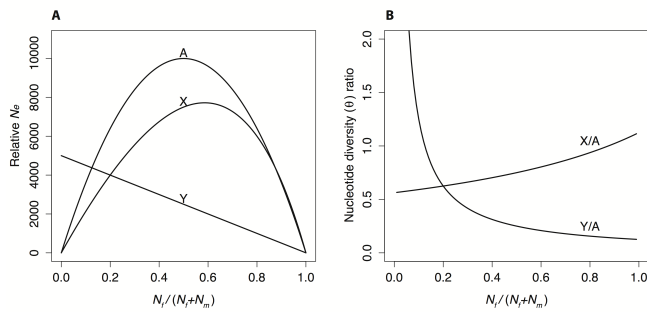
Figure 1 A: The relation between relative effective population size and sex ratio bias for genes on autosomes (A), X chromosomes (X), and Y chromosomes (Y). The sex ratio is shown as the proportion of females, $N_f/(N_f + N_m) = 0$, ranging from 0 to 1, where N_m and N_f are the effective number of breeding males and females, respectively. Calculations assume a constant population size, non-overlapping generations, and a Poisson distribution of offspring number (Wright 1931; Charlesworth 2009). The N_e for each chromosome type is calculated relative to the predicted N_e for genes on autosomes with an equal sex ratio. **B:** The corresponding expected relation between sex ratio bias and the sex-chromosome-to-autosome diversity ratio, assuming $\theta = 4N_e\mu$ and equal neutral mutation rates for all genes.

calculated the average θ over all simulations, and obtained the Y/A ratio of neutral diversity under the model and compared this to our empirical data (**X** new figure). Confidence intervals were calculated from bootstrapping 10000 replicate simulations using the BCa method (Efron 1987) implemented in the Boot package in R (Canty and Ripley 2012; R Core Team 2011).

$$S_n = \frac{X_1 + X_2 + \cdots + X_n}{n} = \frac{1}{n}\sum_i^n X_i \qquad (6)$$

Results and Discussion

Evolutionary relationships of sex chromosome races in R. hastatulus

Male-to-female sex ratio of N_e

Background Selection and Selective Sweeps

Conclusions

Acknowledgments

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