Supplemental Information

Supplemental Table Legends

Table S1. Sequence capture probe locations relative to the *S. purpurea* genome V1.1 This array is available from Arbor Biosciences (Ref#170623-30).

Table S2. Primers targeting genes in the sex-linked region of *Salix nigra*.

Table S3. Read depths for filtered BAM files (on-target) and filtered VCF (whole genome).

Table S4. All SNPs on Chr7, Sc197, and sc257 with associations with sex greater than on any other scaffold (highest on other chromosomes was Chr8:6232700, log10P=6.42). SNPs with Bonferroni-adjusted $P_{FDR} > 0.05$ (= $\log_{10} P_{FDR} > 7.9$) are noted in column 2.

Table S5. Patterns of heterozygosity for males and females for SNPs with significant sex association on chromosome 7.

Table S6. Results of amplicon sequencing of 16 male and 16 female *S. nigra* trees from New York and West Virginia. Results are shown only for loci that had a minor allele frequency > 0.2.

Table S7. Genes between 4.88MB and 6.88 MB on chromosome 7 and on scaffold 197 and 257 in the S. purpurea genome based on homology with the *S. purpurea* V5 genome. This region is the location of the non-recombining sex-linked region in *Salix nigra*. **= Genes with at least one SNP with Bonferroni-adjusted $P_{FDR} < 0.05$; *= genes with at least one SNP with sex associations greater than on any chromosome except chr7, sc197, and sc257 (but which were not statistically significant). The probes were designed based on the *S. purpurea* V1.1 genome, for which gene names are not always translatable to the V5.1 genome, which was used as a reference for mapping the sequence capture probes. Thus, the presences of probes for each gene in the v5.1 genome was based on the presence of a V5.1 synonym in the Spurpurea_519_v5.1.synonym.txt file prepared by JGI and/or the successful hit after blasting the probe sequences onto the *S. purpurea* V5.1 transcripts.

Table S8. Genotypes of all individuals for the 38 significant SNPs on Chromosome 7. A) All genotype calls. 0 = homozygote reference allele, 1 = heterozygote, 2 = homozygote alternate allele, -1 = not called. Because position 6

122 911 had 3 alleles, exact genotypes were called. B) sums across heterozygotes and homozygotes.

Supplemental Figures

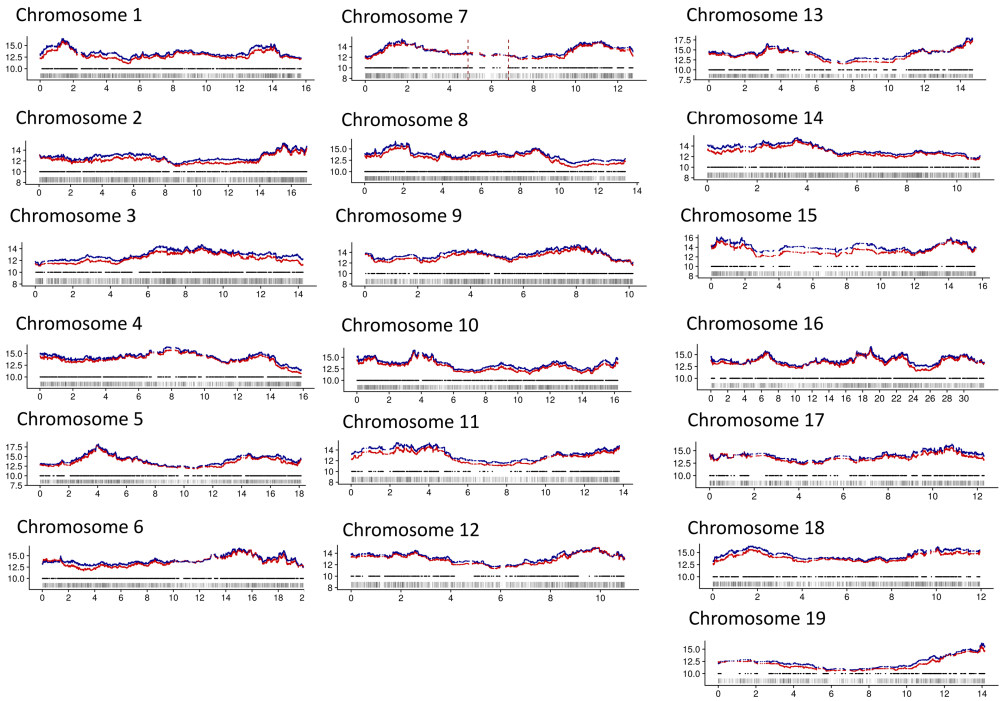

Figure S1. Sequence capture probes and sequencing depth across the 19 chromosomes of Salix nigra. Sequencing reads were mapped to the *Salix purpurea* v5.1 genome. The bottom dashes indicate locations for all genes from the *S. purpurea* v5 annotation. The middle row of black dots are the locations where the sequence capture probes map onto genome using bwa mem. The blue (male) and red (female) dots at the top represent mean read depths for male and female libraries respectively. Depths are plotted as means over 5000 bp windows for all individuals within each sex. Vertical dashed lines on chr7 indicate the proposed location of the SLR. Note that the Y-axis depth values are only meaningful for the blue and red mean depth lines.

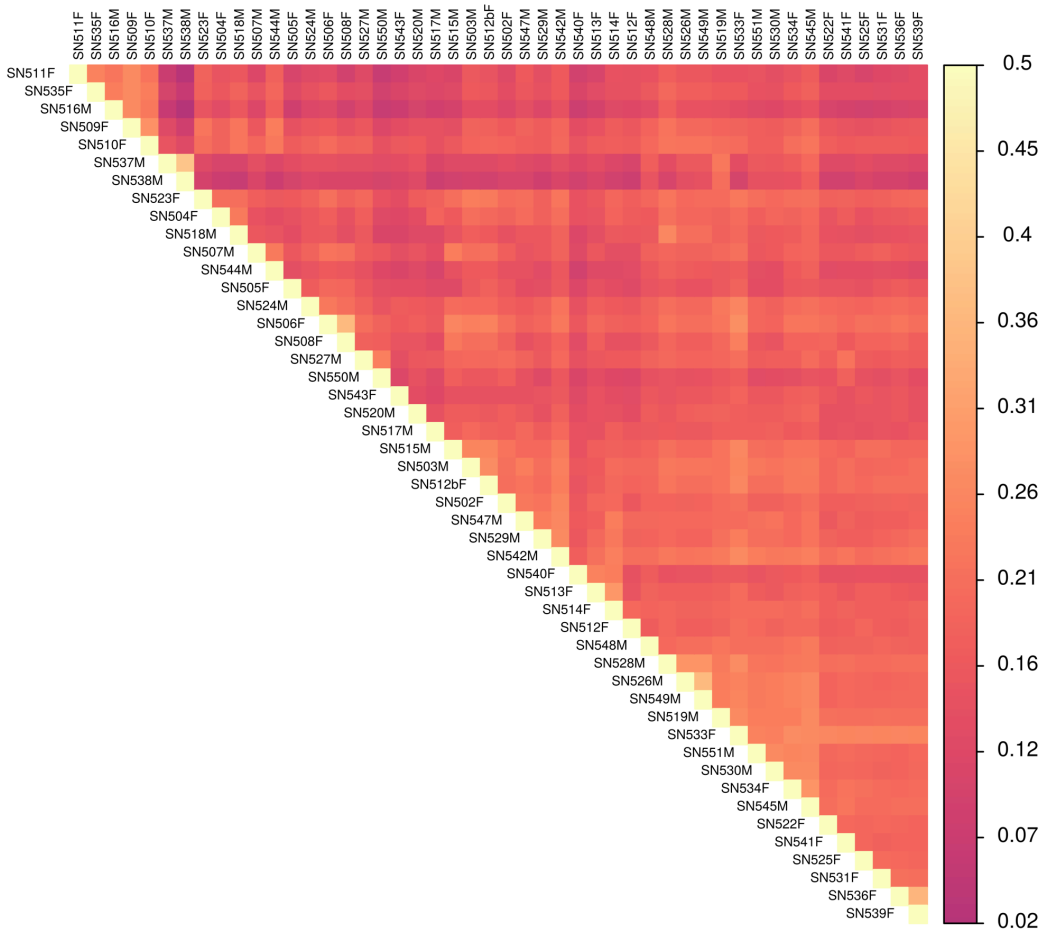

Figure S2. Pairwise kinship estimates between males and female *Salix nigra* in this study. Kinship was estimated using the relatedness2 algorithm in vcftools, which applies the methods used in KING (Manuchaikul et al. 2010).

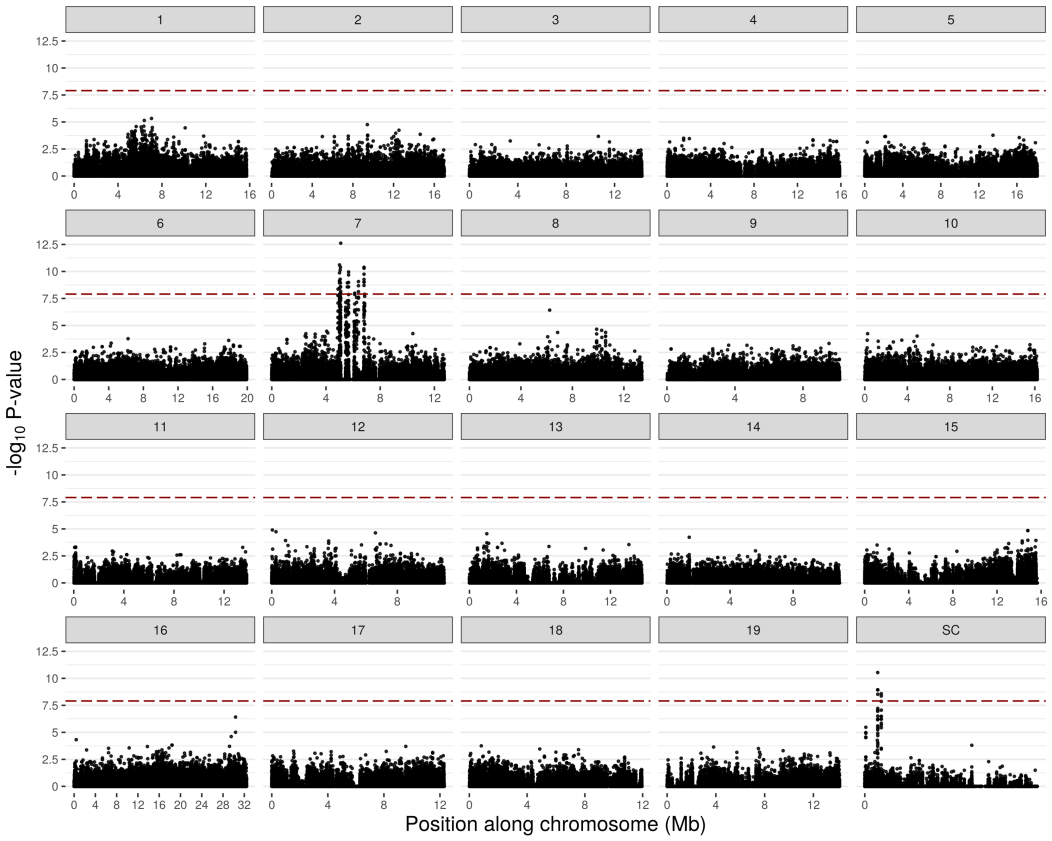

Figure S3. Genome wide associations with sex for 24 male and 24 female *Salix nigra* individuals, expressed in terms of $-\log_{10}$ *P*-values plotted for each chromosome. The relative positions of each SNP on each chromosome (numbers in grey titles above each panel) and scaffold (SC) as mapped against the *S. purpurea* genome are shown on the x-axis. The red dashed line indicates a Bonferroni-adjusted $P_{FDR} = 0.05$ ($-\log_{10} P_{FDR} = 7.91$).

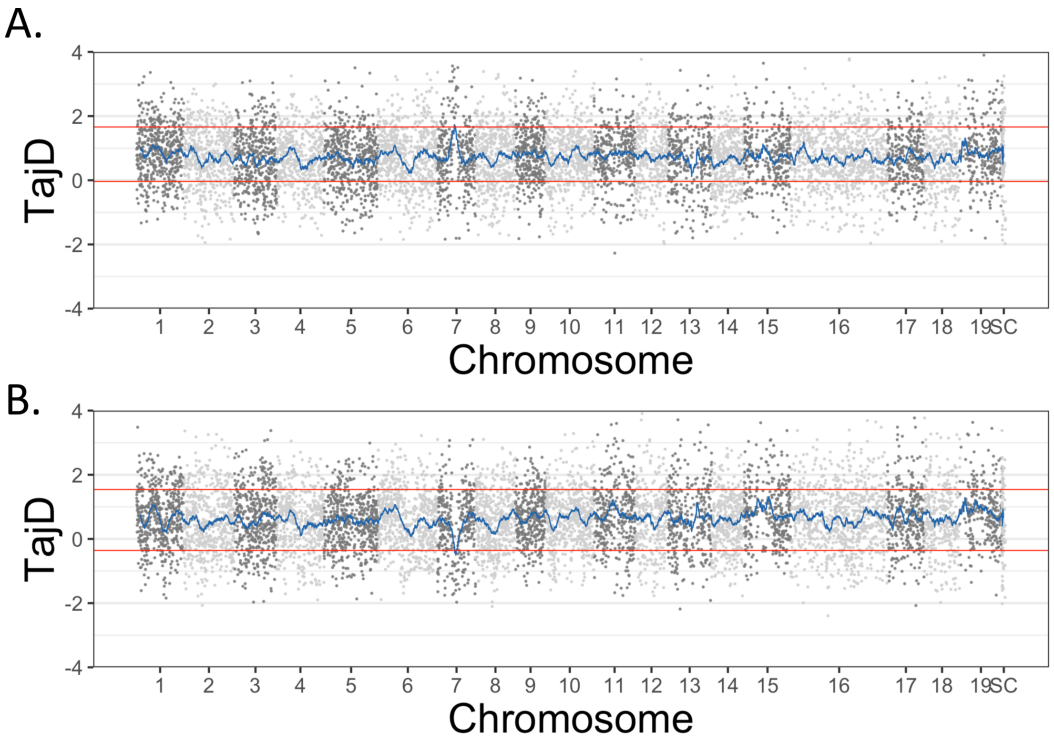

Figure S4. Patterns of Tajima's D (TajD) across the *Salix nigra* genome. A) Tajima's D for males only. Dots represent values of Tajima's D within 25kb across the genome each of the 19 chromosomes and unplaced scaffolds (SC). The blue line is the rolling mean of 22 25kb windows, the approximate size of the non-recombining sex-linked region (SLR). Red horizontal lines indicate the 99% and 1% quantiles of the Tajima's D calculated from non-overlapping 25kb windows across the genome including all of chr7. B) Tajima's D for females only.