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Genome structure and emerging evidence of an incipient sex chromosome in *Populus*

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The genus *Populus* consists of dioecious woody species with largely unknown genetic mechanisms for gender determination. We have discovered genetic and genomic features in the peritelomeric region of chromosome XIX that suggest this region of the *Populus* genome is in the process of developing characteristics of a sex chromosome. We have identified a gender-associated locus that consistently maps to this region. Furthermore, comparison of genetic maps across multiple *Populus* families reveals consistently distorted segregation within this region. We have intensively characterized this region using an F_1 interspecific cross involving the female genotype that was used for genome sequencing. This region shows suppressed recombination and high divergence between the alternate haplotypes, as revealed by dense map-based genome assembly using microsatellite markers. The suppressed recombination, distorted segregation, and haplotype divergence were observed only for the maternal parent in this cross. Furthermore, the progeny of this cross showed a strongly male-biased sex ratio, in agreement with Haldane's rule that postulates that the heterogametic sex is more likely to be absent, rare, or sterile in interspecific crosses. Together, these results support the role of chromosome XIX in sex determination and suggest that sex determination in *Populus* occurs through a ZW system in which the female is the heterogametic gender.

[Supplemental material is available online at www.genome.org.]

The genus *Populus* is composed of dioecious woody plants that arose during a period of global cooling in the late Miocene (5–10 million years ago [Mya]) (Schoell et al. 1994; Shevenell et al. 2004) and consists of six subgenera—*Abaso*, *Leuce*, *Leucoides*, *Aigeiros*, *Turanga*, and *Tacamahaca* (Eckenwalder 1996). Cytological studies reveal that all *Populus* exist in the diploid form with a haploid number of chromosomes equal to 19, although triploid and tetraploid individuals are commonly observed as well (Smith 1943; Bradshaw and Stettler 1993). The chromosomes of *Populus* are metacentric, small, and of uniform size (Blackburn and Harrison 1924; Meurman 1925; Erlanson and Hermann 1927; Nakajima 1937). Moreover, there have been no definite sex chromosomes found in any *Populus* species (Peto 1938; van Buijtenen and Einspahr 1959).

Mechanisms of sex determination in plants are extremely heterogeneous, ranging from XY systems common in many animal species to completely autosomal determination of gender (Ainsworth 2000; Ming et al. 2007). There is evidence that sex chromosomes originate from autosomes, and dioecy almost certainly evolves from ancestral hermaphrodites that lacked sex chromosomes (Muller 1914; Liu et al. 2004). Although *Populus* trees are normally dioecious, they show evidence of labile sex expression (Stettler 1971; Rowland et al. 2002). The observed sex ratios in certain genetic backgrounds of *Salix viminalis*, a close dioecious relative of *Populus*, suggest a multilocus epistatic model of gender determination (Alstrom-Rapaport et al. 1998). However, the genetic mechanism for gender determination in *Populus* remains largely unknown (McLetchie and Tuskan 1994).

There has been considerable interest recently in the evolution of sex chromosomes, and major insights have emerged from genomic investigations of sex chromosomes in model organisms in recent years, including several dioecious plants (for review, see Charlesworth et al. 2005; Ming and Moore 2007). One salient characteristic of all sex chromosomes described thus far is suppressed recombination, which is seen as essential to the evolution of separate sexes from hermaphroditic organisms when multiple loci are involved in gender determination (Nei 1969; Charlesworth et al. 2005). Furthermore, following suppression of recombination, the chromosomes will begin to diverge in the gender determination region, and one chromosome (the Y in heteromorphic males or the W in heteromorphic females) tends to degrade due to the accumulation of deleterious mutations and large-scale rearrangements and insertions (Charlesworth et al. 2005; Nicolas et al. 2005). Therefore, incipient sex chromosomes should show regions of suppressed recombination with high levels of haplotype divergence.

Due to its experimental tractability and abundant genetic and genomic resources, the genus *Populus* is widely used in basic

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scientific investigations geared toward perennial woody plant biology, biofuels production, carbon sequestration, and production of forest products (Tuskan et al. 2004a). *Populus* genomic resources have grown rapidly in recent years, culminating in the recent release of the whole-genome sequence database (Tuskan et al. 2006). As a sequenced dioecious plant, the genus *Populus* provides an excellent model and a rare opportunity to study chromosomal-scale evolution, including the transition of autosomes into sex chromosomes. However, to allow inferences about genome structure and chromosomal evolution, multiple genomes from closely related species must be examined. The main objectives of this article are to (1) elucidate the genetic mechanism for gender determination in *Populus* and (2) determine whether there is a sex chromosome in *Populus*. To achieve these goals, we established a comprehensive consensus genetic map and conducted extensive map comparisons among several pedigrees, whose parents involve alternate species and the sequenced genotype "Nisqually-1."

Results

Map construction, chromosome-level assembly, and map comparison

A comprehensive consensus genetic map containing 1138 markers was established for Family 13 and Family 331 (Fig. 1). The overall average genetic length between markers was 2.1 cM, and the average physical length between the mapped simple sequence repeats (SSRs) was <1 Mb. This map achieved inclusive coverage of the entire 480-Mb *Populus* genome with linkage groups corresponding to the 19 haploid chromosomes. Based on the mapped primer sequence tags, 162 large sequence scaffolds representing a physical distance of 385 Mb were mapped unambiguously along the 19 *Populus* chromosomes. The vast majority (91%) of the mapped microsatellite markers were colinear with the sequence assembly, indicating that both the genetic map and sequence scaffolds achieved high fidelity. There was a high degree of marker colinearity among the genetic maps built for alternate pedigrees (Supplemental Figs. 2, 3). However, there were some discrepancies in marker order observed among different genetic maps. Scaffold assembly was verified by referencing the marker order derived in Family 545, whose maternal genotype is Nisqually-1. For example, there is a marker, P_2861, in Family 545 on chromosome IX that mapped in divergent order on the consensus map. This marker initially caused a discrepancy in the scaffold assembly for chromosome IX; yet, its position on the Nisqually-1 genetic map was identical to that revealed in the sequence assembly. The alternate position of this marker suggests that there is a chromosomal rearrangement event on chromosome IX in the maternal parent of Family 13 relative to Nisqually-1.

Consistent segregation distortion and mapping of the gender determination locus

Based on a genome-wide clustering test for SSR position by chromosome, the average density of SSR was relatively uniform across the *Populus* genome. However, SSR frequency was significantly higher on chromosomes II and X and significantly lower on chromosome XIX. Thirty-five SSR markers were mapped to the 44.5-cM (~5.1-Mb) peritelomeric portion of chromosome XIX. In contrast, in the distal portion of chromosome XIX, only four SSR markers were mapped when 21 were expected by chance alone (based on a Poisson distribution with $P[b_{ij} \geq \lambda_{ij}] = 0.0005$). However, based on the Nisqually-1 assembly, SSRs are distributed relatively evenly across chromosome XIX; no substantial SSR clusters or deserts were observed (Supplemental Fig. 4). Together, these findings suggested that physical distance in the peritelomeric portion of chromosome XIX is larger than what the genetic distance indicates and that recombination is suppressed in this region.

Map comparisons among the different mapping pedigrees demonstrated high marker colinearity on chromosome XIX (Supplemental Figs. 2, 3). However, a striking feature occurred consistently among the examined genomes, i.e., a region of segregation distortion extending over very large portions of chromosome XIX within and among the interspecific mapping pedigrees. Sequence annotation revealed that multiple members of a nucleotide-binding site/leucine rich repeat (*NBS-LRR*) gene family occur in the peritelomeric region of chromosome XIX. There are 192 members of this gene family in the mapped scaffolds of Nisqually-1, of which 47 are located in a 2.45-Mb segment (composed of 1.77 Mb of assembled reads and 0.68 Mb of captured gaps) occurring at the peritelomeric region on chromosome XIX (Fig. 2). This region contains significantly more *NBS-LRR* genes than expected by chance alone ($P[b_{ij} \geq \lambda_{ij}] \simeq 0.0$ based on genome-wide average *NBS-LRR* distribution within a random 2.45-Mb region).

Utilizing the consensus map and a 14-yr-old, flowering *Populus deltoides* × *Populus nigra* BC_1 pedigree (Supplemental Fig. 1), we colocated SSR markers and a locus associated with gender determination to the peritelomeric region of chromosome XIX. This pedigree also showed a significantly skewed gender ratio in favor of male progeny (197 males vs. 115 females). Sequence annotations for the assembled scaffolds mapped on chromosome XIX revealed no obvious candidate genes for gender determination.

Recombination suppression revealed by fine-scale mapping

As mentioned above, consistent segregation distortion occurs across the peritelomeric region of chromosome XIX, a region that contains a higher than expected SSR frequency, an overabundance of *NBS-LRR* genes, and a gender-associated locus. From these observations, we hypothesized that chromosome XIX is an incipient sex chromosome. To resolve the issue of suppressed recombination and to lend support to the hypothesis that chromosome XIX is evolving into a sex chromosome, we created a new integrated assembly for scaffold 117, a single haplotype of chromosome XIX, which was not included in the original map-based assembly (Table 1; Fig. 3; Supplemental Fig. 5). By using Family 545, scaffold 117 was mapped to the peritelomeric region of chromosome XIX using 27 microsatellite markers. However, based on gene content comparisons, we detected substantial divergence between the alternate haplotypes in this region. There were 107 genes on scaffold 117, of which only 30 were shared with chromosome XIX. Strikingly, 26 of these are *NBS-LRR* genes.

Fine-scale mapping for scaffold 117 within Family 545 indicated that there is no recombination within the upper 706-kb region of chromosome XIX (Fig. 3). In contrast, the lower portion (a 257-kb region) included (1) several recombination positions, (2) significant segregation distortion ($\chi^2 \geq 58.3$) for alleles located on the maternal haplotypes, and (3) no distortion within the paternal haplotypes. Moreover, recombination suppression

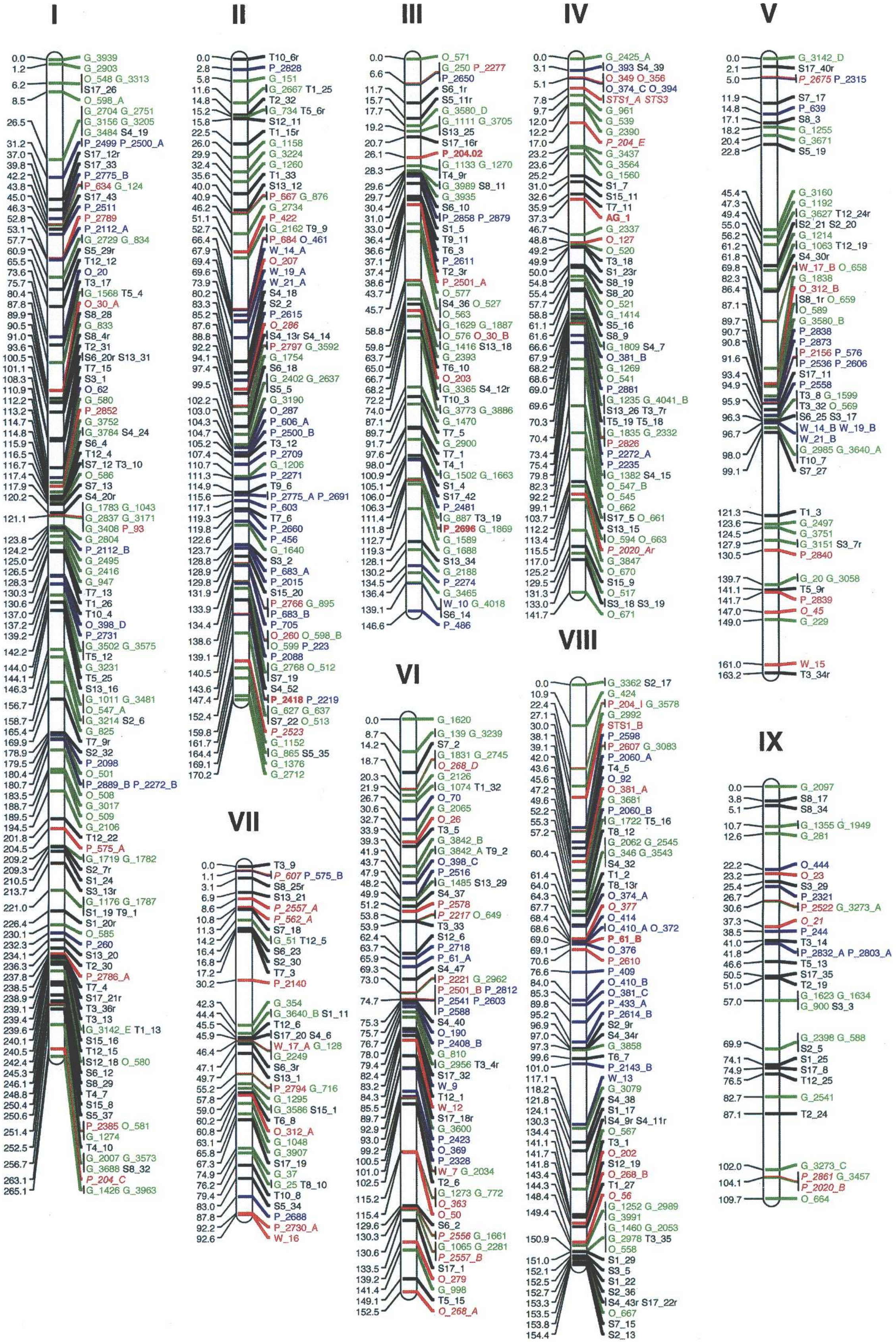

Figure 1. (Continued on next page)

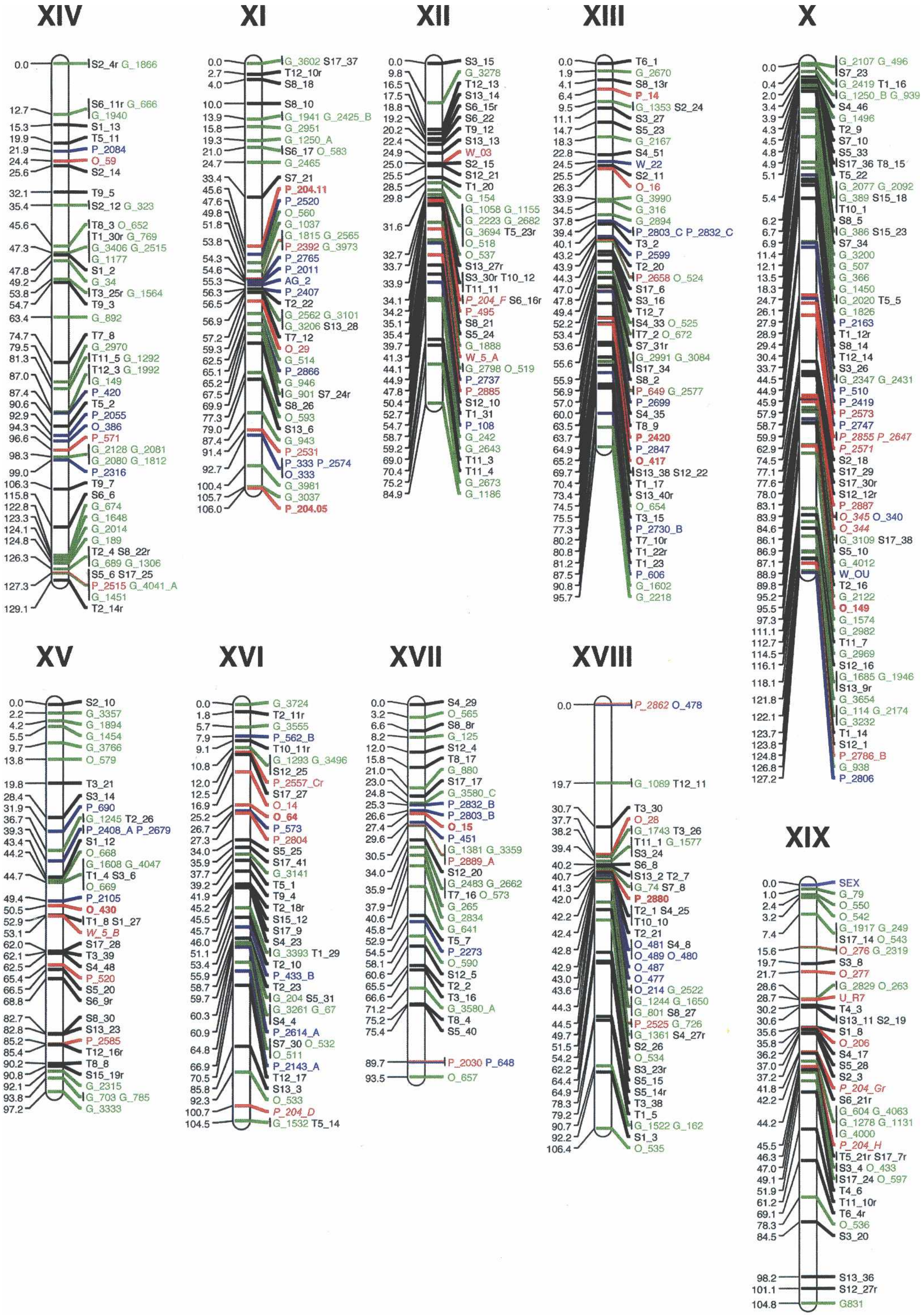

Figure 1. The consensus genetic map for *Populus*. (Black) AFLP markers; (red) the framework SSR markers in Family 13 and Family 331; (green) the alternative SSR markers genotyped in Family 13; (blue) the alternative SSR markers genotyped in Family 331; (SEX) the gender determination locus. The markers in bins have the same consensus position. The consensus map was established from genotypes of different pedigrees, and thus, the genetic distance does not reflect the recombination rate between markers but rather only marker orders on the consensus map. Note SSR primers were developed from different sequence data (for marker nomenclature, see text).

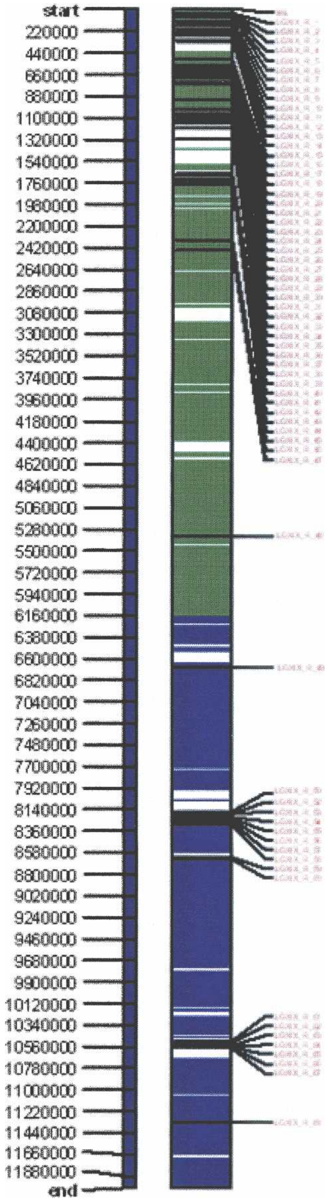

Figure 2. Forty-seven *NBS-LRR* genes localize to a 2.45-Mb region at the peritelomeric end of chromosome XIX. The physical length is estimated by the assembled sequence scaffold lengths plus the averaged gap length among scaffolds. The green portion of the vertical bar indicates the distorted region; the blue portion indicates the nondistorted region; the white portion indicates sequence gaps; the horizontal black bars show the physical positions of *NBS-LRR* genes; and the red letters are the serial number of *NBS-LRR* genes based on their physical orders along the chromosome. The ruler to the *left* of the chromosomal bar indicates the physical length in base pairs. Only one *NBS-LRR* gene would be expected in the corresponding 2.45-Mb region based on a uniform average genome-wide distribution assumption.

extends along the maternal haplotype into a region homologous to the paternal haplotype (i.e., 257 kb representing 6.8 cM in the maternal parent vs. 231 kb representing 15.8 cM in the paternal parent). Therefore, it appears that recombination suppression is confined to the peritelomeric portion of the chromosome XIX containing the gender-association locus.

Discussion

In a previous study, high conservation of SSR priming sequences was observed across *Populus* species (Tuskan et al. 2004b). In this study, near-complete marker colinearity was observed in pedigrees established from multiple species within the *Tacamahaca* and *Aigeiros* subgenera. The comparative mapping results demonstrate that the tested *Populus* species, and other members of the *Salicaceae* (Hanley et al. 2006), have maintained the basic genome structure formed following a whole-genome duplication event (Tuskan et al. 2006). Thus, our consensus map extends the sequence database of *Populus trichocarpa* Nisqually-1 to other *Populus* and *Salix* species, supplying a bridge between genetic and genomic information related to candidate genes.

A provocative characteristic of chromosome XIX is the location of a gender-determination locus, which we mapped to the peritelomeric region of chromosome XIX and which has recently been reported in a similar position in a *P. nigra* pedigree (Gaudet et al. 2007). Markussen et al. (2007) also reported mapping of a gender-determination locus in a *Populus tremula* × *Populus tremuloides* pedigree. However, they did not align this locus to chromosome XIX. We were able to align SSR from their map to our consensus map and the primer sequences to the Nisqually-1 genome and confirmed that this locus also maps to the peritelomeric chromosome XIX (Supplemental Fig. 6). Therefore, all three mapping studies validate the position of the gender-determination locus in *Populus* genome to the peritelomeric region of chromosome XIX.

Although the mapping studies revealed that there is a single locus that controls gender determination, recombination suppression would render all genes within this region as one locus. Thus the gender locus might encompass several genes underlying gender determination. Multigene-based gender determination can be used to explain labile sex expression in *Populus*. If there is more than one gene determining gender, recombination would impair sexual differentiation. When the recombination suppression is relaxed or translocation of sex-determination loci occurs, hermaphrodites will be generated. To maintain separate sexes, the genes determining maleness or femaleness would have to be closely linked on opposing haplotypes of a single chromosome, and this region would have to develop local mechanisms to prevent recombination (Ohno 1967; Nei 1969). The conclusions of Ohno (1967) and Nei (1969) are consistent with our findings for the female genetic maps and genome assemblies of chromosome XIX. Interestingly, incipient sex chromosomes have also been reported in papaya, and recombination was found to be severely suppressed in the vicinity of the sex determination locus (Liu et al. 2004). Furthermore, Bergero et al. (2007) report several linked sex-related genes in the dioecious plant *Silene latifolia*.

Multiple lines of evidence point to a ZW sex determination system in *Populus*, with the female being the heterogametic sex. First, the sequenced tree, Nisqually-1, is a female, and it showed highly divergent haplotypes in the sex determination region. Second, suppressed recombination in this region was only observed in the female parent. Finally, we have observed distorted segregation ratios in only the maternal genotypes of several mapping populations in *Populus*, including Family 545 and Family 13 (Yin et al. 2004).

The complex haplotype divergence observed in the sex determination region on chromosome XIX could be a contributing mechanism that reinforces recombination suppression (Rick 1969; Chetelat and Meglic 2000). It is also possible that gene

Table 1. Comparison of SSR markers on the consensus linkage map, sequence scaffold 117, and genome assembly of chromosome XIX

Marker	Linkage group/ scaffold	Position (cM)	Start position (bp)^a	End position (bp)^a	Expected Length (bp)	χ^2
G_79	XIX	1.0	235,624	235,834	210	10.54
O_542	XIX	3.2	834,243	834,396	153	11.27
G_249	XIX	7.4	318,318	318,475	157	11.62
	XIX		1,330,889	1,331,054	165	
	XIX		1,165,560	1,165,662	102	
O_276	XIX	15.0	52,837	53,019	182	6.86
	XIX		87,493	87,672	179	
	Scaffold 117		309,886	310,090	204	
G_2319	Scaffold 117	15.6	310,111	310,311	200	4.38
O_277	XIX	21.7	87,291	87,472	197	10.16
	Scaffold 117		817,253	817,153	138	
G_2829	XIX	28.6	1,435,402	1,435,566	164	12.78
O_263	XIX	28.6	1,627,995	1,628,257	262	6.86
O_206	XIX	35.8	2,309,017	2,309,177	160	9.11
G_604	XIX	44.2	3,377,531	3,377,662	131	5.83
G_4063	XIX	44.2	4,274,020	4,274,168	148	6.32
G_1131	XIX	44.2	6,172,808	6,172,948	140	3.55
G_4000	XIX	44.2	6,183,340	6,183,464	124	4.46
O_433	XIX	47.0	3,732,913	3,733,100	187	3.75
	XIX		7,097,848	7,098,044	196	
O_597	XIX	49.1	7,656,813	7,657,009	196	2.77
O_536	XIX	78.3	8,056,011	8,056,267	256	0.31
	XIX		10,126,963	10,127,036	73	
G_831	XIX	104.8	11,270,441	11,271,290	849	0.07

Assembly inconsistencies were observed within the first 28 cM in the distorted region where the gender-determination locus was located. Note that individual SSR markers align to multiple positions in the scaffold assemblies of Nisqually-1 genome. For most markers, a least one marker position is syntenic with the assembly. Also, most physical and genetic order discrepancies occur in the peritelomeric region, demonstrating the divergence of haplotype structure in this region.

[a]Start and stop position estimates include an estimated genome-wide average gap of 100 kb between mapped scaffolds (Tuskan et al. 2006).

content in this region plays a direct role in suppressing recombination, thus contributing to the evolution of a sex chromosome in *Populus*. There is a distinct overabundance of *NBS-LRR* genes in this region, and these may play a role as recombination suppressors. Previous work has demonstrated recombination rates that were 20 times lower than expected around *MXC3*, a locus conferring disease resistance in *Populus* (Stirling et al. 2001). Recombination suppression has also been observed around the *Mla* resistance cluster in barley (Wei et al. 1999), the *Tm-2a* virus resistance gene, the *Mi* nematode resistance gene in tomato (Ganal et al. 1989), the *RPP5* gene conferring resistance to the oomycete pathogen in *Arabidopsis* (Yang and Hua 2004), and the Hessian fly resistance locus in wheat (Behura et al. 2004). Therefore, recombination suppression seems to be a relatively common feature of *R*-gene clusters. It is unclear if this is due to structural effects of the complex tandem repeats, if this is due to some direct action of the gene products themselves, or if there is a selective advantage to accumulating tandem clusters of these genes in regions of suppressed recombination.

Consistent segregation distortion was also observed within the peritelomeric region on chromosome XIX across pedigrees, indicating the existence of genomic segments with unequal transmission of the parental alleles to their progeny. This pattern was not due to interspecific differences or selective primer amplification. That is, segregation distortion and recombination suppression were observed for this region of chromosome XIX in Family 545, an F_1 pedigree involving a pure *P. trichocarpa* mother and a pure *P. deltoides* father. This suggests that the causal mechanism is an inherent characteristic of the alternative haplotypes of chromosome XIX. The relationship between gender determination and segregation distortion, if any, is currently unclear. However, it is interesting to note that *NBS-LRR* genes have recently been shown to be involved in hybrid necrosis, which is emerging as a primary mechanism for the establishment and maintenance of barriers to gene flow among related species (Bomblies and Weigel 2007). Related mechanisms may create negative genetic interactions between different haplotypes in this region of suppressed recombination, thus contributing to the observed patterns of segregation distortion.

An additional piece of evidence from natural populations is the extensive occurrence of skewed sex ratios in *Populus* in favor of male trees (Grant and Mitton 1979; Rottenberg et al. 2000). There is some evidence that sex ratios are male-biased in more severe environments in *Populus* (Farmer 1964; Grant and Mitton 1979; Rottenberg et al. 2000), perhaps driven by differential resource usage by the genders (Casper and Charnov 1982; Bierzychudek and Eckhart 1988). However, male-biased sex ratios were also observed for our pedigree for which gender has been determined in *Populus*. This suggests that male-biased sex ratios may have a genetic basis in *Populus*. Haldane's rule (1922) postulates that in hybrids, the heterogametic sex is more likely to be absent, rare, or sterile (Coyne and Orr 2004). This phenomenon is widespread in plants and animals and is probably due to the effects of deleterious recessive alleles that accumulate on sex chromosomes (Coyne and Orr 2004), coupled with the faster rate of evolution (and divergence) of alleles on sex chromosomes (Charlesworth et al. 1987). Perhaps the incipient heterogametic sex chromosomes in female *Populus* trees harbor deleterious polymorphisms in linkage disequilibrium with positively selected alleles, which result in reduced average fitness and skewed sex ratios. With the availability of the whole-genome sequence and the initiation of a number of efforts to characterize adaptive polymorphisms and gender determination in natural populations, answers to this and other intriguing questions about

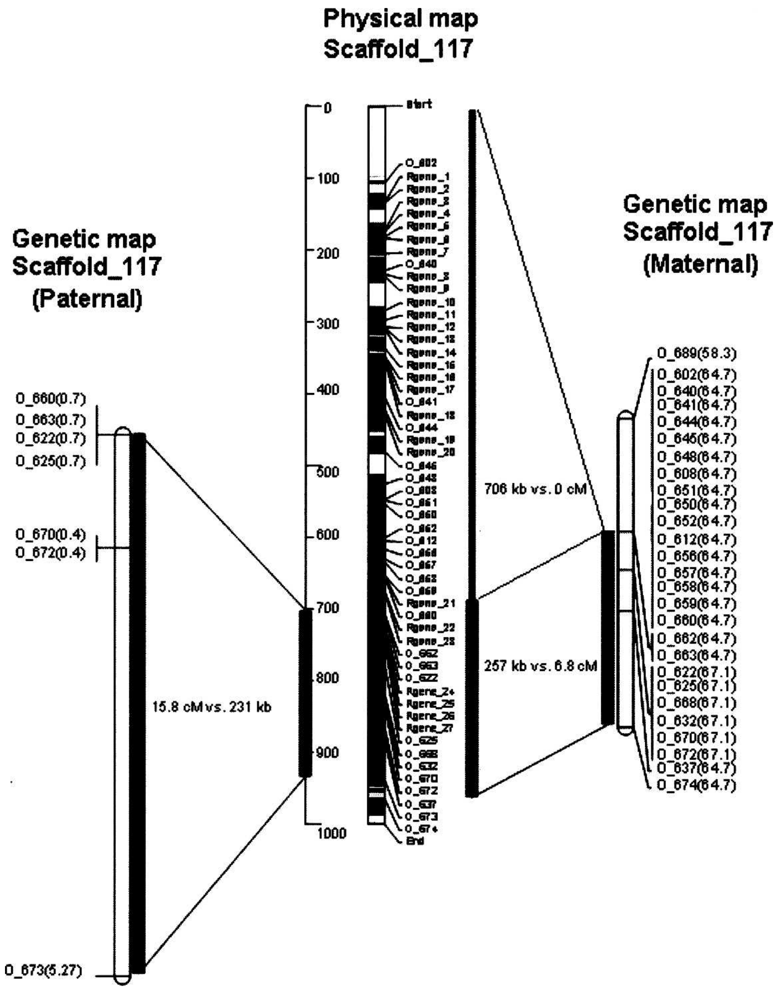

Figure 3. Relationship between the physical (*middle*) and gender-specific genetic maps (*left* and *right*) of scaffold 117 in Family 545. This scaffold was positioned in the peritelomeric region of chromosome XIX. We detected recombination suppression in the upper 706-kb region of this scaffold containing an overabundant number of *NBS-LRR* genes. In contrast, the lower 257-kb region is rich with recombination hotspots in both the maternal and paternal parents. Moreover, genotyping revealed more heterozygous loci in the maternal genotype than in the paternal genotype on this scaffold. Values in parentheses are χ^2 statistics for tests of segregation distortion. Alleles from the maternal parent showed strong and consistent segregation distortion, whereas alleles from the paternal parent generally conformed to Mendelian expectations (χ^2 critical value = 3.84 for $\alpha \leq 0.05$).

the evolutionary biology of this model genus are rapidly emerging.

Methods

Plant materials and mapping of the gender locus

Five mapping pedigrees were used in this study (Supplemental Fig. 1). Family 13 is an interspecific backcross involving female parent 52–225, an F_1 hybrid derived from *P. trichocarpa* (T), 93–968 × *P. deltoides* (D) (ILL-101), back-crossed to a male parent (F109R6P15), a pure *P. deltoides* (Yin et al. 2004). Family 331 is an F_2 (T×TD) inbred interspecific hybrid family arising from sib-mating of female 53–246 and male 53–242 of F_1 hybrid (T×D) Family 53, which is a cross between a female *P. trichocarpa* (93–968) and a male *P. deltoides* (ILL-129). Thus, Family 13 and Family 331 share the same grandmother, clone 93–968, and were used to construct the consensus map. A total of 171 progeny were genotyped in Family 13, while 310 progeny were genotyped in Family 331. Family BCN is a cross between a *Populus angustifolia* (clone 996) and a natural hybrid (clone WSU-6, a *Populus fremontii* × *P. angustifolia*). None of these pedigrees involved the sequenced individual, Nisqually-1. To compare the genetic map and genome sequence of Nisqually-1 directly, we created a genetic map for Family 545, an interspecific F_1 pedigree obtained from a cross between *P. trichocarpa* (clone Nisqually-1) and *P. deltoides* (clone ILL-101). Among the parental species, *P. fremontii*, *P. deltoides*, and *P. nigra* belong to the *Aigeiros* subgenus, and *P. angustifolia* and *P. trichocarpa* belong to the *Tacamahaca* subgenus.

The *Populus* gender-determination locus was mapped in a cross between a female *P. deltoides* and a male hybrid derived from *P. deltoides* and *P. nigra*. The paternal parent was a natural hybrid, whereas the female parent is a pure *P. deltoides* tree originating from a natural population in Illinois. Among the 312 progeny investigated, 115 were females and 197 were males. A χ^2 test was used to test for bias in the sex ratio.

Consensus map construction and genome assembly

Marker generation, genotyping, and nomenclature were as described in Tuskan et al. (2004b) and Yin et al. (2004). Four sources of SSR primers were used for map construction in this study: ORNL primers (O_), PMGC primers (P_), WPMS primers (W_) (Smulders et al. 2001), and GCPM primers (G_). The majority of the G_ SSR primers were designed based on end-sequences of BAC contigs used for *Populus* physical map construction (Kelleher et al. 2007). The microsatellite locations on scaffold 117 were identified using the Sputnik program (C. Abajian, University of Washington, Seattle). Additional primer information is available at http://www.ornl.gov/sci/ipgc/ssr_resource.htm. In total, we examined 640 SSR primer pairs, selected from 1230 pairs, and generated data for 701 loci for the consensus map construction.

Maternally informative testcross locus data in Family 13 and fully informative markers in Family 331 were linked using JoinMap 3.0 (Van Ooijen and Voorrips, 2001). We first analyzed each population data set separately and then applied the "Combine Groups for Map Integration" command to integrate linkage groups based on shared orthologs. When shared orthologs, defined as a common interval in both pedigrees, were detected, the combined value of the recombination rate was replaced by a weighted average value (Sewell et al. 1999; Stam 2004). The consensus position for the alternative SSR markers was derived by the following formula:

$$P_i = P_u + \frac{D_{fram}}{D_{alt}} L_{iu},$$

where P_i is the consensus position of the *i*th alternative SSR marker, D_{fram} is the size of the framework interval, D_{alt} is the size of the new interval after new alternative markers added in, P_u is the position of the upper boundary of the framework interval, and L_{iu} is the distance between the *i*th alternative markers to the upper boundary of the new interval.

Scaffold assembly was conducted with the *Populus* genome assembly (06252004 version compiled using the JAZZ assembler (Aparicio et al. 2002) at the U.S. Department of Energy Joint Genome Institute [JGI]). SSR priming site sequences were used as queries, and then the genome sequence location was determined based on "best" hits against the sequence scaffolds using the BLASTN (Altschul et al. 1997). Primer hits were accepted only if priming sites were in the correct orientation and separated by the expected physical distance. Multiple SSR were used to map scaffolds to linkage groups in cases where multiple priming sites were present on alternate scaffolds.

Discovery of the genetic features of the incipient sex chromosome

Segregation distortion in the progeny was tested using a χ^2 test. *NBS-LRR* genes were identified using the Advanced Search feature of the poplar genome portal (http://genome.jgi-psf.org/poplar), based on identification of genes with *NBS, LRR, TIR*, and *CNL* domains. A total of 528 genes were found, which were subsequently manually curated using the Gene Ontology tool. Genes containing just a *TIR* or *CNL* domain were excluded. *NBS-LRR* gene distribution was visualized by MapChart 2.2 (Voorrips, 2006). Thereafter, *NBS-LRR* gene clusters were evaluated by the observed number in each cluster compared with the whole-genome average number under an assumed Poisson distribution. The average number of *NBS-LRR* genes in 1-Mb DNA sequence, λ, was calculated as

$$\lambda = \sum_i m_i \Big/ \sum_i L_i \times 1{,}000{,}000,$$

where m_i is the number of *NBS-LRR* genes detected on chromosome or scaffold *i*; L_i is the base pair length of chromosome or scaffold *i*. Gene content was analyzed based on gene annotation by Eugene, Grail, Genewise, and FgenesH predictors available at http://genome.jgi-psf.org/cgi-bin/browserLoad/474c6f8a3e06fd24124c70ab. The survey of recombination suppression was conducted using Family 545. The maternal genotype of this pedigree is Nisqually-1, the sequenced individual, so genetic and genomic comparisons were conducted on the same individual.

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