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ORIGINAL ARTICLE

A dense linkage map of hybrid cottonwood (*Populus fremontii* × *P. angustifolia*) contributes to long-term ecological research and comparison mapping in a model forest tree

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Cottonwoods are foundation riparian species, and hybridization among species is known to produce ecological effects at levels higher than the population, including effects on dependent species, communities and ecosystems. Because these patterns result from increased genetic variation in key cottonwood traits, novel applications of genetic tools (for example, QTL mapping) could be used to place broad-scale ecological research into a genomic perspective. In addition, linkage maps have been produced for numerous species within the genus, and, coupled with the recent publication of the *Populus* genome sequence, these maps present a unique opportunity for genome comparisons in a model system. Here, we conducted linkage analyses in order to (1) create a platform for QTL and candidate gene studies of ecologically important traits, (2) create a framework for chromosomal-scale perspectives of introgression in a natural population, and (3) enhance genome-wide comparisons using two previously unmapped species. We produced 246 backcross mapping (BC_1) progeny by crossing a naturally occurring F_1 hybrid (*Populus fremontii* × *P. angustifolia*) to a pure *P. angustifolia* from the same population. Linkage analysis resulted in a dense linkage map of 541 AFLP and 111 SSR markers distributed across 19 linkage groups. These results compared favorably with other *Populus* linkage studies, and addition of SSR loci from the poplar genome project provided coarse alignment with the genome sequence. Preliminary applications of the data suggest that our map represents a useful framework for applying genomic research to ecological questions in a well-studied system, and has enhanced genome-wide comparisons in a model tree.
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Introduction

Over the past decade, advances in DNA marker technology have provided a glimpse into the genetic bases of ecological processes, expanding our knowledge of ecological genetics (reviewed in Via, 2002) and leading to the emerging field of community and ecosystem genomics (see Feder and Mitchell-Olds, 2003; Thomas and Klaper, 2004; Mauricio, 2005; Whitham *et al*., 2006). The ability to identify and characterize quantitative trait loci (QTL) associated with traits of ecological significance continues to be an important task and has contributed significantly to an understanding of ecological processes from a genomic perspective (Jackson *et al*., 2002). Marker techniques such as amplified fragment length polymorphisms (AFLP, Vos *et al*., 1995) have made linkage and QTL mapping possible for virtually any organism by overcoming the major barrier to such studies in the past (that is, lack of sufficient markers, see review by Doerge (2002)). In addition to AFLP based maps, over 4000 SSR markers have been developed for the genus *Populus* (http://www.ornl.gov/sci/ipgc/ssr_resource.htm), many of which have been used for genetic mapping in both *Populus* (discussed below) and the related genus *Salix* (Hanley *et al*., 2006, see also the above website). These markers provide a unique opportunity for wide-ranging comparative genomic studies across diverse taxa.

Beginning with Keim *et al*. (1989) and Whitham (1989), 18 years of research on a cottonwood hybrid zone (*Populus fremontii* × *P. angustifolia*) in northern Utah has revealed numerous relationships between genetic variation (via hybridization) in a foundation tree and higher order processes. Genetically based variation in cottonwood phytochemical (for example, Driebe and Whitham, 2000; Schweitzer *et al*., 2004; Bailey *et al*., 2004, 2005;

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Rehill *et al*., 2006), morphological (for example, Floate and Whitham, 1995; Larson and Whitham, 1997) and phenological traits (for example, Floate *et al*., 1993) has been shown to affect populations, communities and ecosystem processes at multiple scales including individual trees, stands, rivers and the western US region (Bangert *et al*., 2006a, b). These patterns suggest that novel applications of population and quantitative genetic tools may provide unprecedented opportunities to link genetic factors (for example, QTL and genes) with ecological patterns. This would help fulfill a major goal of placing community and ecosystem ecology within an evolutionary framework (Mitton, 2003; Whitham *et al*., 2003, 2006).

In addition to its potential as a model organism for ecological research, *Populus* has recently emerged as a premier model for forest tree biology and improvement (Taylor, 2002; Wullschleger *et al*., 2002). Characteristics such as ease of vegetative and seed propagation, fast growth rate, small genome size, and conservation of chromosome number across the genus ($n = 19$) make *Populus* ideal for experimental research, and mapping populations have been produced for numerous geographically and ecologically distinct species from diverse sections within the genus (see Table 2 below). These experimental populations provide a unique opportunity for comparative linkage mapping in a model system (for example, Yin *et al*., in review)—an opportunity that has been further enhanced by the recent publication of the *P. trichocarpa* genome sequence (Tuskan *et al*., 2006).

Here, we have created a high-density AFLP linkage map from a segregating interspecific backcross population of hybrid cottonwoods (*P. fremontii* × *P. angustifolia*). We chose a backcross design for four reasons: first, because few codominant markers had been developed for *Populus* at the beginning of our study, we used a dominant marker system (AFLP) which is best served by a backcross design (that is, few repulsion phase markers); second, our study was aimed at identifying QTL of ecological importance in a hybrid system where F_2's (but not backcrosses) are apparently rare (Keim *et al*., 1989); third, introgression in the natural population occurs unidirectionally (*P. fremontii* alleles to *P. angustifolia*) (Keim *et al*., 1989; Martinsen *et al*., 2001); and fourth, $F_1 \times F_1$ crosses in the greenhouse showed decreased success relative to backcrosses, suggesting negative interactions in the F_2 generation (G Martinsen, unpublished data). We aligned our map with Yin *et al*.'s (2004) map using SSR markers from the poplar genome sequence project (Tuskan *et al*., 2006) in order to link our data with the poplar genome sequence. The specific objectives of this study were to (1) create a linkage map for future QTL and candidate gene studies of ecologically important traits, (2) provide a framework for a chromosomal scale perspective of introgression in a natural system, and (3) to enhance comparisons of genome structure among multiple species within the genus.

Materials and methods

Mapping pedigree and DNA extraction

Parents for a segregating backcross mapping population were chosen from a naturally occurring hybrid zone on the Weber River in northern Utah. Parental species/hybrid class was determined using preliminary marker data from 33 nuclear RFLP loci (detailed in Martinsen *et al*., 2001). Using the technique of Stanton and Villar (1996), we crossed a *P. angustifolia* female clone (#996) with a male F_1 hybrid (*P. fremontii* × *P. angustifolia*, clone WSU-6) resulting in 246 full-sib backcross progeny. The seed progeny were germinated under a misting bench within 2 weeks of dehiscence and planted in standard potting mix. Cuttings of the parental clones were made at the same time. Cuttings from the parent clones and hybrid progeny were grown in a greenhouse for 2 years under uniform conditions. Fresh leaves were collected from parents and progeny at the height of the growing season, frozen on dry ice, and in some cases lyophilized. DNA was extracted as per Martinsen *et al*. (2001), or using the Qiagen DNeasy plant miniprep kit (Qiagen, Helden, Germany). Reanalysis of RFLP markers subsequent to the cross confirmed WSU-6 as an F_1 hybrid, but showed *P. angustifolia* clone #996 to likely be an advanced backcross hybrid/introgressant (see Martinsen *et al*., 2001) heterozygous for *P. fremontii* and *P. angustifolia* alleles at a single locus (RFLP probe p1254, Bradshaw and Stettler, 1993).

AFLP analysis

AFLP analysis was performed using the method of Vos *et al*. (1995) with modifications from Travis *et al*. (1996). Preselective amplification was conducted using adenine (A) as the first selective base in all cases. Forty-five $3 + 3$ primer combinations (*Eco*RI + AXX/*Mse*I + AXX) were chosen at random, and used to generate marker data. Marker names include the second and third selective bases for the *Eco*RI enzyme followed by the second and third *Mse*I selective bases, and finally by the approximate marker size in base pairs. For example, GGCC150 represents a 150 bp marker generated from an *Eco*RI + AGG/*Mse*I + ACC primer combination.

SSR analysis

A subset of 46 individuals from our mapping population were screened with 341 SSR markers that were derived from the *Populus trichocarpa* whole-genome sequencing project, and mapped in a *P. trichocarpa* × *P. deltoides* (TD) pedigree to enhance genome assembly (Tuskan *et al*., 2006). These SSR markers were selected at regular intervals throughout the genome to allow integration of the *P. angustifolia* × *P. fremontii* map with the whole-genome sequence, and to enhance comparisons of genome structure among multiple members of the genus. Initial screening was conducted with both parents and six progeny, and loci that appeared to be segregating in both parents were selected for mapping. SSR amplification and genotyping was performed as described elsewhere (Yin *et al*., 2004), except loci were analyzed on an ABI3730 automated capillary electrophoresis instrument, and amplification was performed with 10 pmol fluorescein 12-dUTP (Roche Diagnostics, Indianapolis, IN, USA), rather than end-labeled primers.

Marker segregation and map construction

Linkage analysis was restricted exclusively to markers with expected segregation ratios of 1:1 (that is, testcross markers where the F_1 parent was $+/-$ and the

P. angustifolia parent −/−, see Supplementary Table S1, electronic Supplementary Material). Segregation distortion in the testcross markers was assessed using a χ^2 analysis, and was identified as significant ($P<0.05$) deviation from expected Mendelian segregation. Distorted markers were not excluded from the linkage analysis (discussed below). Species origins of markers were inferred primarily on the assumption that markers that were homozygous null in *P. angustifolia* and heterozygous in the F_1 were likely fixed absent or rare in *P. angustifolia* and present in *P. fremontii*. Furthermore, markers that were putatively derived from the same species were consistently in the same linkage phase, and were generally fixed in wild populations (M Zinkgraf, S Woolbright and G Allan, unpublished data), thus lending support to our assumptions.

The linkage map was created using MAPMAKER 3.0 (Lander *et al*., 1987). Given the number of framework markers from preliminary results (Woolbright, 2001), the estimated genome size from Bradshaw *et al*. (1994) and whole-genome sequence assembly (Tuskan *et al*., 2006), and simulations from Yin *et al*. (2004), we chose an LOD score of 8.0 for linkage analysis. We then determined the appropriate recombination fraction (rf = 0.37) using the relationship between LOD score and population size described in Cervera *et al*. (2001) (see also Yin *et al*., 2004). Using these values as the 'default linkage criteria', preliminary linkage groups were identified with the 'groups' command. Marker data were then inverted for the entire dataset in order to place possible repulsion phase markers. Once initial groups were identified, one or two anchor loci were chosen to begin map construction. Markers within each group were ordered using the 'Order' command, and initial orders checked using the 'ripple' command again with an LOD threshold of 8.0. Additional markers were added using the 'build' command and checked with the 'ripple' command. When markers could not be ordered unambiguously, the marker with the least amount of missing data was usually chosen as a framework marker and the rest added as accessory markers using the 'try' command. Occasionally, markers that resulted in the least number of likely scoring errors or in the least amount of map expansion were chosen for the framework map.

SSR were also placed in the framework AFLP map using the 'try' command. Because SSR markers were mapped using a much smaller population size, distances between framework AFLP markers were fixed, and SSR positions were determined by interpolation between framework positions. Codominant SSR were used to infer alignment with other Populus maps, and each linkage group was reoriented and assigned a name according to the convention of the International Populus Genome Consortium (http://www.ornl.gov/sci/ipgc/) and Cervera *et al*. (2001).

Once linkage groups were characterized, the size of blocks showing segregation distortion in favor of a particular allele was estimated as per Yin *et al*. (2004). These values were then used to calculate the ratio of distorted regions to total length of the chromosome.

Marker distribution

The distribution of markers among linkage groups was calculated using the method from Remington *et al*. (1999). Using the Poisson distribution, we evaluated the probabilities $P(m \leqslant \lambda)$ and $P(m \geqslant \lambda)$ at $\alpha \leqslant 0.05$ where m and λ are the total and expected marker numbers, respectively, for each linkage group.

We also looked for regions of clustering and dispersion within each linkage group using the method from Yin *et al*. (2004). By sliding along each linkage group, 'windows' for clustering analysis were identified as consecutive intervals where marker spacing was less than the average spacing for the entire map. Windows for testing marker dispersion were defined by consecutive intervals with spacing greater than the average. The number of markers within each window was counted, and compared to the null expectation for evenly spaced markers for a particular window size. Significant departures from expectation were tested under a cumulative Poisson distribution using a one-tailed test ($\alpha \leqslant 0.05$).

Genome length and coverage

Observed genome length was calculated as the sum (cM) of all linkage groups for both the complete (all markers) and framework maps. Only framework markers were used to estimate genome length in order to avoid problems associated with marker clustering (see Cervera *et al*., 2001). Estimated genome length was calculated using the method from Hulbert *et al*. (1988), which provides an estimate based on partial linkage data. We also used the method from Nelson *et al*. (1994), which incorporates information from all linked and unlinked markers.

Observed map coverage was calculated as the ratio of observed map length to the estimated map length (G_e from Hulbert *et al*., 1988) for both the complete and framework maps. Theoretical map coverages were estimated for the framework map as per Lange and Boenke (1982), which accounts for chromosomal ends; and using the method from Bishop *et al*. (1983), which accounts for linear chromosomes.

Results

Marker analysis

Forty-five AFLP primer combinations yielded a total of 809 scorable polymorphic markers, with an average of 18 polymorphisms per primer combination. Of these, 564 were 'pseudo-testcross' markers with the F_1 parent heterozygous (+/−), and the recurrent parent carrying only the null allele (−/−). Of the remaining markers, 97 were 'intercross' markers (+−/+−), and 148 were heterozygous in the recurrent parent (−−/+−, see Supplementary Table S1, ESM). These were excluded from linkage analysis. A total of 790 monomorphic fragments (average 17.6 per primer combination) were also identified. Of the 341 SSR markers tested, 89 failed to amplify, 35 were monomorphic, 24 were intercross informative, 86 were paternally informative, 32 were maternally informative and 75 were both paternally and maternally informative.

Segregation distortion

Chi-square analysis of the raw AFLP marker data revealed significant ($P \leqslant 0.05$) deviation from the expected 1:1 segregation pattern in 113 of the 541 (21%)

Table 1 Segregation distortion by linkage group

Linkage group	*Length (cM)*	*Distorted block(s) (cM)*	*Percent distorted*	*Favored allele*
I	216.2	58.6	27.1	F
III	149.6	35.9, 10.0	30.7	N, N
IV	144.3	48.7	33.7	N
VI	159.7	8.6	5.4	N
X	142.4	30.2	21.2	F
XIII	64.1	7.9, 16.8, 4.9	46.2	N, N, N
XIV	88.2	10.2	11.6	N
XV	73.3	18.2	24.8	N
XVI	81.1	9.1, 14.7	29.3	F, F
XVIII	89	54.2	60.9	N
XIX	66.5	59.7	89.8	N

Length is the total length of each linkage group. The length of the distorted block(s) is given in centimorgans (Kosambi). Percent distorted is the ratio of the sum of the lengths of distorted blocks to total length. Favored allele: F = *P. fremontii*; N = *P. angustifolia*.

mapped AFLP testcross markers. Table 1 summarizes genome-wide segregation distortion at the level of individual linkage groups. Fifteen distinct regions or blocks of distortion occurred on 11 of 20 linkage groups, with two linkage groups (XVIII and XIX) exhibiting distortion across more than half their lengths. The size of the distorted regions varied among linkage groups. Distortion occurred more often in the direction of the recurrent allele (*P. angustifolia*), with 276.1 cM distorted (13.4% of the genome) vs 112.6 cM for the donor allele (*P. fremontii* 5.5% of the genome).

Map construction genome length, and coverage

MAPMAKER grouped the 564 AFLP testcross markers into 19 linkage groups, one triplet and nine unlinked markers. Twenty-four markers were removed from the analysis due to unnecessary map expansion or linkage to multiple groups. This was most often caused by the inclusion of faint markers that were difficult to score, and/or by extreme segregation distortion that may have been the result of comigration of separate loci. One hundred eleven SSR markers were placed in interpolated positions (see Materials and methods), including a minimum of 2 and a maximum of 11 markers on each of the 19 *Populus* linkage groups (Figure 1).

A total of 328 framework AFLP markers were identified and used to create a framework map spanning a distance of 2030.6 cM. Table 2 summarizes the results of our linkage and genome analyses, comparing them with results from other recent *Populus* mapping efforts. All results are within the range reported from other studies, except for Bishop *et al*.'s (1983) method for theoretical map coverage, which was slightly higher than the others.

Table 3 summarizes AFLP marker distribution when all markers are considered. Three linkage groups (XIII, XV, and XIX) contained more markers than expected, and four (IX, XI, XII and XVIII) contained less. At the level of individual linkage groups, significant clustering occurred within all but one (VII) major linkage group.

Discussion

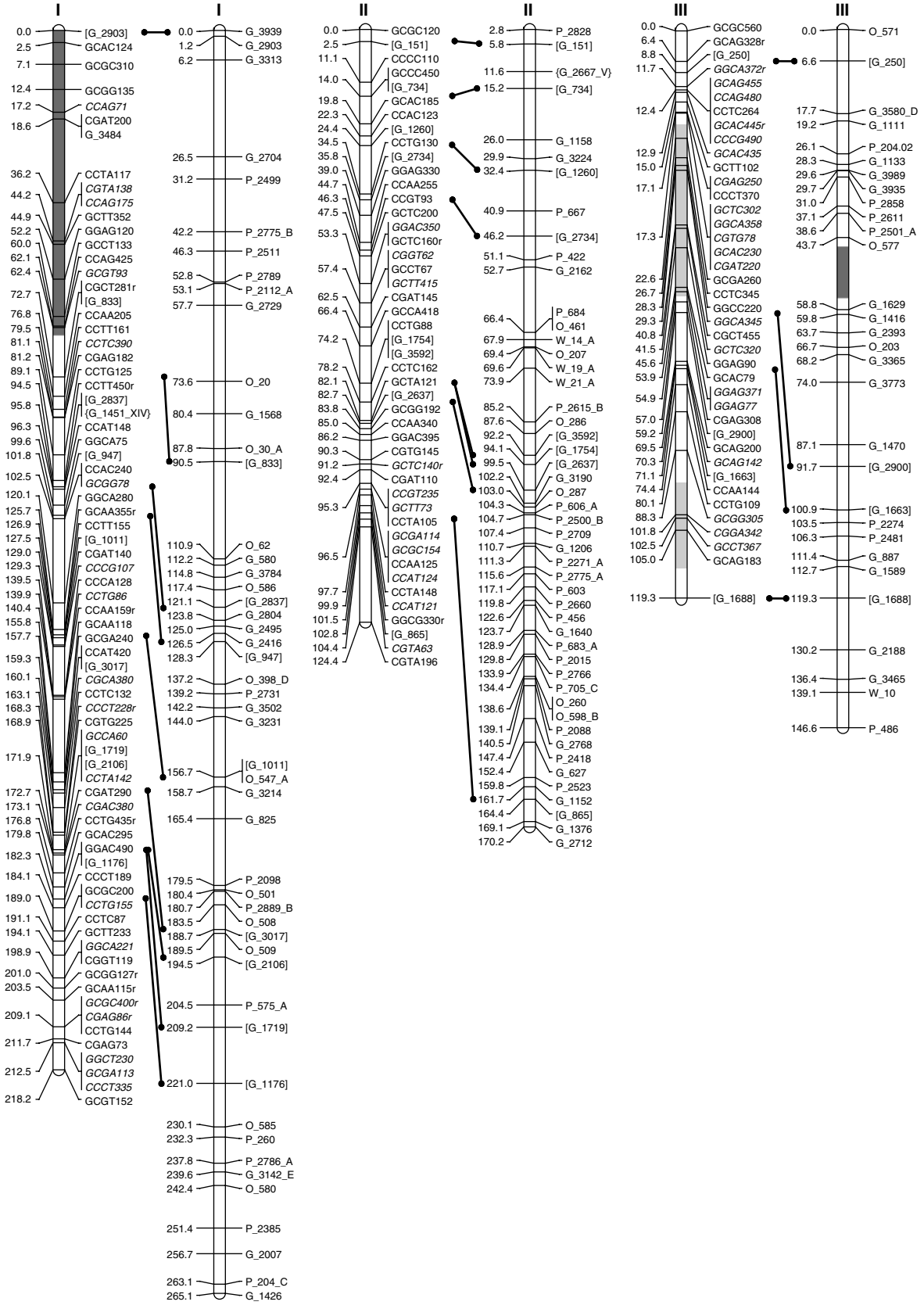
Our experimental design yielded a genetic map of comparable quality to other *Populus* maps (see Table 2). The number of major linkage groups was equal to that of the haploid chromosome number in *Populus* ($n = 19$). Our estimate of genome length falls within the range observed from other studies and is near the original estimate of 2400–2800 cM set by Bradshaw *et al*. (1994), which has been verified through simulation studies (Yin *et al*., 2004). Observed map length was also within the range of other *Populus* maps but lower than our estimated length, and lower than the more robust map of Yin *et al*. (2004). The discrepancy between estimated and observed lengths has been observed in other studies (Table 2), and can be explained by problems with marker clustering or dispersion due to map expansion caused by cosegregation of AFLP bands and other genotyping errors.

Marker clustering and dispersion were also comparable to other *Populus* maps (for example, Yin *et al*., 2004). Explanations for dispersion include regions of increased recombination and missing markers that have not been identified, perhaps due to gaps in occurrence of restriction enzyme recognition sites (Supplementary Figure S1, ESM). The addition of multiallelic SSR markers has helped to alleviate these problems and the availability of a map-linked genome sequence (Tuskan *et al*., 2006) will allow future targeted design of SSR and single nucleotide polymorphic (SNP) markers specifically for dispersed regions.

Targeted SSR and SNP markers should also be useful for characterizing problem areas arising from the presence of *P. fremontii* alleles in the genome of the *P. angustifolia* parent (for example, RFLP p1254, Martinsen

Figure 1 Genetic linkage map of male clone WSU-6, a *Populus. fremontii* × *P. angustifolia* F_1 hybrid, as determined by 246 progeny from a backcross to *P. angustifolia* clone #996. Linkage maps were drawn using the MapChart software (Voorrips, 2002). Linkage groups were compared with groups from Yin *et al*. (2004) (shown at right of each pair) using homologous SSR markers and named as per Cervera *et al*. (2001). Bars between each pair of linkage groups show the relative position of homologous SSR. Numbers at left of each group show absolute marker position in Kosambi map units. Marker names are to the right. Accessory markers are in italics. Names ending in 'r' represent inverted markers. Markers in square brackets, [], indicate microsatellite loci used to compare the two maps. Markers in brackets,{}, indicate possible translocations and include the alternate linkage group. Shaded regions indicate blocks of distortion. Light gray shading indicates distortion toward the recurrent (*P. angustifolia*) allele, dark gray distortion toward the donor *(P. fremontii*) allele.

et al., 2001). The presence of introgressed (that is, intercross, Supplementary Table S1, ESM) fragments are indistinguishable from shared parental alleles and both likely lead to 'blind spots' when using dominant markers to search for ecologically relevant QTL. If introgression is the result of positive selection, ecologically important



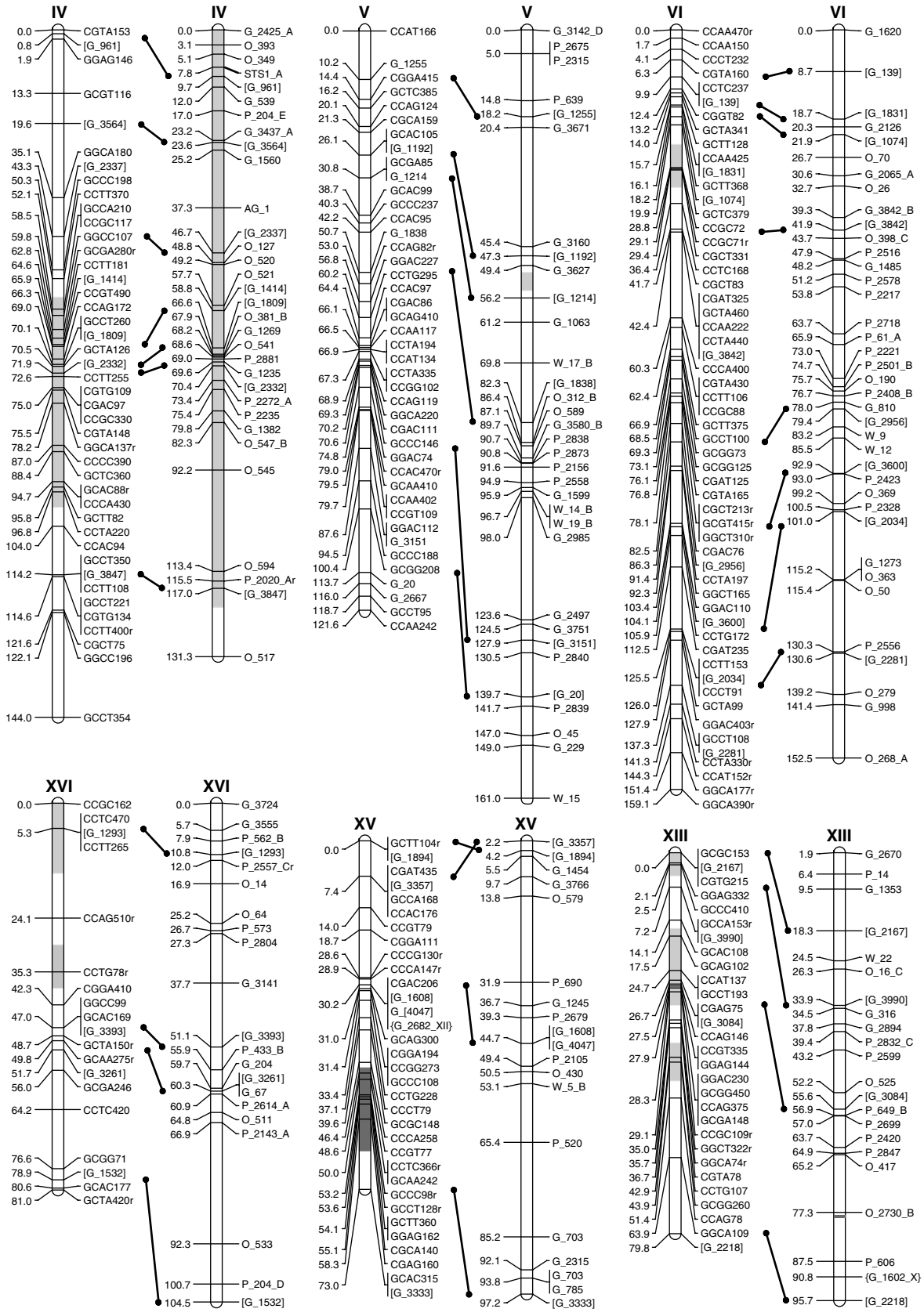


Figure 1 Continued

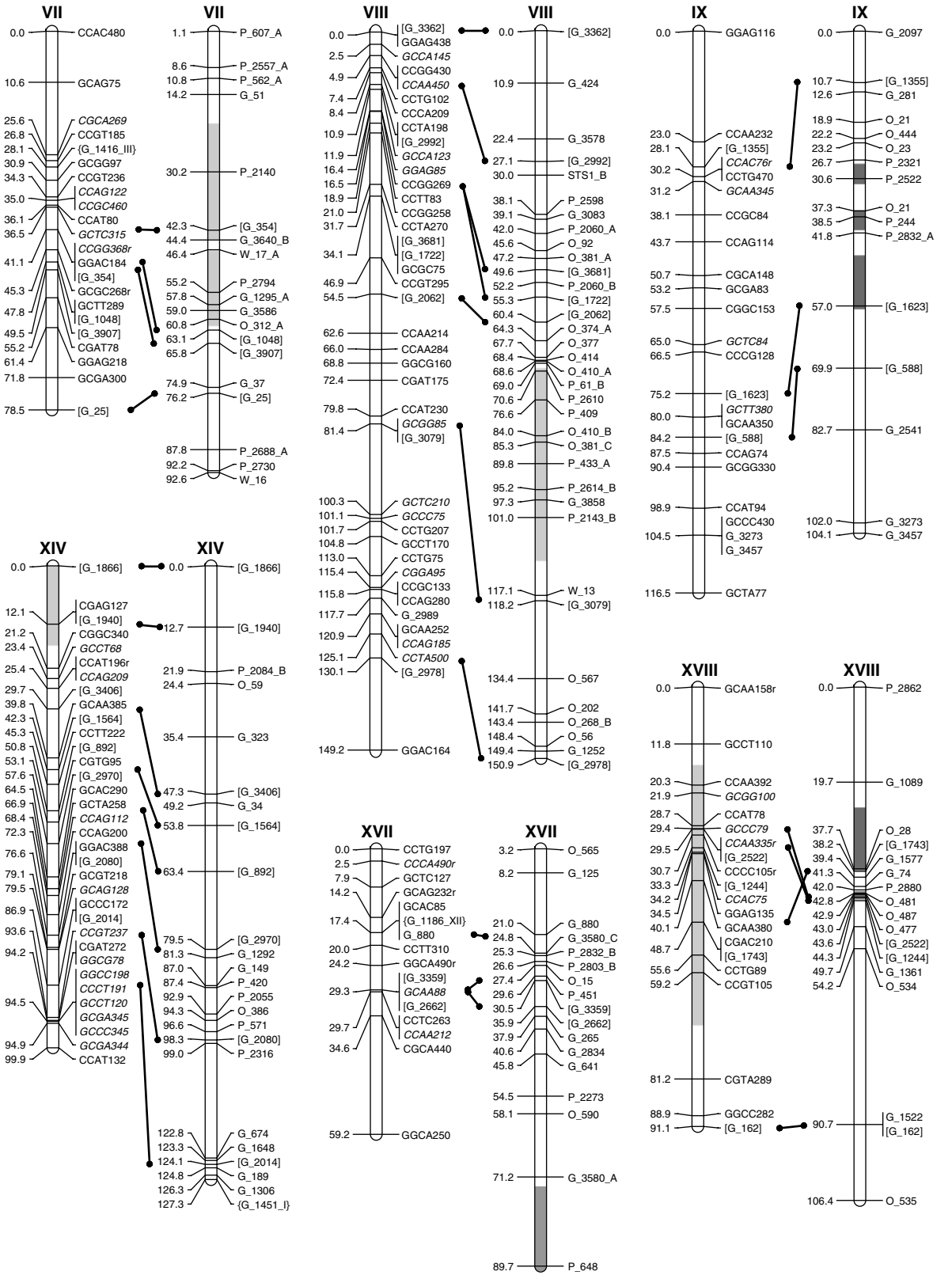


Figure 1 Continued

regions of the genome could therefore be missed due to poor linkage data when codominant markers are unavailable. Future addition of evenly distributed, targeted SSRs, combined with introgression studies in natural populations should help alleviate this problem.

In contrast to dispersed regions, we observed marker clusters for all but one (VII) of the 19 major linkage

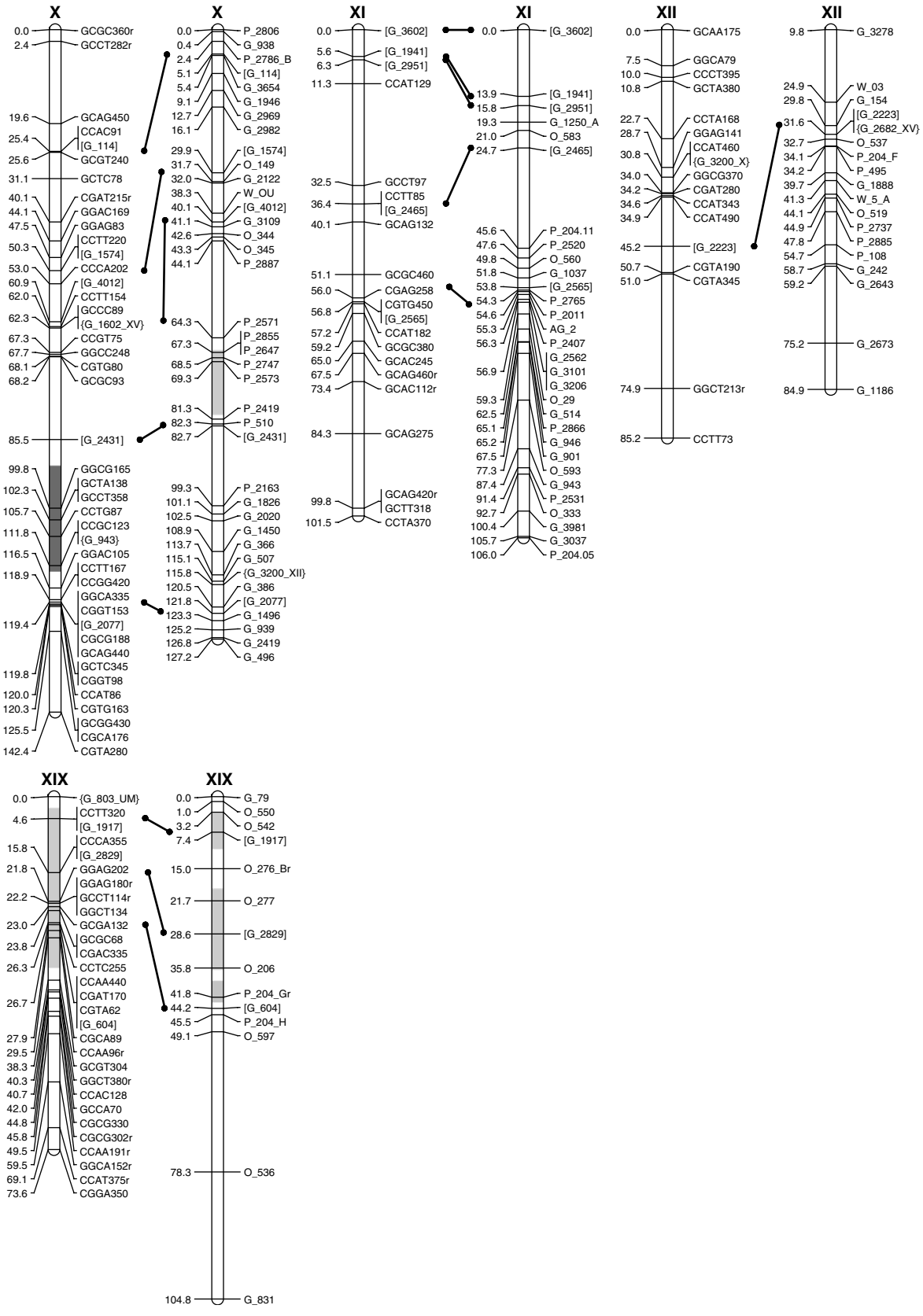


Figure 1 Continued

Table 2 Map data and comparisons with other Populus maps (adapted from Cervera *et al*., 2001)

Species	*This paper*	*Cervera et al. (2001)*				*Yin et al. (2004)*	*Yin et al. (2002)*		*Frewen et al. (2001)*		*Wu et al. (2000)*
	F × A	*D (87001)*	*D (87002)*	*N*	*T*	*T × D*	*D*	*N × D*	*D*	*T*	*D*
Population size	246	127	105	127	105	180	93	93	346	346	93
No. of linkage groups	20	21	23	34	23	19	31	34	24	26	19[a]
No. of framework markers	330	238	147	179	194	445	—	—	—	—	—
Average no. per group	16.5	11.3	6.4	5.3	8.4	23.4	—	—	—	—	—
Average length (cM)	102.9	103.7	70.7	69.3	83.5	—	—	—	—	—	154
Smallest group (cM)	5.9	7	8.9	10.5	9	73	—	—	—	—	53
Largest group (cM)	218.7	183.2	169.3	252.1	178.3	262.5	278.7	313.1	—	—	295
Average marker spacing (cM)	6.9	10	12.5	10.4	11.2	5.2	13.6	16	—	—	23.3
G_{of} (cM)[b]	2058	2178	1626	2356	1920	2313.5	—	—	1778	2002	2927
G_{oa} (cM)[c]	2100.4	2304	1838	2791	2326	—	3801	3452	—	—	—
G_{on} (cM)[d]	2729.7	2932	2618	3760	2590	—	—	—	—	—	—
G_e (cM)[e]	2693.9	2520	2375	3869	2616	2478.9	—	—	—	—	—
C_{of} (%)[f]	76.4	86.4	68.5	60.9	73.4	93.3	—	—	74.1[g]	83.4[g]	—
C_{oa} (%)[h]	78	91.4	77.4	72.1	88.9	—	—	—	—	—	—
C_{eb} (%)[i]	98	92.8	90.6	84.8	90.5	99.9	—	—	—	—	—
C_{el} (%)[j]	97	94.8	87.6	79.9	87.2	—	—	—	—	—	—

F = *P. Fremontii*; A = *P. angustifolia*; D = *P. deltoides*; T = *P. trichocarpa*; N = *P. nigra*.
[a]Data restricted to the 19 largest linkage groups.
[b]Framework map length.
[c]Map length using all markers.
[d]Estimated map length (Nelson *et al*., 1994).
[e]Estimated map length (Hulbert *et al*., 1988).
[f]Observed map coverage using framework markers (G_{of}/G_e).
[g]Map coverage is based on Bradshaw and Stettler's (1994) G_e of 2600 cM.
[h]Observed map coverage using all markers (G_{oa}/G_e).
[i]Theoretical map coverage (Lange and Boenke, 1982).
[j]Theoretical map coverage (Bishop *et al*., 1983).

Table 3 Clustering and dispersion in the complete map

	Length	*Experimental AFLP*	*Observed AFLP*	*Clustered*		*Dispersed*	
				Number	*%*	*Number*	*%*
I	216.2	53.06	63	4	9.4	0	—
II	126.6	31.68	39	2	27.1	0	—
III	149.6	37.54	37	2	5.3	1	25.9
IV	144.3	36.27	36	3	14.6	1	31.8
V	121.5	30.59	35	1	20.8	0	—
VI	159.7	39.59	48	5	25.9	0	—
VII	72	19.26	17	1	13.5	1	31.8
VIII	149.4	37.74	33	2	15	2	33.2
IX	116.8	30.86	19	1	0.8	1	23.4
X	142.4	35.79	36	2	1.5	1	21
XI	90	24.25	16	1	3.2	0	—
XII	85.2	23.15	15*	1	6.4	1	35.4
XIII	64.1	16.51	25*	1	6.4	0	—
XIV	88.2	22.65	26	1	13.5	0	—
XV	73.3	18.77	27*	2	27.8	0	—
XVI	81.1	22.04	15	1	3	0	—
XVII	59.2	16.63	12	1	0.6	1	29.7
XVIII	89	23.98	16	1	5.8	1	35.6
XIX	66.5	17.13	25*	2	26.3	0	—
Triplet 1	5.3	2.52	3	0	—	0	—

LG is linkage group. L_i is the length of each group (in Kosambi units) plus twice the average marker spacing for the group. Expected marker numbers were calculated for each linkage group taking into consideration the total number of markers and the lengths of each linkage group. Departure from the expected marker number was assessed under a cumulative Poisson distribution using a two-tailed test (*significant at $\alpha \leqslant 0.05$). Clustered regions are the number of blocks per group showing significant clustering. The percentage of clustering is the ratio of the sum of the lengths of clustered blocks to the total length of the group. Dispersed regions were calculated in the same manner but for regions of dispersion.

groups. Some marker clusters (that is, 5% at $\alpha = 0.05$) are expected due to random chance. Clustering also occurs in regions of the genome with reduced recombination and has been used to describe structural features of chromosomes. Young *et al*. (1999) were able to identify likely positions of centromeres in soybean linkage groups by comparing the distribution of a methylation sensitive (*Pst*I) vs an insensitive restriction enzyme (*Eco*R1). In the analysis, *Pst*I markers were under-represented in marker clusters thought to occur in

cytosine methylated heterochromatic regions surrounding the centromere. Thus, enzyme choice in AFLP analyses might be used to produce more uniformly distributed maps. Finally, clustering may arise from problems with meiotic pairing due to divergence of parental chromosomes, particularly when using interspecific crosses between highly divergent species.

Map comparisons

Our map showed a high degree of marker colinearity with the map of Yin *et al.* (2004); however, map alignment using SSR markers identified two putative inversions, and eight putative translocations (Figure 1). Given the divergent species used in the comparison, chromosomal rearrangements are not necessarily unexpected. Alternatively, the inversions on linkage groups XV and XVIII could be the result of errors in map order due to the small sample size used in the SSR analysis. Marker translocations could also be the result of multiple and divergent SSR priming sites arising from recent or ancient genome duplications (see Tuskan *et al*., 2006). These issues are being addressed by enhancing the resolution of our SSR map, by performing comparative analyses with additional *Populus* genetic maps (Yin *et al*., in review), and by resequencing and reassembling problematic areas of the genome (GA Tuskan, personal communication).

The absence of major chromosomal rearrangements in this and other comparative mapping (for example, Cervera *et al*., 2001), coupled with the shared areas of segregation distortion and recombination repression (Yin *et al*., in review) suggest that genic interactions are mostly responsible for species barriers between *P. fremontii* and *P. angustifolia*. These barriers likely resulted in decreased success observed in experimental $F_1 \times F_1$ crosses and backcrosses to *P. fremontii* (G Martinsen, unpublished data), as well as unidirectional introgression in the natural system (Keim *et al*., 1989; Martinsen *et al*., 2001). Similar patterns have been observed in other species from sections Tacamahaca and Aigeiros (Floate, 2004), and likely indicate shared barriers at the section level. Molecular data have contributed to the characterization of such barriers by revealing 'hallmarks' such as segregation distortion (discussed below) and recombination repression. Linkage analyses, QTL studies and candidate gene surveys have been useful for identifying traits and genes underlying these phenomena (for example, Bradshaw and Stettler, 1994; Cervera *et al*., 2001; Yin *et al*., 2004).

Our sample size allowed for only coarse map alignment, and reliable statistical tests for shared segregation distortion were not feasible. However, we did notice large areas of shared distortion favoring Tacamahaca alleles on at least two linkage groups (IV and XIX). One of these (XIX) was used in a recent study by Yin *et al*. (in review) that identified similarities in recombination repression and segregation distortion across multiple families. These patterns have provided insight into potential species barriers (for example, R genes), and suggest the evolution of a primitive *Populus* sex chromosome (Yin *et al*., in review). Thus, the data revealing segregation distortion in *P. fremontii* × *P. angustifolia* hybrids have contributed to our understanding of *Populus* at levels exceeding our original intention (that is, section vs species).

In contrast to genic interactions contributing to species barriers, genetic admixture may also lead to the adaptive introgression of alleles, an important but largely understudied aspect of plant evolution (Grant, 1971; Martinsen *et al*., 2001; Whitney *et al*., 2006). Recently, Lexer *et al*. (2007) used map-based SSR to avoid tightly linked markers when surveying for introgression and linkage disequilibrium in European hybrid zones of *P. alba* and *P. tremula*. Primers for the loci they used are known to amplify in multiple species across several sections within the genus (see also Rahman and Rajora, 2002 and citations therein). SSR markers developed for *Populus* have also been used for mapping in *Salix* (Hanley *et al*., 2006). Thus, map-based genetic markers provide a unique (but untested) opportunity for comparative studies of introgression across multiple taxonomic levels. Furthermore, these studies demonstrate how research questions aimed at specific populations or species can contribute to a larger focus (that is, evolution in the Salicacae), arguing for the continued use of map-based markers across broad areas of inquiry.

Segregation distortion

Segregation distortion is common in mapping studies of forest trees and has been documented in most if not all *Populus* mapping efforts. While distortion can influence map construction (Zhang *et al*., 2002 and citations theirin) and may affect QTL detection through spurious associations, exclusion of distorted markers is not necessarily warranted as they may be linked to genes or traits of interest. For example, both Cervera *et al*. (2001) and Yin *et al*. (2004) found that segregation distortion in some markers may have resulted from susceptibility to *Melamspora* rust or other selective forces acting during generation of the hybrid pedigree. Bradshaw and Stettler (1994) found that a recessive pollen lethal allele tightly linked to a mapped RFLP marker (p1054) in a *P. trichocarpa* × *P. deltoides* cross was the most likely cause of distortion in their mapping population. These results suggest markers showing segregation distortion due to linkage with genes under selection may have important ecological consequences, and should therefore be included in mapping studies of natural populations. Caution should be exercised however, when making conclusions involving QTL linked to distorted markers.

Assigning species status to dominant marker alleles is problematic given the difficulty distinguishing introgression from coancestry. Assuming most alleles segregate in both species, we would have expected to see a more-or-less equal distribution of coupling- vs repulsion phase (that is, inverted markers). In our study, most mapped markers ($n = 495$ or 92%) were in coupling phase, and were likely donated by *P. fremontii* chromosomes carried by the F_1. Furthermore, in a survey of individuals from multiple populations of each species, 71 of 100 mapped markers (71%) were fixed absent or rare (allele frequencies $\leqslant 0.05$) in *P. angustifolia* relative to *P. fremontii* (M Zinkgraf, S Woolbright and G Allan unpublished data). Similarly, Martinsen *et al*. (2001) found that *P. fremontii*-specific alleles at 26 of 33 RFLP markers (78.8%) were absent from the *P. angustifolia* zone. Given these data, the difference in marker phase likely reflects a high level of divergence among the species, and AFLP alleles segregating in both species could indicate introgression.

Conclusions and future research

Given the extensive amount of ecological research on the Weber River hybrid zone (Whitham *et al*., 2003, 2006), our map represents a unique opportunity to combine long-term ecological research with map-based genetic techniques. For example, we have begun to identify QTL associated with a number of ecologically important traits such as condensed tannins, which have important community and ecosystem phenotypes (Woolbright, 2001; Whitham *et al*., 2003). Foliar condensed tannin concentrations have been linked to arthropod communities (Whitham *et al*., 2006), aquatic and terrestrial litter decomposition (Schweitzer *et al*., 2005; LeRoy *et al*., 2006), root production (Fischer *et al*., 2006) and nutrient cycling (Schweitzer *et al*., 2004). Using the recently completed *Populus* genome sequence (Tuskan *et al*., 2006), we have begun to build candidate gene lists for a number of ecologically relevant QTL. The ability to link genetic-level factors with community composition and ecosystem-level processes is unprecedented, and demonstrates the potential of genetic mapping in ecological genetic/genomic research.

The original aim of our study was to describe broad-scale ecological processes in terms of the genetic variation within a foundation species. Historically, *P. fremontii* and *P. angustifolia* have played little if any commercial role, and have been studied primarily for their ecological importance. Here, we have shown that research focusing on specific ecological questions in two largely overlooked species contributes to much larger questions relating to evolution in a model system, and studies such as those by Rahman and Rajora (2002); Hanley *et al*. (2006)and Lexer *et al*. (2007) demonstrate the potential for comparative studies across even broader taxonomic levels. In light of these results, future genetic studies of *Populus* and its relatives should capitalize on the availability of shared SSR and other markers.

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