A Sugar Pine Consensus Map: Comparative Mapping Between the *Pinus* Subgenus *Pinus* and the Subgenus *Strobus*

Extended Abstract

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Abstract—We have constructed a consensus genetic linkage map for sugar pine using three mapping populations that segregate for resistance to white pine blister rust, a disease caused by the fungal pathogen *Cronartium ribicola*. The major gene of resistance, *Cr1*, was mapped in two of the populations and included in the consensus map, which contains 400 markers organized into 19 linkage groups. All 19 linkage groups align with the 12 linkage groups of the loblolly pine reference map. This work provides the foundation for comparative genomics and mapping within the *Pinus* subgenus *Strobus*.

Genetic Mapping in Sugar Pine

Genetic maps are useful integrative tools in genomic research in many crop species (Kole 2007) and have also been constructed for several species within the *Pinus* subgenus *Pinus* and within other genera of the Pinaceae family. The majority of genetic maps constructed in pines thus far have been to species belonging to the *Pinus* sub-genus *Pinus* (commonly referred to as hard pines; Table 1) because of their wide economic importance, with relatively little progress being made in species from the subgenus *Strobus* (commonly referred to as soft pines). A genetic map was constructed in eastern white pine (*P. strobus* L., Echt and others 1997) using random amplified polymorphic DNA (RAPD) markers. Some progress has been made in western white pine (*P. monticola* Dougl. ex D. Don) and in sugar pine (*P. lambertiana* Dougl.) for mapping the major gene of resistance (MGR) to *Cronartium ribicola*, the fungal pathogen that causes white pine blister rust (Liu and others 2006; Devey et al. 1995; Harkins et al. 1998). In Harkins et al. (1998), *Cr1* (the MGR) was positioned on a linkage group in five sugar pine trees with RAPD (OPG_16_950) markers that were segregating for the hypersensitive response. A RAPD marker that was positioned at 1.2 cM from *Cr1* was converted to a sequence characterized amplified region (SCAR) marker for use in constructing a full genome consensus map (Jermstad et al. 2010). In the mapping study presented here, three sugar pine mapping populations were used for constructing individual and consensus maps with single nucleotide polymorphisms (SNPs) derived from genes that were originally amplified and sequenced in loblolly pine. The amplicons represent annotated genes that 1) are related

Table 1. Genetic maps constructed in hard pines and other genera of the Pinaceae. A comprehensive list of maps in conifers can be viewed at http://www.pierroton.inra.fr/genetics/labo/mapreview.html

Common Name	Taxonomic Name	Map Publication
—Subgenus *Pinus*		
Loblolly pine	*Pinus taeda* L	Devey et al. (1994) Theor Appl Genet 83:238-242
Longleaf pine	*Pinus elliotti* Engel	Nelson et al. (1994) J Hered 85: 433-439
Slash pine	*Pinus palustris* Mill	Nelson et al. (1993) Theor Appl Genet 87: 145-151
Monterey pine	*Pinus radiata* L	Devey et al. (1996) Theor Appl Genet 99 : 656-662
Turkish red pine	*Pinus brutia* Ten	Kaya and Neale (1995) Silvae Genet 44: 110-116
Maritime pine	*Pinus pinaster* Aiton	Plomion et al. (1995) Heredity 74:661-668
Japanese black pine	*Pinus thunbergii* Parl	Hayashi et al. (2001) Theor Appl Genet 102: 871-875
Scots pine	*Pinus sylvestris* L	Lerceteau et al. (2000) Mol Breeding 6: 451-458
Japanese red pine	*Pinus densiflora* Sieb. et Zucc.	Yong-Yul Kim et al. (2005) Mol Cells 20: 201-209
—Other genera		
Douglas fir	*Pseudotsuga menziesii* [Mirb.] Franco	Jermstad et al. (1998) Theor Appl Genet 97:762-770
Norway spruce	*Picea abies* [L] Karst	Binelli and Bucci (1994) Theor Appl Genet 88: 283-288
White spruce	*Picea glauca* Moench	Tulsieram et al. (1992) BioTechnology 10: 686-690
Japanese Cedar (Sugi)	*Cryptomeria japonica* D. Don	Mukai et al. (1995) Theor Appl Genet 90: 835-840

In: Keane, Robert E.; Tomback, Diana F.; Murray, Michael P.; and Smith, Cyndi M., eds. 2011. **The future of high-elevation, five-needle white pines in Western North America: Proceedings of the High Five Symposium.** 28-30 June 2010; Missoula, MT. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 376 p. Online at http://www.fs.fed.us/rm/pubs/rmrs_p063.html

Table 2. A summary of sugar pine linkage analyses. Maps 5701 and 6000 were derived from megagametophytes (n=95) from adult open-pollinated trees that are heterozygous for *Cr1*. Maps 5038 and 5500 are derived from the parents of the QTL population, while the sex-averaged map is derived from the progeny (n=94) and the two parents of the QTL population. The consensus map is derived from the four adult trees.

	Tree 5701 (TG101)	Tree 6000 (TG102)	Tree 5038 (♀) (TG103)	Tree 5500 (♂) (TG104)	QTL sex-averaged (TG105)	Consensus (TG106)
No. markers in JM input file	192	190	165	165	282[a]	457[b]
No. markers unmapped[c]	9	4	43	48	69	57
No. markers mapped	183	186	122	117	213	400
No. LGs	16	17	19	19	23	19
Map length (cM)	822.1	849.1	642.8	883.8	1142.7	1230.9
Cr1 mapped	√	√	-	-	-	√
*scar*OPG_16	√	√	-	√	√	√

[a] 165 markers segregated in the maternal parent and 165 markers segregated in the paternal parent. Forty-eight of these markers were in the intercross configuration and should only be counted once in the sex-averaged linkage analysis because the marker data merge and map to a single position. [165+165 = 330 - 48 (IC) = 282]

[b] Although the sum of segregating markers for the individual adult trees = 712, the number of markers recognized and analyzed by JoinMap = 457. Similar to the way JoinMap analyzed IC loci in the sex-averaged linkage analysis, TYPE II COS marker data found in > one tree were merged and analyzed as a single locus, and thus, counted only once. Among the four trees, there were 255 TYPE II COS markers (712-255= 457)

[c] these are markers that linked to ≤ 1 marker (s)

to stress responses, 2) are transcription factors. Two of the populations consist of megagametophytes from open-pollinated trees (5701 and 6000) that are heterozygous for *Cr1*. A third mapping population (*QTL*) consists of progeny (needle tissue) from a controlled-cross that is segregating for partial resistance to white pine blister rust. JoinMap v. 1.4 software (Stam 1993) was used for linkage analysis with the following parameters: LinkLOD 4.0, MapLOD 0.1, and Kosambi mapping function. *Cr1* was positioned in the 5701 and 6000 maps, and the SCAR linked to *Cr1* was positioned in 5701, 6000 and 5500 (the male parent of the QTL population). Segregation data from the parents of the QTL population (5038 and 5500) and from trees 5701 and 6000 were combined in order to construct a consensus map for sugar pine (Jermstad et al. 2010). The consensus map consisted of 400 markers organized into 19 linkage groups (Table 2), which is seven more linkage groups than what is expected for pine (n=12). In total, six maps were constructed: 1) 5701, 2) 6000, 3) 5038, 4) 5500, 5) a sex-averaged map (5038 x 5500), and 6) a consensus map (5701, 6000, 5038 and 5500) (Table 2). These genetic linkage maps (TG101-106) are recorded in the TreeGenes Comparative Mapping Database (Wegrzyn et al. 2008) and can be viewed at http://dendrome.ucdavis.edu/cmap/. The amplicons used for mapping in sugar pine were developed in the ADEPT2 project (http://dendrome.ucdavis.edu/NealeLab/adept2/overview.php/).

Comparative Mapping

Several comparative mapping studies have reported syntenic relationships among the subgenus *Pinus* (Devey et al. 1999; Brown et al. 2001; Chagne et al. 2003; Komulainen et al. 2003). Synteny has also been observed between loblolly pine and conifers from other genera of the Pinaceae, i.e., *Pseudotsuga* (Krutovsky et al. 2004) and *Picea* (Neale and Krutovsky 2004). Therefore, we hypothesized that synteny would be found not only between the novel sugar pine maps, but, also observed between loblolly pine (subgenus *Pinus*) and sugar pine (subgenus *Strobus*).

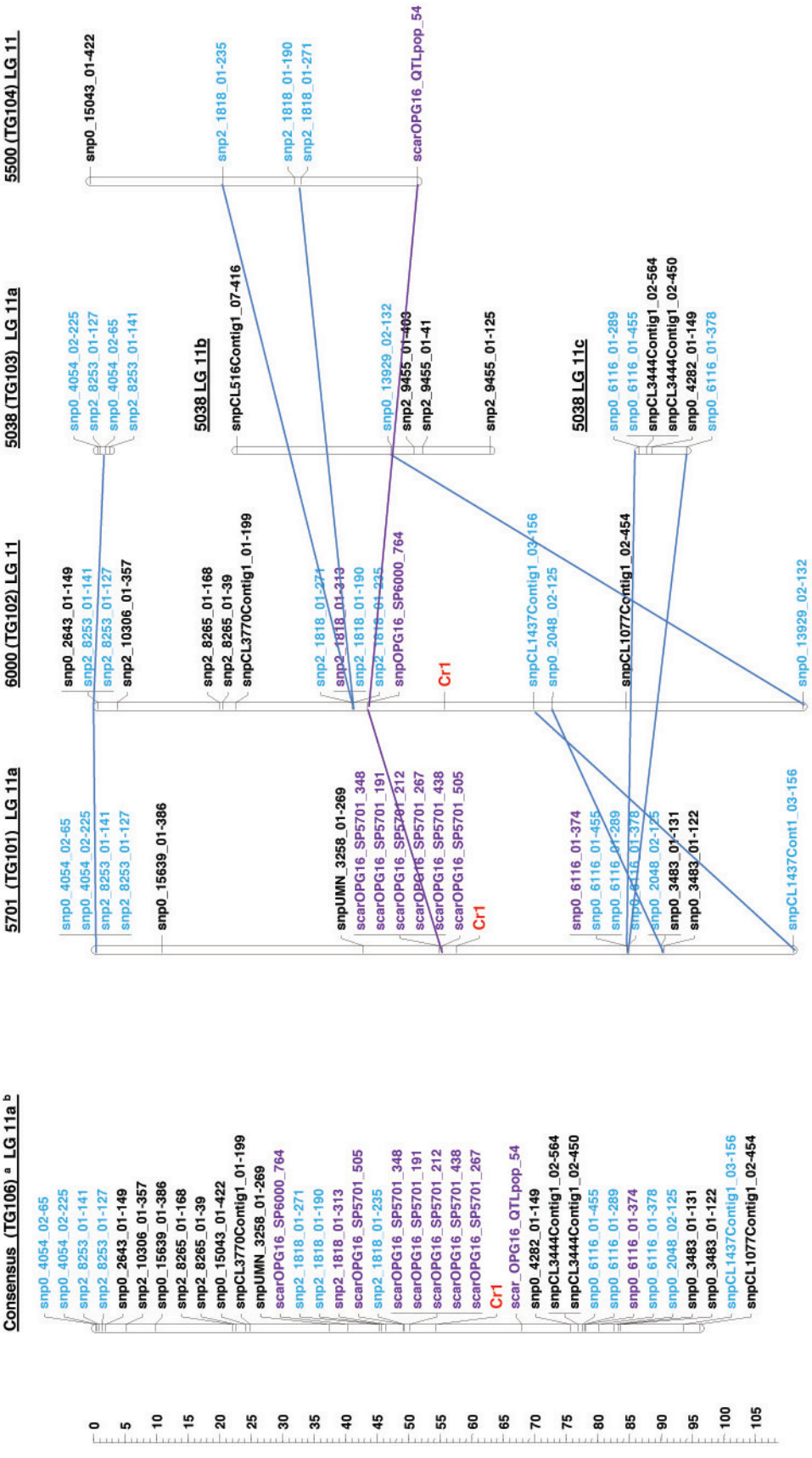
In our mapping study, two types of conserved orthologous sequence (COS) markers are observed: 1) TYPE I = the same amplicon but different SNPs within the amplicon, and 2) TYPE II = precise SNP location within the amplicon. When making intra-specific comparisons, both types of COS markers were available. However, in inter-specific comparative mapping, TYPE II COS markers are rare, as SNPs are usually not conserved across species. We first aligned the consensus map (TG106) with the four maps constructed for the individual trees 5701 (TG101), 6000 (TG102), 5038 (TG103), and 5500 (TG105). Subsequently, we aligned the consensus map with the loblolly pine reference map (TG091).

Intra-specific

Through comparative mapping within species, the integrity of the map can be determined by examination of marker groups and positions. Also, through comparative mapping, the position of genes/loci can be inferred from one map to another when orthologous markers align. For example, the hypersensitive response (i.e. *Cr1*) is not expressed in the parents of the *QTL* population (5038 x 5500) that is segregating for partial resistance. However, by aligning markers that are in common among the various trees (including the SCAR marker linked to *Cr1*), the mapped position of *Cr1* in trees 5701 and 6000 is inferred upon the maps constructed from the *QTL* population, even though *Cr1* is not expressed in this population (Fig. 1).

Inter-specific

We identified 60 TYPE I COS markers (amplicons) between sugar pine and loblolly pine, with 56 of them (93 percent) showing alignment to the loblolly pine map. Four of the markers were not collinear. A plausible explanation for this is that these markers are paralogs (alternate member



[a] Accession number assigned to maps in the TreeGenes Comparative Mapping Database (http://dendrome.ucdavis.edu/cmap/)

[b] Maps with > 1 linkage group aligning with the *P. taeda* map are given letter suffixes, such as "a", "b", etc.

Figure 1. Linkage group 11a of the sugar pine consensus map (TG106) aligned with the same linkage group from the four individual tree maps derived from three mapping populations. TYPE I COS markers (amplicons) that coaligned among two or three trees are shown in blue font. TYPE II COS markers (SNP) that coaligned among two or three trees are shown in purple font. The major gene for resistance to white pine blister rust (*Cr1*) is shown in red font.

Table 3. Summary of 56 collinear Type I COS markers in loblolly and sugar pine maps (Jermstad et al. 2010). Annotations are derived from BLAST queries of non-redundant (nr) plant protein sequences (BLASTx; http://www.ncbi.nlm.nih.gov).

Pt LG	Type i COS	*Pl* LG	GenBank GI	Annotation
1	*snp*CL3036Contig1_01	1a	255547830	Signal recognition particle subunit srp72, putative *[Ricinus communis]*
	*snp*UMN-1609-01	1a	N/A	-
	*snp*0-7471-01	1b	N/A	-
	*snp*0-18587-01	1b	115450977	Conserved hypothetical protein *[Oryza sativa]*
	*snp*0-18261-01	1b	18394104	Oxireductase *[Arabidopsis thaliana]*
2	*snp*2-374-01	2	N/A	-
	*snp*0-18470-01	2	N/A	-
	*snp*0-13565-01	2	255585824	small heat-shock protein *[Ricinus communis]*
	*snp*0-1453-01	2	255538284	fms interacting protein *[Ricinus communis]*
	*snp*UMN-915-01	2	42562204	senescence-associated E3 unbiquitin ligase 1 *[Arabidopsis thaliana]*
	*snp*CL572Contig1-04	2	255585914	alcohol dehydrogenase *[Ricinus communis]*
3	*snp*CL1530Contig1-04	3a	15237148	HTB2; DNA-binding; histone H2b *[Arabidopsis ricinus]*
	*snp*0-9922-01	3a	30689298	UBX domain-containing protein *[Arabidopsis thaliana]*
	*snp*CL1209Contig1-02	3b	15240918	transferase family protein *[Arabidopsis thaliana]*
4	*snp*0-5204-01	4	255570480	sec15, putative *[Ricinus communis]*
	*snp*0-17247-02	4	115459326	Armadillo-like helical domain containing protein *[Oryza sativa]*
	*snp*2-4011-03	4	15234240	HSP21 heat shock protein 21 *[Arabidopsis thaliana]*
	*snp*2-7808—01	4	115483694	Conserved hypothetical protein *[oryza sativa]*
	*snp*0-11649-03	4	224104341	beta tubulin *[Populus trichocarpa]*
5	*snp*CL4432Contig-04	5	42570490	AFC1 (ARABIDOPSIS FUS3-COMPLEMENTING GENE 1)
	*snp*CL544Contig1-03	5	15235213	Caffeoyl-CoA-O-methyltransferase *[Arabidopsis thaliana]*
	*snp*0-744-01	5	255550431	Xylem seine proteinase 1 precursor *[Ricinus communis]*
	*snp*2-5064-01	5	255551501	Big map kinase/bmk *[Ricinus commnunis]*
	*snp*0-12929-02	5	255551669	Receptor serine/threonine protein kinase *[Ricinus commnunis]*
6	*snp*0-806-01	6	115447491	GRAM domain contining protein *[Oryza sativa]*
7	*snp5488-02*	7a	255569410	Peroxidase 44 precursor *[Ricinus communis]*
	*snp*CL3162Contig1-02	7b	15238392	AtRAB4A GTP-binding protein *[Arabidopsis thaliana]*
	*snp*CL1698Contig1-01	7b	N/A	-
8	*snp*CL3037Contig1-06	8a	115455427	Similar to 60s ribosomal protein L13a-4 *[Oryza sativa]*
	*snp*CL3758Contig1-05	8a	115462873	Similar to TGF-beta receptor-interacting protein 1 [*Oryza sativa]*
	*snp*2-5724-02	8b	115472857	Homeodomain-related containing protein *[Oryza sativa]*
	*snp*CL363Contig1-04	8b	255564363	Rhicadhesin receptor precursor *[Ricinus communis]*
	*snp*2-5962-01	8b	30689268	PFT1 (PHYTOCHROM AND FLOWERING TIME 1) *[Arabidopsis thaliana]*
	*snp*CL2117Contig1-03	8b	255553619	Receptor protein kinase CLAVATA1 precursor *[Ricinus communis]*
9	*snp*0-12156-02	9	255543198	ATP binding protein *[Ricinus communis]*
	*snp*2-6541-01	9	N/A	-
10	*snp*CL1694Contig1-04	10a	115468878	Similar to Small nuclear ribonucleoprot4ein component [*Oryza sativa]*
	*snp*0-7321-01	10b	226531267	LOC100286137 *[Zea mays]*
	*snp*2-684-01	10b	168023746	LRR receptor-like protein [*Physcomitrella patens*]
	*snp*UMN-CL228Contig1-03	10b	255558550	40s ribosomal protein S26 *[Ricinus communis]*
	*snp*CL3116Contig1-03	10b	255548998	ran-family (Ras-related nuclear proteins) small gtpase *[Ricinus communis]*
	*snp*2-7852-01	10b	N/A	-
	*snp*2-8491-01	10b	25587817	acyl-CoA thioeserasse *[Ricinus communis]*
	*snp*CL305Contig1-05	10b	255556504	dihydrolipoamide dehydrogenase *[Ricinus communis]*
11	*snp*2-10306-01	11a	159469223	hydroxyproline-rich glycoprotein *[Chlamydomonas reinhardtii]*
	*snp*UMN-3258-01	11a	N/A	-
	*snp*0-13929-02	11b	115466184	GAGA binding- like family protein *[Oryza sativa]*
	*snp*2-9455-01	11b	190612857	pentatricopeptide repeat protein *[Picea abies]*
	*snp*2-3141-01	11c	15240885	disease resistsance protein (TIR-NBS-LRR class) *[Arabidopsis thaliana]*
12	*snp*0-17197-01	12	115474617	slu7a_arath pre-mRNA splicing Prp18-interacting factor [*Arabidosis thaliana*]
	*snp*0-16860-01	12	115436956	Armadillo-like helical domain containing protein *[Oryza sativa]*
	*snp*0-13058-01	12	255550387	polygalacturonase *[Ricinus communis]*
	snp0-489-01	12	N/A	-
	*snp*2-4724-01	12	115447049	Similar to protein kinase ATN1 *[Oryza sativa]*
	*snp*UMN-5833-01	12	255585558	S-adenosylmethionine-dependent methyltransferase *[Ricinus communis]*
	*snp*CL1052Contig-03	12	115443669	YqeH GTP-binding protein; nitric oxide synthase

of the same gene-family). All 19 linkage groups of the consensus sugar pine map found alignment with the 12 linkage groups of the loblolly pine reference map (Table 3). In some cases, multiple sugar pine linkage groups aligned to a single loblolly pine linkage group due to gaps in the sugar pine map. Where gaps exist in the map, a well-defined pine reference map can serve as a scaffold onto which other pine maps can align and be ordered. The sugar pine linkage group containing *Cr1*, aligned to linkage group 11 in loblolly pine (Table 3). Although loblolly pine is not susceptible to white pine blister rust and *Cr1* expression is not observed, a locus resembling *Cr1* might be present in all pines at this location, perhaps in a cluster of *R* genes, a phenomenon commonly observed in plants (Michelmore and Meyers 1998). It will be interesting to see what genes reside on this linkage group when the loblolly pine genome sequence becomes available.

Summary

We present the first genome-scale genetic map for sugar pine using several populations, two of which are segregating for the major gene resistance conferred by the *Cr1* locus. Because of marker collinearity and successful RAPD-to-SCAR conversion, we were able to infer the position of *Cr1* on the consensus map. We also present here the first comparative mapping study to show syntenic relationships between hard pines (subgenus *Pinus*) and soft pines (subgenus *Strobus*). Because we observe synteny between subgenus *Pinus* and subgenus *Strobus*, we anticipate an even greater degree of synteny among the members of the subgenus *Strobus*. The ability to transfer information regarding gene sequence (amplicon) and function across taxonomic boundaries will be invaluable, saving time and effort for future studies in the soft pines. This becomes particularly relevant as forests and landscapes are challenged by rapidly changing climate.

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