A Consensus Linkage Map for Sugi (*Cryptomeria japonica*) From Two Pedigrees, Based on Microsatellites and Expressed Sequence Tags

Naoki Tani,* Tomokazu Takahashi,† Hiroyoshi Iwata,*,[1] Yuzuru Mukai,‡ Tokuko Ujino-Ihara,* Asako Matsumoto,* Kensuke Yoshimura,* Hiroshi Yoshimaru,* Masafumi Murai,* Kazutoshi Nagasaka* and Yoshihiko Tsumura*,[2]

**Department of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan, †Graduate School of Science and Technology, Niigata University, Igarashi, Niigata 950-2181, Japan and ‡Faculty of Agriculture, Shizuoka University, Ohya, Shizuoka 422-8529, Japan*

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ABSTRACT

A consensus map for sugi (*Cryptomeria japonica*) was constructed by integrating linkage data from two unrelated third-generation pedigrees, one derived from a full-sib cross and the other by self-pollination of F_1 individuals. The progeny segregation data of the first pedigree were derived from cleaved amplified polymorphic sequences, microsatellites, restriction fragment length polymorphisms, and single nucleotide polymorphisms. The data of the second pedigree were derived from cleaved amplified polymorphic sequences, isozyme markers, morphological traits, random amplified polymorphic DNA markers, and restriction fragment length polymorphisms. Linkage analyses were done for the first pedigree with JoinMap 3.0, using its parameter set for progeny derived by cross-pollination, and for the second pedigree with the parameter set for progeny derived from selfing of F_1 individuals. The 11 chromosomes of *C. japonica* are represented in the consensus map. A total of 438 markers were assigned to 11 large linkage groups, 1 small linkage group, and 1 nonintegrated linkage group from the second pedigree; their total length was 1372.2 cM. On average, the consensus map showed 1 marker every 3.0 cM. PCR-based codominant DNA markers such as cleaved amplified polymorphic sequences and microsatellite markers were distributed in all linkage groups and occupied about half of mapped loci. These markers are very useful for integration of different linkage maps, QTL mapping, and comparative mapping for evolutional study, especially for species with a large genome size such as conifers.

TREE breeding is a time-consuming process, mainly because of the long intervals between generations, which has prevented tree breeders from using crossbreeding effectively. However, the presence of many molecular markers and use of quantitative trait locus (QTL) analysis make it possible to construct genetic maps, to detect QTL, and subsequently to perform marker-assisted selection for molecular breeding (Staub *et al.* 1996). A double pseudo-testcross strategy has generally been adopted for constructing genetic maps in conifers having allogamous characteristics (Grattapaglia and Sederoff 1994). Although this strategy exploits one of the characteristics of conifers, namely high heterozygosity within species, the average estimate of gene diversity within species in gymnosperms does not exceed 28.1% (Hamrick *et al.* 1992). Therefore, QTL analyses using multiple pedigrees should be important for understanding QTL within conifer species. However, difficulties of usage may be encountered when some types of genetic markers, such as randomly amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP), are used to function as a "bridge" marker to merge linkage groups or QTL derived from different pedigrees. For map comparisons to be meaningful, a detection of the orthologous locus in each pedigree can be achieved by DNA sequence homology and conserved map location (Brown *et al.* 2001). Thus, markers based on expressed sequences, such as cDNA-based restriction fragment length polymorphisms (RFLPs) and cleaved amplified polymorphic sequences (CAPS) derived from expressed sequence tags (ESTs), should be used as bridge markers for integrating maps from different pedigrees. Especially in species with large genomes, such as conifers, the signal of a single-copy gene in RFLP analysis is generally weak, and the DNA is not usually well digested because it is methylated (Iwata *et al.* 2001). Therefore, a large number of CAPS markers derived from ESTs are especially valuable as bridge markers between multiple pedigrees. In addition, mapping with multiple populations provides several advantages over mapping based on a single population. In particular, many candidates for bridge loci derived from ESTs can be placed on a single map. Therefore, dense consensus maps including numerous EST-based markers be-

[1]*Present address:* National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan.

[2]*Corresponding author:* Department of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan. E-mail: ytsumu@ffpri.affrc.go.jp

come fundamental tools for comparing linkage groups and QTL derived from different pedigrees.

Sugi (Japanese cedar), *Cryptomeria japonica* D. Don, is an important forest tree, because of its excellent characteristics, including rapid growth, straight bole, ready regeneration, and soft wood with a pleasant color and scent. Several projects to map the sugi genome have been undertaken on the basis of different marker systems and types of segregating populations, such as a full-sib F_1 population (Kuramoto *et al.* 2000; Nikaido *et al.* 2000) and a three-generation pedigree derived from self-pollination of F_1 individuals (Mukai *et al.* 1995; Iwata *et al.* 2001). Some loci were identified relating to quantitative traits such as juvenile growth rate, profusion of flowering, rooting ability of cuttings (Yoshimaru *et al.* 1998), and modulus of elasticity of the wood (Kuramoto *et al.* 2000). The information contained in these maps is sufficient for integration to correlate the loci identified on them.

In the Pinaceae, intensive genome studies have been conducted on *Pinus teada* (*e.g.*, Sewell *et al.* 1999; Brown *et al.* 2001; Temesgen *et al.* 2001). Genome studies have been extended to other pine species, such as *P. radiata* (Devey *et al.* 1999) and *P. elliottii* (Brown *et al.* 2001), and have resulted in the partial construction of comparative maps. However, pine genomes are large (*e.g.*, the estimated *C*-value for loblolly pine is 21–23 pg; Wakamiya *et al.* 1993) and contain complex gene families (Kinlaw and Neale 1997). The large genome has caused some problems, such as nonidentical allelic association of RFLP patterns (*e.g.*, Jermstad *et al.* 1994; Devey *et al.* 1996; Sewell *et al.* 1999). In contrast, *C. japonica* has several advantages in genome studies relative to the Pinaceae. First, Tsumura *et al.* (1997) developed EST markers derived from *C. japonica* cDNA, used them as markers in PCR amplification in related families, and showed that more than half of these EST markers, including single and multiple fragments, were also targetable under low-stringency conditions in DNA from other members of the Taxodiaceae, Cupressaceae, Sciadopitaceae, and Pinaceae. Second, the estimated *C*-value for *C. japonica* is only ~11 pg (Hizume *et al.* 2001), approximately half the genome size of pine. Third, gibberellin treatment promotes flower-bud formation, which accelerates production of the next generation. *C. japonica* can also be rapidly propagated by cuttings.

This article presents the results of integrating the linkage data from two independent pedigrees into a single consensus map. This consensus map will serve as a fundamental tool for molecular breeding in *C. japonica* and related species and a basis for studies of genome organization and evolution in conifers.

MATERIALS AND METHODS

Mapping populations: Two unrelated native cultivars, Yabukuguri and Iwao, were used for the first generation of the first pedigree (referred to as YI). These two cultivars were selected for QTL analysis as to growth patterns. Growth patterns of Yabukuguri and Iwao were slow and quick growers, respectively. Furthermore, Yabukuguri shows a trait for poor male flower fertility. Two unrelated native cultivars, Kumotooshi and Okinoyama, were used for the first generation of the second pedigree (referred to as KO). These two cultivars were selected by Ohba and co-workers (Kawasaki *et al.* 1984; Ohba *et al.* 1988) as part of an effort to clarify heritable traits of heartwood color (red and black). In the KO pedigree, 73 self-pollinated progeny of the third generation were derived from self-fertilization of an F_1 plant from Kumotooshi × Okinoyama. The KO was previously used to construct a linkage map based on RFLP, RAPD, and isozyme markers and a morphological trait (Mukai *et al.* 1995); later it was used to add CAPS markers to the linkage map (Iwata *et al.* 2001). Because high segregation distortion ratios were detected when these former linkage maps were constructed (Mukai *et al.* 1995; Iwata *et al.* 2001), sib-cross strategy of the two F_1 plants of Yabukuguri × Iwao was adapted to obtain 150 full-sib progeny of the third generation in the YI pedigree (Figure 1).

Genetic markers: For the YI pedigree, four kinds of genetic markers were used to construct the linkage map: 146 CAPS markers, 133 RFLP markers obtained with 119 cDNA probes, 42 microsatellites, and five single nucleotide polymorphisms (SNPs) in three genes. For the KO pedigree, we used 96 CAPS markers, 122 RFLP markers (117 probes derived from cDNA and 3 probes derived from genomic DNA libraries), 33 RAPD markers with dominant manner, one isozyme, and one morphological trait. Of the 96 CAPS markers in KO, segregation data for 68 were obtained, and 46 have already been assigned to positions on the KO linkage map (Iwata *et al.* 2001). We investigated the segregation patterns of 28 additional CAPS markers in the KO pedigree (appendix a). In all, the segregation patterns of 326 genetic markers in the YI pedigree and 253 in the KO pedigree were determined (Table 1).

The primary source of RFLP probes was the *C. japonica* cDNA libraries constructed by Mukai *et al* (1995) and Ujino-Ihara *et al.* (2000). These probes were used in combination with six restriction enzymes (*Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, and *Hin*dIII). The primer pairs for CAPS included those used by Tsumura *et al.* (1997) and Iwata *et al.* (2001) and the continuously developed primer pairs listed in appendix a, which were derived from the sequence information of two cDNA libraries (Mukai *et al.* 1995; Ujino-Ihara *et al.* 2000). Microsatellite markers were derived from three different microsatellite-enriched genomic libraries developed by Moriguchi *et al.* (2003) and N. Tani, T. Takahashi, T. Ujino-Ihara, H. Iwata, K. Yoshimura and Y. Tsumura (unpublished data; listed in appendix b). Genotypes of 42 microsatellite loci were determined by electrophoresis on 7.5% polyacrylamide gels (ethidium bromide stain). To determine SNP genotypes, PCR products were purified and sequenced using Big Dye terminator cycle sequencing kits (Perkin-Elmer, Foster City, CA), following the manufacturer's recommendations and the corresponding primer for each gene on an Applied Biosystems (Foster City, CA) model 3100 automated sequencer.

Identification of orthologous markers: We detected 45 orthologous CAPS markers between the KO and YI pedigrees, which were found by the coincidental existence of polymorphisms between the pedigrees in the second generation. To increase the number of orthologous markers, we screened for polymorphisms in the second-generation individuals of the YI pedigree by using probes for all RFLP markers found on the KO linkage map. We found that 29 RFLP loci yielded polymorphisms between the two full-sib individuals of the second generation of the YI pedigree. In all, 70 orthologous markers were used to integrate the two independent linkage maps.

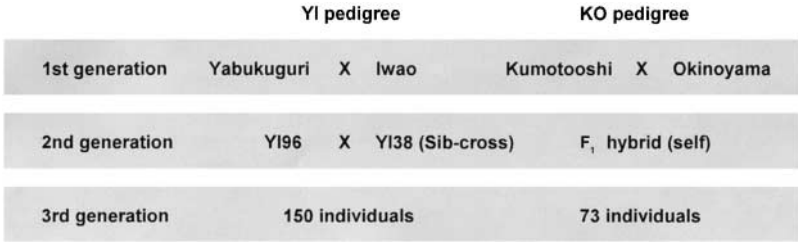


FIGURE 1.—Two three-generation pedigrees used for Cryptomeria genetic mapping. The third-generation progeny of YI and of KO were derived from sib-crosses and self-fertilization in the second generation, respectively.

Genetic linkage analysis and map construction: Because the segregating generation in the YI pedigree was produced by sib crossing in the second generation, a double pseudo-testcross strategy was adopted for linkage analysis (GRATTAPAGLIA and SEDEROFF 1994). Each segregating marker was scored individually for all configuration types defined in Figure 2 (RITTER *et al.* 1990). The segregation ratio of each marker was tested with a chi-square test for goodness of fit to the expected 1:1 ratio when the marker was present in one of the two parents or to the expected 3:1 ratio when the marker was present in both parents. In the first round of analysis, segregation data were used to construct two maps based on meiosis in both F_1 parents, YI96 and YI38. Two data matrices, therefore, were designed to construct the two parental linkage maps. Markers belonging to configuration type b (Figure 2) were ignored in this round of analysis. For markers belonging to configuration type c, the data from heterozygous individuals were ignored, because the parental origin could not be deduced. Parental maps were then constructed with MAPMAKER 3.0 (LANDER *et al.* 1987) using the backcross option. The linkage phase was deduced statistically from two-point linkage data. The highest two-point linkage LOD value indicates the putative correct linkage phase in the reciprocal data set (given phase and reverse phase). Markers were initially associated by using the "Group" command (two-point comparison). For each linkage group, marker orders were then defined by using the "Order" command. Three different orders were compared for three different information values in MAPMAKER (1, 2, and 5 cM) at LOD = 2.0. Other markers were then added with the "Try" command. The "Ripple" command was employed at LOD $\geq$ 2.0 to assess the robustness of the marker order.

In the second round of analysis, linkage analysis for the YI pedigree was done with JoinMap 3.0 (VAN OOIJEN and VOORRIPS 2001), using the parameter set for progeny derived by cross-pollination (CP). In this analysis, all the configuration patterns shown in Figure 2 were used. Two recombination data sets derived from the parental meiosis were analyzed together. Linkage groups were assigned with a minimum LOD threshold of 3.8. Loci that were completely linked were identified and removed from the data set before the marker order within groups was determined. Map distances were calculated with KOSAMBI's (1944) mapping function.

The two data sets were merged for linkage groups that retained markers orthologous to each other. The YI linkage map was also integrated with the previously constructed KO linkage map by using JoinMap 3.0. Highly skewed marker-segregation ratios ($P < 0.001$) were removed when the integrated map was constructed. The integrated map was constructed on the basis of the mean recombination frequency and the combined LOD scores. The images of the linkage groups were drawn with MAPCHART (VOORRIPS 2002).

Estimation of genome length and map coverage: The estimated genome length G_e was determined from the partial linkage data according to $G_e = N(N-1)X_e/K$ with a confidence interval of $G_e/(1 \pm 1.96/\sqrt{K})$, where N is the number of markers and thus $N(N-1)$ is the number of pairwise

TABLE 1

Number of genetic markers used for map construction

Mapping population	CAPS	RFLP	Microsatellite	SNP	RAPD	Isozyme	Morphological trait	Total
Total no. of loci per marker type								
YI	146	133	42	5	0	0	0	326
KO	96	122	0	0	33	1	1	253
Orthologous locus	45	29	0	0	0	0	0	74
Population total	197	226	42	5	33	1	1	505
Total no. of loci used for map construction per marker type								
YI	130	125	38	5	0	0	0	298
KO	94	114	0	0	33	1	1	243
Orthologous locus	44	26	0	0	0	0	0	70
Population total	180	213	38	5	33	1	1	471
No. of mapped loci per marker type[a]								
YI	128	121	37	5	0	0	0	291
KO	96	116	0	0	31	1	1	245
Orthologous locus	41	24	0	0	0	0	0	65
Population total	181	213	37	5	31	1	1	469
Consensus map	172	200	37	5	22	1	1	438

[a] Number of loci were counted on the second-round analysis maps (constructed with JoinMap 3.0).

comparisons. X_e is the maximum distance between two adjacent markers in centimorgans at a certain minimum LOD score, and K is the number of marker pairs with the same minimum LOD score (Hulbert *et al.* 1988; Chakravarti *et al.* 1991). A minimum LOD score of 3.8 was chosen to estimate the genome.

To calculate the observed genome length, the total length of the map G_t was calculated. In addition, the observed genome length G_o was calculated by the formula of Nelson *et al.* (1994), which takes into account all markers, linked and unlinked: $G_o = G_t + X_o(L - R)$, where X_o is the observed maximum distance between two framework markers; L is the total number of linkage groups, triplets, doublets, and unlinked markers; and R is the haploid number of chromosomes.

The expected genome map coverage C_e was calculated from the equation $C_e = 1 - e^{-XeN/1.25Ge}$ (Lange and Boehnke 1982), adjusted for chromosome ends. In this equation, N is the number of framework markers; X_e is the maximum distance between two adjacent framework markers in centimorgans at a certain minimum LOD score; and G_e is the estimated genome length. Only framework markers were considered, because these equations refer to randomly distributed markers. The observed map coverage C_o is defined as the ratio of the observed genome length G_o to the estimated genome length G_e.

RESULTS

Genetic markers: From 187 CAPS primer pairs, 210 probes derived from three cDNA libraries, 42 microsatellite markers, five SNPs in three genes, 26 RAPD primers, one isozyme stain, and one morphological trait, a total of 505 genetic markers that segregated among the progeny of the segregating generation in the two independent pedigrees of *C. japonica* were identified. Of these 505 markers, a total of 444 markers (176 CAPSs, 197 RFLPs, 42 microsatellites, five SNPs, 22 RAPDs, one isozyme, and one morphological trait) were identified as unique markers. The remaining 61 markers yielded 2–4 loci per marker and were restricted to the categories of gene-based markers (CAPS and RFLP) and RAPD. On average, between the two pedigrees, each marker type yielded 1.15 (YI) and 1.08 (KO) unique segregation loci, except for RAPD. The maximum numbers of scorable segregation loci per marker type were 4 (CAPS), 3 (RFLP), and 3 (RAPD). Although some previous studies in conifers reported that allelic associations among RFLP fragments could not be identified for some loci because of too many bands per single gel image (Devey *et al.* 1996; Jermstad *et al.* 1998; Sewell *et al.* 1999), our RFLP image allowed allelic association among RFLP fragments to be deduced, owing to fewer bands per single image.

Segregation distortion: A chi-square test was performed to test the null hypotheses of segregation ratios of 1:1, 1:2:1, and 3:1 for markers in the YI pedigree and of 1:2:1 and 3:1 for markers in the KO pedigree. The segregation ratios of 58 (17.8%) and 61 (25.1%) markers were significantly distorted ($P \leq 0.05$) from the expected Mendelian ratios in the YI and KO pedigrees, respectively. For 37 (11.3%) and 32 (13.2%) markers in the YI and KO pedigrees, respectively, the differences from the expected Mendelian ratios were even more significant ($P \leq 0.01$; Table 2). When we ignored the results for SNP, morphological trait, and isozyme markers (owing to their small numbers), the CAPS markers had the highest percentages of distorted segregation in both pedigrees (19.9% in YI, 31.3% in KO). The percentage of RFLP markers with distorted segregation ratios was slightly lower than that of the CAPS markers. However, microsatellite markers, on the basis of noncoding regions of the genome, represented only a small percentage of markers with distorted segregation, compared with the CAPS and RFLP markers (7.1% of microsatellite markers in YI). The RAPD markers, on the basis of bands randomly extracted from the entire genome, indicated that 19.4% of markers were distorted in the KO pedigree.

These markers with highly distorted segregation ratios at the 0.1% level were excluded from linkage analysis. However, we included the KO markers showing distorted segregation ratios in the linkage analysis, because Mukai *et al.* (1995) and Iwata *et al.* (2001) used them in linkage analyses and obtained plausible maps for the KO pedigree. They observed that most of these markers were clustered on the linkage maps, which led them to speculate that the main cause of the segregation-ratio distortions was linkage with deleterious or lethal alleles (Mukai *et al.* 1995; Iwata *et al.* 2001). If we exclude these markers with distorted segregation ratios, it would be difficult to obtain long enough linkage groups, because the cluster of ignored markers would hamper making connections between linkage groups on both sides of the cluster. We therefore included these markers with distorted segregation ratios in the linkage analysis of the KO pedigree.

First round of linkage analysis: Linkage analysis in the YI pedigree was based on 130 CAPS markers, 125 RFLP markers, 38 microsatellite markers, and 5 SNP markers. When we did a first-round analysis with MAPMAKER software, we split the data set into separate subsets of data for constructing linkage maps corresponding to parental meiosis. Seventy-seven CAPS markers, 86 RFLP markers, 21 microsatellites, and 4 SNP markers segregated in the gametes of the YI96 parent. A scaffold map was obtained at a LOD of 3.8 and a distance-linkage criterion, θ, value of 0.3. Twelve major linkage groups and 1 unlinked marker were found. During marker ordering, 141 markers were placed in the linkage groups, but 46 other markers could not be placed. The observed and estimated map lengths were estimated to be 1650.9 and 2168.5 cM, respectively, at a LOD score of 3.8, and 95.9% of the genome was estimated to be covered by the linkage map of YI96 (Table 3).

In the YI38 parent's meiosis, 83 CAPS markers, 26 microsatellite markers, 64 RFLP markers, and 4 SNP markers segregated in the gametes. A scaffold map was also obtained at a LOD of 3.8 and a θ of 0.3. Sixteen

TABLE 2

Numbers of markers with distorted segregation ratios for each marker type

Mapping population	Marker type	No. of distorted loci[a]				
		$P < 5$	$P < 1$	$P < 0.1$	$P < 0.01$	Total
YI	Total	21 (6.4)	12 (3.7)	5 (1.5)	20 (6.1)	58 (17.8)
	CAPS	11 (7.5)	4 (2.7)	1 (0.7)	13 (8.9)	29 (19.9)
	Microsatellite	0 (0.0)	1 (2.4)	0 (0.0)	2 (4.8)	3 (7.1)
	RFLP	9 (6.8)	6 (4.5)	4 (3.0)	5 (3.8)	24 (18.0)
	SNP	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)	2 (40.0)
KO	Total	29 (11.9)	14 (5.8)	6 (2.5)	12 (4.9)	61 (25.1)
	CAPS	13 (13.5)	7 (7.3)	1 (1.0)	9 (9.4)	30 (31.3)
	Morphological traits	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Isozyme	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
	RAPD	3 (9.7)	2 (6.5)	1 (3.2)	0 (0.0)	6 (19.4)
	RFLP	13 (10.5)	4 (3.2)	4 (3.2)	3 (2.4)	24 (19.4)

[a] Values in parentheses are percentages representing the ratio of distorted markers at each significance level to total of significantly distorted markers.

major linkage groups and 4 unlinked markers were obtained in the first-round analysis. During marker ordering, 137 markers were placed in linkage groups, but 32 other markers could not be placed. The observed and expected map length estimates were 1584.8 and 1810.1 cM, respectively. The expected map coverage estimate indicated that 96.1% of the genome was covered by the linkage map based on YI38 meiosis (Table 3).

We obtained one more linkage map, based on F_1 hybrid meiosis in the KO pedigree. Twenty-eight additional CAPS markers were added to the data set for linkage analysis. A scaffold map was obtained at a LOD of 4.0 and a θ of 0.3. Ninety-seven CAPS markers, 123 RFLP markers, 31 RAPD markers, one isozyme, and one morphological trait segregated in the gametes of these F_1 hybrids. Twelve major linkage groups were recognized; no unlinked markers were observed. Upon ordering of these markers, locations of 193 markers were determined on the KO linkage map; 60 other markers, however, could not be placed. The observed and expected map length estimates were 1165.0 and 1395.5 cM, respectively. The estimated map coverage rate of the KO linkage map was 96.5% of the genome (Table 3). Clustering of markers resulted in overestimation of the genome size. Therefore, we first evaluated whether the genetic markers were randomly distributed or not; all linkage groups were divided into 5-, 10-, and 20-cM intervals, respectively, following the method of Cervera *et al.* (2001). We detected statistically significant clustering of markers in the YI96 map calculated by MAPMAKER at all intervals. The genetic markers on the YI38 map calculated by JoinMap also were significantly clustered only at 20-cM intervals. It is possible that the genome length estimates of these maps were overestimated.

Second round of linkage analysis: A total of 146 CAPS markers, 133 RFLP markers, 42 microsatellites, and 5 SNP markers segregated in the gametes of the YI96 and YI38 parents, and these were used for synthetic map construction with the CP of JoinMap 3.0 (Van Ooijen

TABLE 3

Observed and expected genome length and map coverage estimates for *Cryptomeria japonica*

Genome length	MapMaker			JoinMap		
	YI96	YI38	KO	YI96	YI38	KO
Observed						
G_o	1650.9	1584.8	1165.0	1325.0	1291.5	929.3
G_t	1567.3	1259.0	1138.6	1037.0	924.3	853.3
Estimated						
Genome length (G_e)	2168.5	1810.1	1395.5	1608.4	1632.4	1121.8
Lower bound	2011.2	1678.3	1299.4	1488.5	1500.2	1056.2
Higher bound	2352.5	1964.4	1507.1	1749.4	1790.1	1196.1
Observed and estimated genome coverage						
C_o (%)	76.1	87.6	83.5	82.4	79.1	82.8
C_e (%)	95.9	96.1	96.5	95.7	94.1	97.7

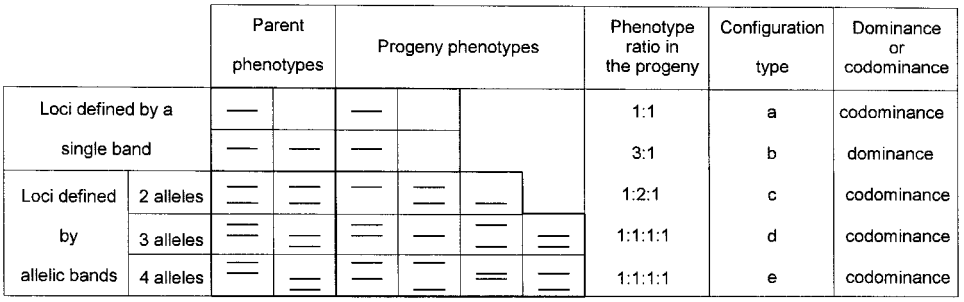


FIGURE 2.—Informative patterns for mapping as defined by RITTER *et al.* (1990). The five configurations presented correspond to segregating loci in the progeny. All the segregating-locus configurations found in an F_2 generation match one of these five configurations. Two kinds of loci are shown: loci defined by a single band, for which only one segregating allele is observed, and loci defined by allelic bands, for which the different segregating alleles are observed.

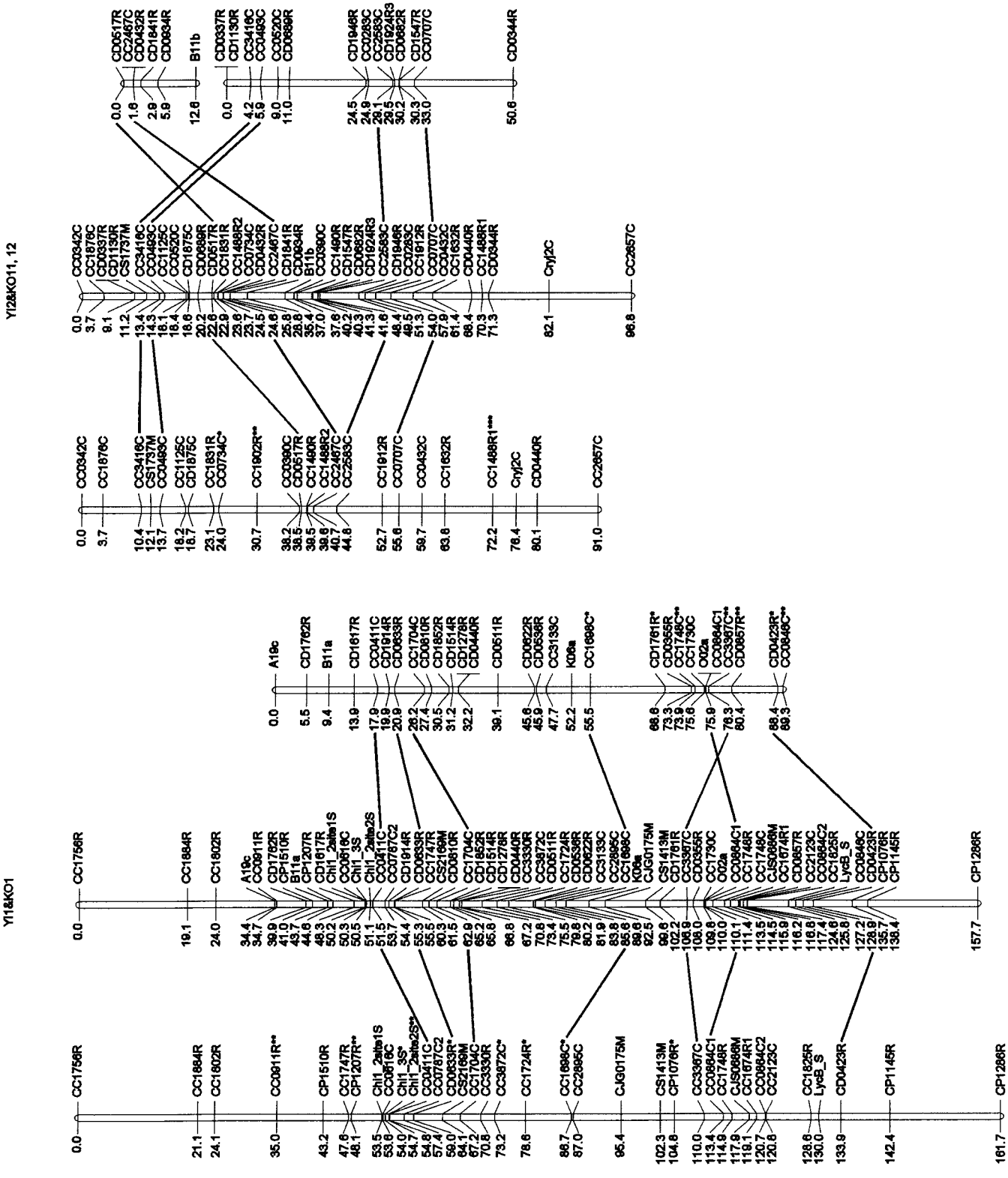
and VOORRIPS 2001). In the second-round analysis, the CP-type data set was used to calculate recombination rates between markers belonging to five configuration types (Figure 2; MALIEPAARD *et al.* 1997; VAN OOIJEN and VOORRIPS 2001): The segregation pattern of 232 markers was configuration type a, 6 was type b, 59 was type c, 23 was type d, and 6 was type e. Twenty-five loci were removed from the data set because of high ($P < 0.1$) distortion in their segregation ratios. The YI map constructed with JoinMap included 301 genetic markers, making a total of 291 loci that were found to be linked, with a LOD of 3.8. The 291 markers were assigned to 12 linkage groups and covered 1294.4 cM. On average, the linkage map of the YI pedigree presented one marker every 4.3 cM (Figure 3).

For the KO pedigree, second-round linkage analysis was also done with JoinMap 3.0 using the F_2 population type code. For linkage analysis, 243 markers were used and 237 markers were found to be linked with a LOD of 4.0 and were assigned to 14 linkage groups, which covered 817.2 cM. On average, the linkage map of the KO pedigree presented 1 marker every 3.0 cM (Figure 3).

Construction of the consensus map: A total of 180 CAPS markers, 213 RFLP markers, 38 microsatellites, 5 SNP markers, 33 RAPD markers, one isozyme, and one morphological trait were used to construct the consensus map. The segregation data from the two independent pedigrees contained 70 orthologous markers. We observed good correlation of the two-point distances between orthologous markers in the KO and YI pedigrees (Figure 4). We used the "Combine Groups for Map Integration" command of JoinMap 3.0. After the multiple linkages containing the same orthologous markers were associated, a consensus map was constructed. We observed 6 markers in which each probe or primer set derived from a single cDNA source belonged to unrelated linkage groups in the consensus map. In these cases, we refer to these markers as putative paralogous markers and omitted them from the list of orthologous markers. The consensus map produced from 65 orthologous markers included 172 CAPS markers, 200 RFLP markers, 37 microsatellites, 5 SNP markers, 22 RAPD markers, one isozyme, and one morphological trait. A total of 438 markers from the KO pedigree spanning 1372.2 cM were assigned to 11 large linkage groups, 1 small linkage group, and 1 unintegrated linkage group. On average, the consensus map presented 1 marker every 3.0 cM (Figure 3).

The KO5 linkage group contained four markers that were orthologous with the YI9&KO3 linkage group in the consensus map. When we included three linkage groups together in calculating a consensus linkage map, the marker ordering in the YI9&KO3&KO5 linkage group was, however, largely contradictory to the marker ordering in YI9. Therefore, we stopped adding the segregation data of KO5 to those of YI9 and KO3. Furthermore, we observed 10 contradictions in orthologous marker ordering between the consensus map and the

FIGURE 3.—Linkage maps for *C. japonica*. The linkage groups on the left were derived from segregation data for the YI pedigree and, on the right, from segregation data for the KO pedigree. The linkage groups in the center were derived from integration analysis of both sets of segregation data with JoinMap 3.0. Markers that are orthologous between the two pedigrees are indicated by allelic bridges. Markers not suitable for integration are indicated by dotted bridges. Loci showing distorted segregation ratios are marked with one ($0.01 < P < 0.05$), two ($0.001 < P < 0.01$), or three ($P < 0.001$) asterisks. The first one to three letters of the locus names indicate the origin of the genetic markers: CC, cDNA library derived from cambium; CD, cDNA library derived from seedlings; CP, cDNA library derived from pollen grains; GD, random genomic library; CS, CJG, and CJS, the three microsatellite-enriched genomic libraries; single letter, RAPD markers derived with the Operon 10mer kit; LAP, leucine aminopeptidase isozyme marker; and MT, morphological trait. Marker types are indicated by the last letter in the locus names: C, CAPS; M, microsatellite; R, RFLP; and S, SNP. Numbers at the end of locus names mean that locus duplication has occurred for that marker.


Yi18&KO1
Yi28&KO11, 12
CC1756R
CC1884R
CC1802R
CC0911R**
CP1510R
CC1747R
CP1207R**
Chl1_2alte1S
CC0516C
Chl1_3S*
Chl1_2alte2S**
CC0411C
CC0787C2
CD0633R*
CS2169M
CC1704C
CC3330R
CC3872C*
CC1724R*
CC1696C*
CC2885C
CJG0175M
CS1413M
CP1076R*
CC3397C
CC0884C1
CC1748R
CJS0886M
CC1674R1
CC1748C
CC0884C2
CC2123C
CC1825R
LycB_S
CD0423R
CP1145R
CP1286R
A19c
CD1762R
B11a
CD1617R
CC0411C
CD1914R
CD0633R
CC1704C
CD0810R
CD1852R
CD1514R
CD1278R
CD0440R
CD0511R
CD0622R
CD0536R
CC3133C
K06a
CC1696C*
CD1761R*
CD0355R
CC1748C**
CC1730C
O02a
CC0884C1
CC3367C**
CD0857R**
CD0423R*
CC0846C**
CC0342C
CC1876C
CD0337R
CD1130R
CS1737M
CC3416C
CC0483C
CC1125C
CC0520C
CD1875C
CD0689R
CD0517R
CC1831R
CC1488R2
CC0734C
CC2467C
CD1841R
CD0834R
B11b
CC0390C
CC1490R
CC1547R
CD0852R
CD1924R3
CC2583C
CD1946R
CC0283C
CC1912R
CC0707C
CC0432C
CC1632R
CD0440R
CC1488R1
CD0344R
Cryj2C
CC2857C
CD0517R
CC2467C
CD0432R
CD1841R
CD0834R
CD0337R
CD1130R
CC3416C
CC0483C
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CD1547R
CC0707C
CD0344R

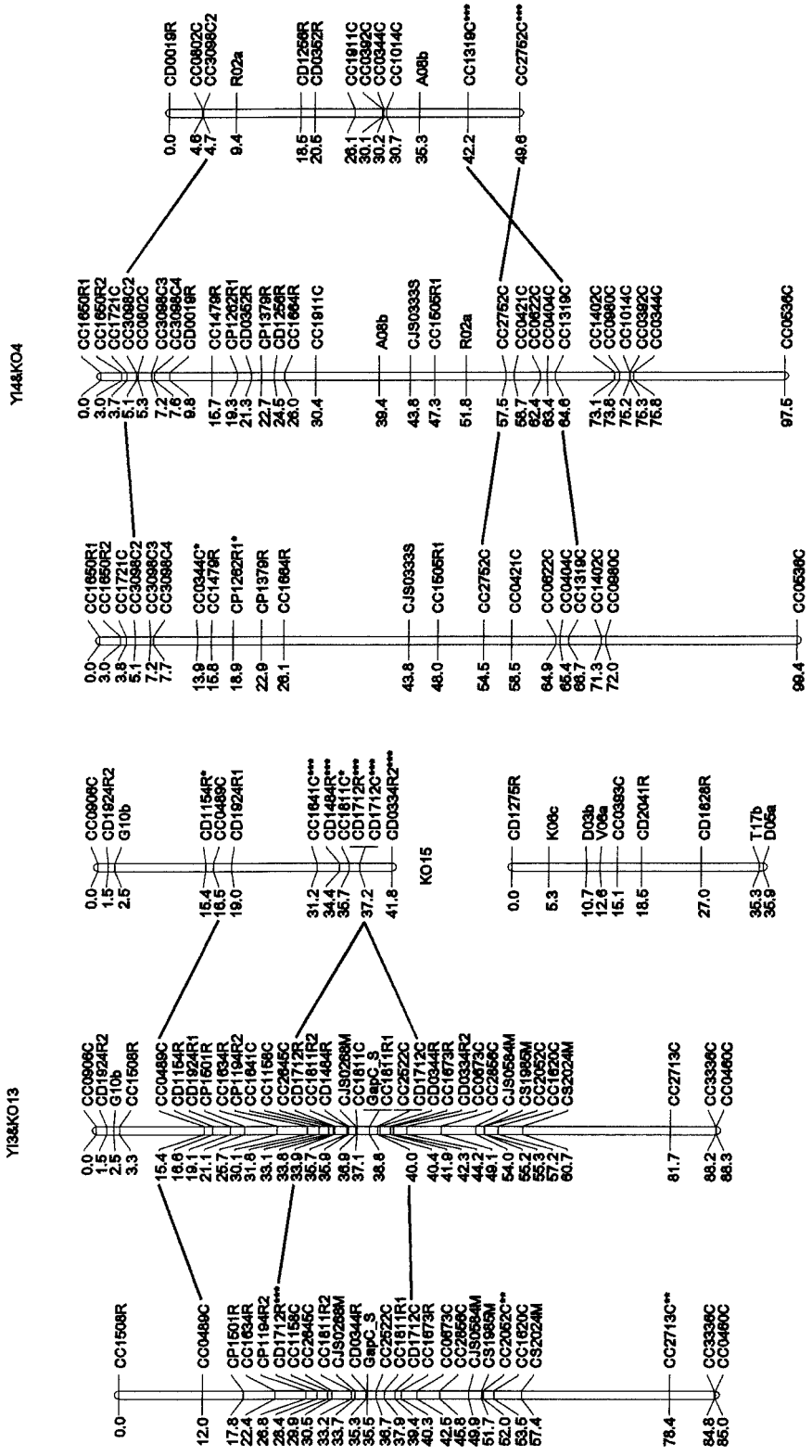

FIGURE 3.—*Continued.*

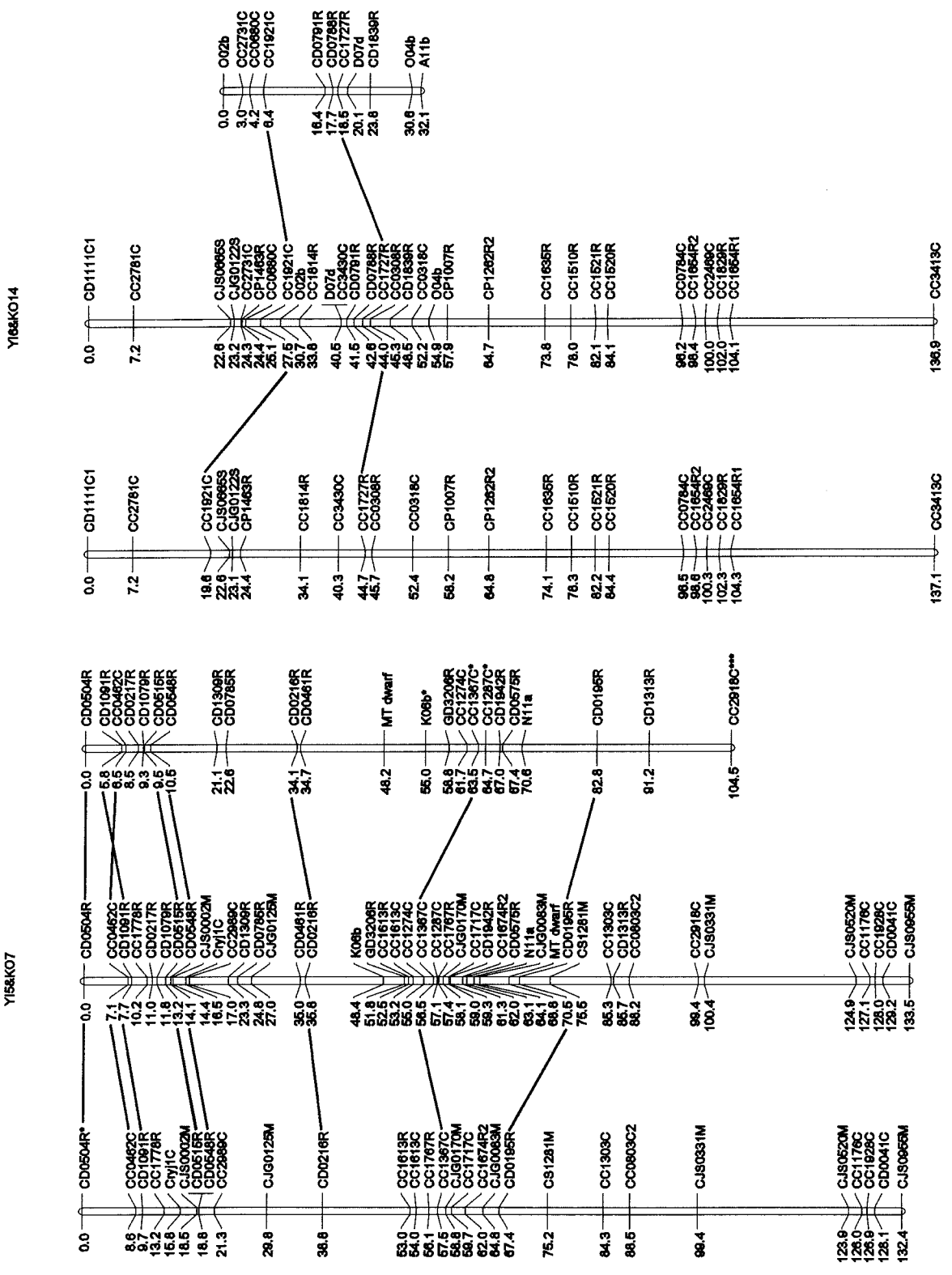

FIGURE 3.—*Continued.*

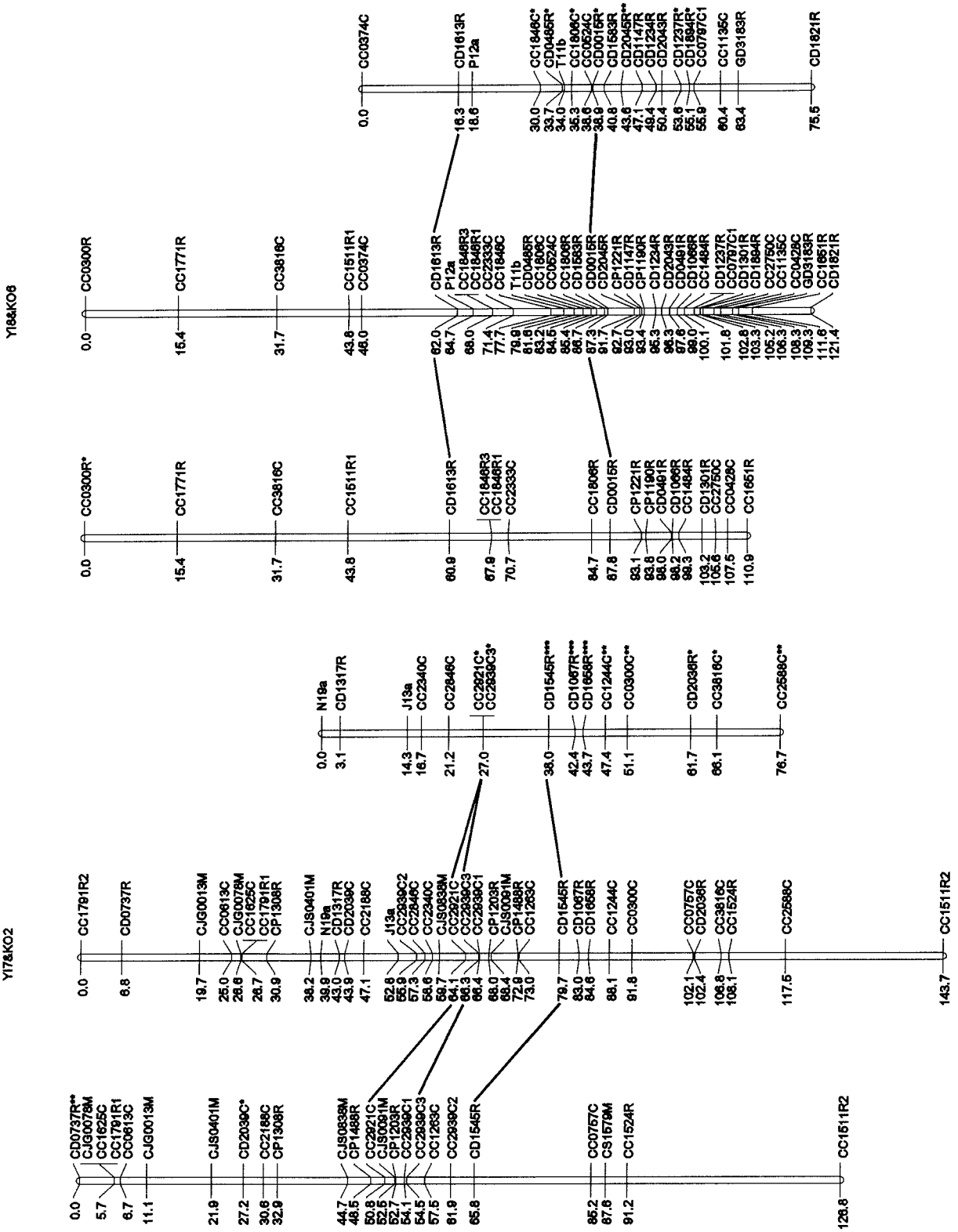

FIGURE 3.—*Continued.*

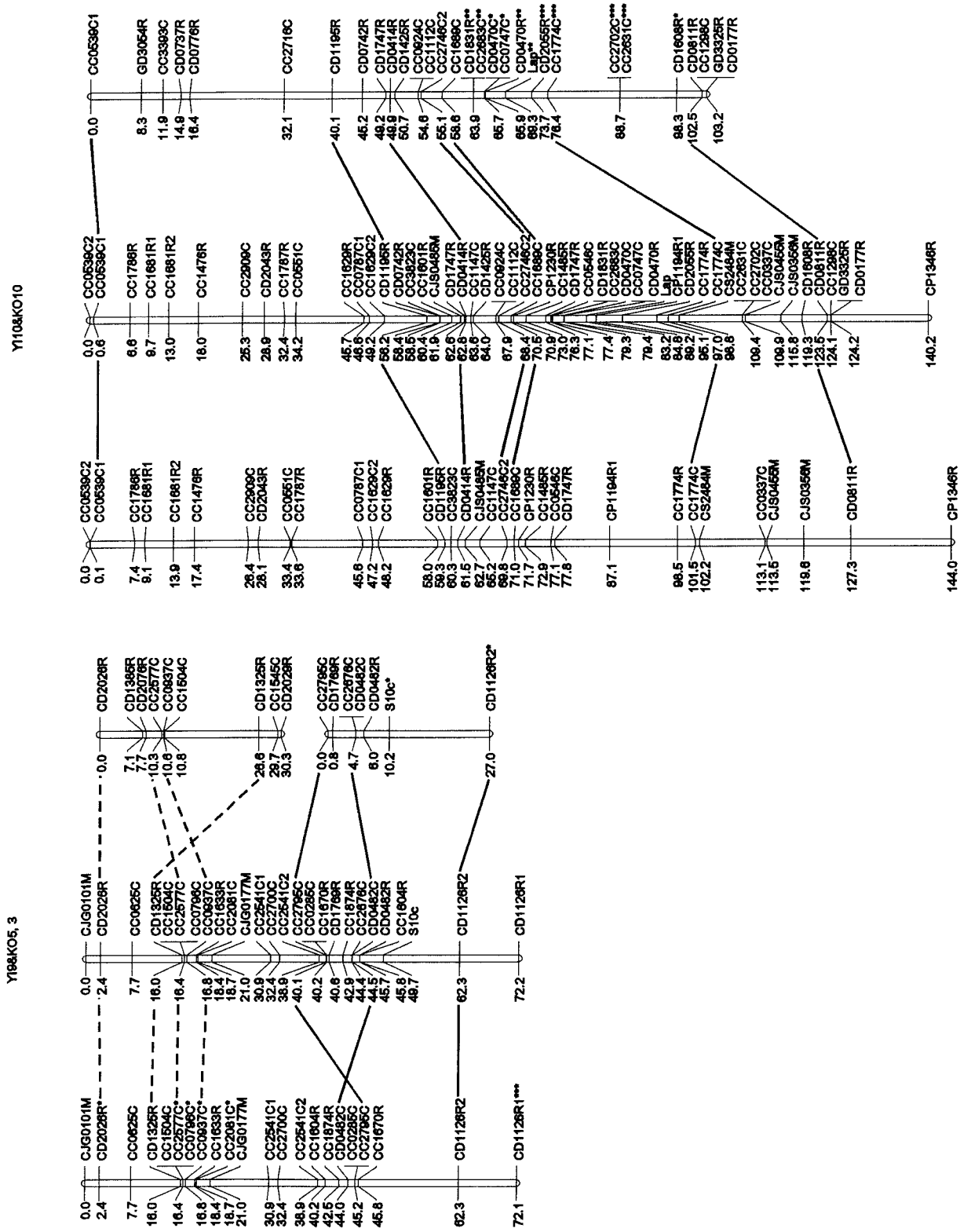

FIGURE 3.—*Continued.*

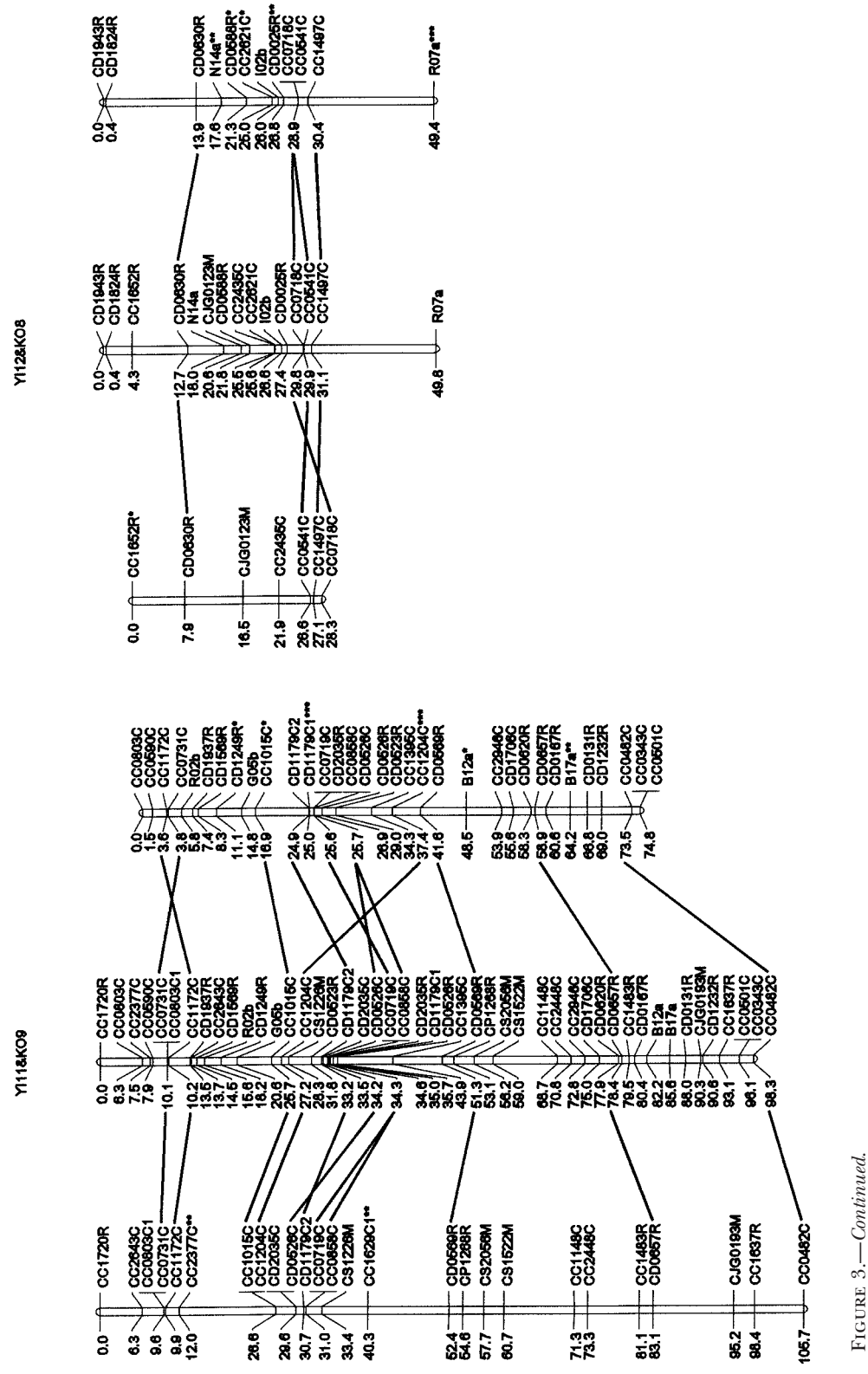

FIGURE 3.—*Continued.*

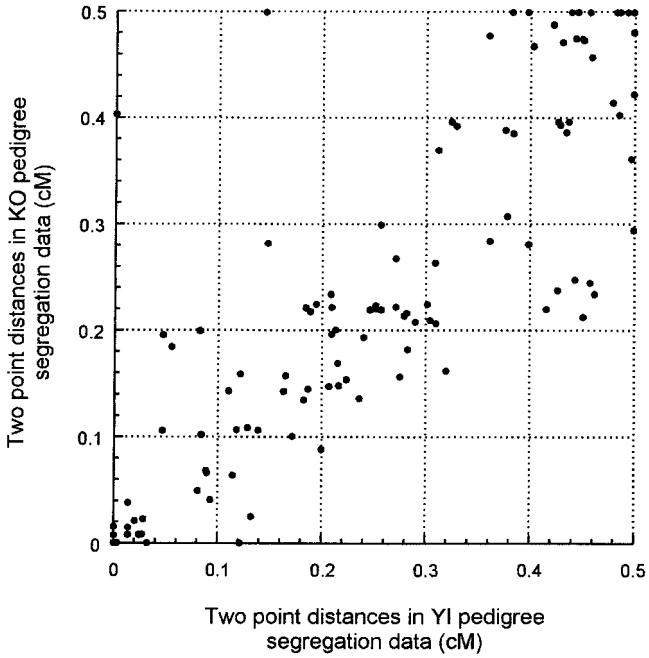

FIGURE 4.—Comparison of two-point distances (in cM) between orthologous markers in YI and KO linkage data.

YI map, and between the consensus map and the KO map, as indicated by crossing of lines connecting the YI, KO, and consensus maps (Figure 3).

DISCUSSION

Segregation ratio distortion: Several reasons for distortion of segregation ratios in plants have been put forth, including such factors as chromosome loss (KASHA and KAO 1970), genetic isolation mechanisms (ZAMIR and TADMOR 1986), and the presence of viability genes (*e.g.*, HENDRICK and MUONA 1990; BEAVIS and GRANT 1991; LIEDL and ANDERSON 1993; BRADSHAW and STETTLER 1994). Nonbiological factors such as scoring errors (DEVEY *et al.* 1994; XU *et al.* 1997; NIKAIDO *et al.* 1999) and sampling errors (PLOMION *et al.* 1995; ECHT and NELSON 1997) can also lead to distortion in segregation ratios. When the KO pedigree was used to construct the first genetic map for *C. japonica*, the segregation ratios of 35 loci were distorted (<5% level of significance) among the 164 segregating loci on the linkage map (MUKAI *et al.* 1995). When the CAPS markers were added to the KO linkage map, IWATA *et al.* (2001) found that 15 out of 60 CAPS markers showed a significant deviation (5% level of significance) from the expected segregation ratio; 11 CAPS markers were distorted from the expected segregation ratio out of 26 additional CAPS markers newly added to the KO linkage map in this study. In total, 25.1% of all markers used for segregation analysis in the KO pedigree showed distorted segregation ratios (Table 2).

MUKAI *et al.* (1995) speculated that distorted segregation ratios of these markers were caused by putative "embryonic lethal gene(s)" (or viability genes), because clustering of genetic markers showing distorted segregation ratios is consistent with the idea that they may be closely linked to a viability gene. If so, the probability of embryonic lethal genes becoming homozygous would be increased, because the third generation in the KO pedigree was formed by self-pollination of F_1 hybrid individuals. On the other hand, we found that 17.8% of genetic markers of the YI pedigree had segregation ratios that deviated from the expected ratios (*i.e.*, those corresponding to the configuration types in the YI pedigree) and were smaller than those in the KO pedigree. We found six conspicuous clusters of markers having distorted segregation ratios in the KO1, KO2, KO6, KO8, KO10, and KO13 linkage groups in the KO linkage map (Figure 3). CD1712R was the only genetic marker showing a distorted segregation ratio in the YI linkage map within regions equivalent to those containing the clusters on the KO linkage map. The sib-cross in the second generation of the YI pedigree could decrease the number of genetic markers whose segregation was skewed from the expected ratios. TSUMURA *et al.* (1989) found that 25% of isozyme loci had distorted segregation ratios when segregation analyses were conducted on progeny of self-pollination, but that no isozyme loci had distorted segregation ratios when segregation analyses were conducted on progeny of sib-crosses. KUANG *et al.* (1999) also detected a high proportion of markers with segregation distortion (34% at 5% significance level) when they analyzed megagametophytes of selfed seeds, except for seeds that died within 1 month of germination. These were derived from a single radiata pine tree. On the other hand, it was reported that only 15% of markers showed segregation distortion (10% significance level) on a genetic linkage map of willow from a full-sib cross of *Salix viminalis* (HANLEY *et al.* 2002). These results supported that sib-crosses could reduce the number of markers with segregation distortion relative to selfed progeny.

The difference in segregation distortion ratios between the two pedigrees should affect map length. REMINGTON and O'MALLEY (2000) analyzed effects of lethal or semilethal loci due to inbreeding on their genetic maps. However, their model was restricted to the segregation progeny derived from selfed progeny of a single tree. In future analysis, we will extend their model to segregation progeny derived from sib-crosses, like the YI pedigree, and compare the effects of lethal or semilethal loci in the KO and YI pedigrees.

Genome length and coverage: Previous studies have used various computer programs for generating genetic maps of forest trees (*e.g.*, BARRENECHE *et al.* 1998; DEVEY *et al.* 1999; SEWELL *et al.* 1999; LESPINASSE *et al.* 2000). In general, maps constructed with JoinMap are shorter than those constructed with a multilocus-likelihood package such as MAPMAKER or OUTMAP (SEWELL *et al.* 1999; BUTCHER *et al.* 2002; GOSSELIN *et al.* 2002).

Our results also showed that all three maps (YI96, YI38, and KO) constructed with JoinMap were shorter than those constructed with MAPMAKER (Table 3). The multilocus-likelihood method used by MAPMAKER assumes an absence of crossover interference; so when interference is present, JoinMap correctly produces shorter maps, even though both programs use the Kosambi mapping function (Stam 1993). This difference was also observed in barley and was attributed to how each program calculates map distance when the actual interference differs from that assumed (Qi *et al.* 1996).

In *C. japonica*, four studies concerned with the construction of genetic maps have been reported (Mukai *et al.* 1995; Kuramoto *et al.* 2000; Nikaido *et al.* 2000; Iwata *et al.* 2001). Using F_1 progeny of unrelated parents, Kuramoto *et al.* (2000) constructed two linkage maps by the two-way pseudo-testcross strategy and estimated the genome length and map coverage statistics by the methods of Hulbert *et al.* (1988). The estimated expected genome length of Iwao-sugi was 2868.0 cM at a LOD score of 4.0, and that of Boka-sugi was 2790.7 cM at a LOD score of 4.0 (Kuramoto *et al.* 2000). On the other hand, our estimates of genome length were between 2168.5 (YI96) and 1395.5 cM (KO) according to MAPMAKER and between 1632.4 (YI38) and 1121.8 cM (KO) according to JoinMap. The reported genome lengths above were outside the confidence intervals (95% criteria) of our data. However, Kuramoto *et al.* (2000) used only RAPD markers to construct genetic maps, in contrast with our linkage map, which was composed mostly of EST markers. RAPD markers could be dispersed throughout the genome more randomly than EST markers. If a majority of EST markers used in our study are clustered, our genome length estimate would be underestimated.

Our expected map coverage estimates ranged from 95.9% (YI96) to 96.5% (KO) according to MAPMAKER. Although the linkage maps for Iwao-sugi based on RAPD markers covered only ~62% of the genome (Kuramoto *et al.* 2000), the total length of the linkage map for Iwao-sugi (1756.4 cM) was longer than those we found (1567.3 cM for YI96 and 1138.6 cM for KO, MAPMAKER analysis). Although many factors can affect marker coverage and genome map density, such as genome length, number of markers, distribution of marker polymorphism, distribution of markers on the genome, crossover distribution on the genome, mapping population size and type, and mapping strategy (Liu 1998), a nonrandom distribution of EST markers might be the main cause of the discrepancy. Our estimates suggest that our linkage maps covered the entire genome of *C. japonica*. However, our linkage map probably does not cover nondense regions of genes in the genome. Extensive microsatellite markers or random genetic markers, such as AFLP and RAPD, would be helpful tools for filling in the nondense regions of genes in the genome.

Construction of the consensus map: The 11 chromosomes of *C. japonica* are represented in the YI map generated with the CP mode of JoinMap and also in the consensus map. The smallest linkage group (YI12& KO8) in the consensus map could belong to any of the 11 linkage groups, but additional genetic markers are needed to make this assignment. It was impossible to find a clear correspondence of the KO15 linkage group with the other consensus map because of a lack of orthologous markers. However, the KO15 linkage group is part of the LG1 linkage group (Iwata *et al.* 2001). The LG1 linkage group was divided into two parts between the CD133 and CD344 markers at the second-round analysis. The interval between these markers was 21.8 cM in the linkage map of Iwata *et al.* (2001). One part of LG1 corresponded to KO13; the other part corresponded to KO15. Therefore, KO15 would be placed downstream of KO13 in the YI3&KO13 consensus linkage group if orthologous markers existed (Figure 3).

Southern blot analyses using cDNA and gene probes have revealed genes that are found in double, and occasionally multiple, copies in many plant species (*e.g.*, Bernatzky and Tanksley 1986; Helentjaris *et al.* 1988), including forest tree species (Kinlaw and Neale 1997; Devey *et al.* 1999; Sewell *et al.* 1999). We found 12 tightly linked clusters of EST markers that came from a single cDNA clone, out of 17 EST markers revealing multiple loci derived from single cDNA clones within one linkage group. Five EST markers from a single cDNA clone were dispersed throughout the genome. Thus, our data demonstrate that >50% of EST markers derived from multigene families were tightly linked or located on the same chromosome.

Some changes in marker order (other than those due to translocation) were observed during construction of consensus maps (Sewell *et al.* 1999; Lespinasse *et al.* 2000; Sebastian *et al.* 2000; Cervera *et al.* 2001; Jeuken *et al.* 2001; Lombard and Delourme 2001). Small discrepancies in marker ordering may be due to mapping imprecision rather than to real rearrangements (Lombard and Delourme 2001). We observed four large and six small differences in marker order between the YI or KO map and the consensus map. One of the reasons for the discrepancies might be due to chance, because LOD score criteria decided arbitrarily were not stringent. Distorted segregation ratios were observed at three loci of the KO pedigree at which we detected large discrepancies in marker ordering. The KO pedigree showed many loci with distorted segregation ratios, which might affect marker order in the linkage map.

One of the main goals of constructing consensus maps is to compare QTL between different genetic backgrounds, especially in allogamous species. We can determine how many and where QTL exist in such species by using multiple pedigrees with different genetic backgrounds. In *C. japonica*, QTL relating to juvenile growth, flower bearing, and rooting ability of cuttings have been

identified in the KO pedigree (Yoshimaru *et al.* 1998), and QTL for the modulus of elasticity of wood have been identified in a different pedigree (Kuramoto *et al.* 2000). We will be able to summarize QTL from different pedigrees on the consensus map and obtain knowledge of how many QTL exist and which QTL are expressed throughout the pedigrees in future analysis.

Our markers and genetic maps should be valuable for researchers studying related species, such as the Taxodiaceae and Cupressaceae, because Tsumura *et al.* (1997) showed that a high proportion of the EST markers could be useful in other species of the Taxodiaceae and Cupressaceae. Therefore, these EST markers will allow studies of genome evolution and comparative mapping between species within the Taxodiaceae and Cupressaceae. The consensus map developed in this study will become the basis of genome studies of the Taxodiaceae and Cupressaceae. Our data are available on our web site (http://www.ffpri.affrc.go.jp/labs/cjgenome/database/cjdatae.html).

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APPENDIX A

Description of additional CAPS markers in sugi

Locus	Forward primer: 5′ to 3′	Reverse primer: 5′ to 3′	Anneal temp.	No. of PCR cycles	Putative size (bp)[a]	Restriction enzyme	
						YI	KO
CC2052C	TGTTGCCGGTAGGGTTTCTA	TTACCGTATTGCTTGCCATTG	55	36	>2000	*Hin*fI	
CC2081C	GCATGGCAGAAGCAGAAG	TTCACATATGCGATGACACAA	60	36	1200	*Sty*I	
CC2123C	CGGCGCTTACCTCATCGTT	CCCTGCTACCGACGGACTCTA	60	36	2000	*Nci*I	
CC2188C	AGCTGTGCGATCAAGTTTCTG	ATGGGCGTGCCTCCTAA	60	40	1400	*Bst*OI	
CC2286C	ATAATGCCACCTCCAGGAC	AGGCCAGTTTAACAAATGTCA	60	36	1800	*Alu*I	
CC2333C	GGTGGACCTTCGTTCTG	AACCCAACTGCACTACTCTT	60	40	1500	*Taq*I	
CC2340C	TACAGGAGGCGGAGGAC	CTCAAACTGCCAAACAACAA	60	35	1000		*Alu*I
CC2377C	GAAGGAGCTGAAGGAGG	CTAAGCGTTGAAACTGAGAA	55	35	1500	*Hae*III	
CC2419C	CAATGAGGAGGTCTGTATG	AAATTTGGAGGATCTCAAC	60	40	1500		*Nde*II
CC2435C	GCAGGCAGTTCAGAGTTTT	TCCCGAAGAGAGTTTTATGG	60	40	900	*Hae*III, *Rsa*I	
CC2448C	ATCCTAAGTCCCCAGAAAGT	GAATTGGAATGGCATAAAGA	60	40	2000	*Rsa*I, *Hha*I	
CC2467C	CGGAGGAGGCGGCTGAGAGT	CGACCCTGAAGATTGTTTGA	60	40	800	*Eco*O109I	*Eco*O109I
CC2469C	TCGACTTCGGTAGCAGCACA	TCATCCGCCTCGTCCTCCTC	62	35	600	*Alu*I	
CC2522C	CGACGAAGAGGATGATGAAC	GCCAGCTGTGATATGATTGT	60	40	2000	*Hin*fI, *Nde*II	
CC2541C	CGCAAGAGAGCTCGTCGTCA	CAAACTTGGAGGATGTGTCA	60	35	2000	*Dde*I, *Hin*fI	
CC2577C	AGGTCTGTAAGGTGTGAGGG	ATAGAAAGGCAACAGTAGCA	60	40	1100	*Dde*I	*Dde*I
CC2583C	AATTATGGGAGAGAACTGGA	ATTAAACCGTACATGGAACT	60	40	1500	*Dde*I	*Dde*I
CC2588C	CTGCCGCTGCCGTTTATTCC	TTATCCACGACGTACACACC	60	40	900		*Ssp*I
CC2621C	GTTGCTGTGGGAGGACTTTG	AGCCCACCTAATAGATGAGA	52	36	700	*Hae*III	*Hae*III
CC2631C	GCATTTGCTCCCATTAGTTC	TTTCTTCCTCGCCATTCTTC	60	36	1300		*Bst*OI
CC2643C	CACGGTGGCATTGACATCTT	ACCTACGCTACAACCCTCCC	62	36	>2000	*Msp*I	
CC2645C	TGTCGGTGTGTTGCCTCTTC	GTGGGCTTCTGCATAATCAT	62	36	1100	*Bgl*II	
CC2657C	ACCTGCCCTCCTTTCCATTC	CAACTGTTACACCGCCCTCC	60	36	2000	*Scr*FI	
CC2674C	CCGACTCACCCTTTCTTCAC	TGCCATATCTCAACAATCTC	52	36	1000	*Alu*I	
CC2676C	CAAGGGTTTGGGAAAGGGAG	CCGATTGAGGAGACTGCTAA	60	36	500		*Bst*OI
CC2683C	TGCGAAATGTTAGCCCTCTG	CCCTCTGTATCATCCCTGTC	60	36	500		*Hae*III
CC2700C	ATTTGTGCAGGTTATTTGTC	TATTCGGTGGAGGAGGTGGT	60	36	700	*Scr*FI	
CC2702C	TTCGCCAAGCCACCATAGAC	CTGCCACCACAACACCCTCC	60	36	500		*Rsa*I
CC2713C	ATCATAGCTGCGAAGAACAC	GTCCCGTCATTGCCACACCA	60	36	350	*Msp*I	*Msp*I
CC2716C	GTTGACATGATCCGAAAGAG	CAAACGCAAATACTGAAAGG	60	36	1000		*Alu*I
CC2731C	CAAGCCCAAGCCCAGGTCGT	TGCAGGGATAGGATAGGTAG	62	36	>2000		*Taq*I
CC2746C	TAGAAATTGCTCATGTGGGT	CCTCTTCTTTCCGCTGCTGT	60	36	2000	*Dde*I, *Rsa*I	*Dde*I
CC2750C	GGCAGCACACAGACAACACA	GATACTTCTCAGGCCCAACT	62	36	1700	*Sin*I	
CC2752C	CCGCACTGCCATCTACGACT	AACCTCTCCTCCAACTCACC	62	36	1000	*Hae*III	ALP (900, 1100)
CC2781C	CAGAGAAACCCAGCGAGGAA	GCAACAATGGCATACAAACT	60	36	1200	*Dra*I	
CC2795C	ATCCAGGAGCAAAGAAAGGT	ATAGCAGCAGAATGGTCAGG	60	36	800	*Dde*I	*Dde*I
CC2831C	GGCGATGGCAGCAAACGAAG	CACGCACCACTCCACCCTAC	62	36	500	*Dra*I, *Hha*I	
CC2846C	AAGTAAGTTGGTCGGTAGGT	AAGAAGGCATTTTGGTGAGG	60	36	1400	*Dde*I	*Msp*I
CC2856C	GACGAAGGCTGAAAAAGGTG	GCATCTAGGCATACGCTGAA	62	36	2000	*Mbo*I	
CC2860C	CTAAAGGGAAACAAATCAGG	TACTCGTCTTCTAACCGTCA	60	36	1100	*Dra*I, *Hin*cII	
CC2895C	TCATGGCATTGCGGAGAGGG	CGGCCTGTAAGACCACCTGA	60	36	1200	*Taq*I	
CC2909C	GCAGCAATCTTTCCTCCTCC	GCATGCATTTAGCCTTCACC	62	36	>2000	*Alu*I	
CC2918C	TTGGCTTCTATGGACCTATG	ACTGGACTTTTGCGATGCTT	60	36	>2000	*Alu*I, *Mbo*I	*Nde*II
CC2921C	TTTTGGCGGTGGGAGGAATG	CAAGAATCGGTGAAGAACAG	60	36	1400	*Rsa*I, *Taq*I	*Taq*I
CC2939C	CTCGCTGAGCAAGACTAGGG	CATGACGAAAATGCCCTGTA	60	40	1000	*Nde*II, *Taq*I	*Taq*I
CC2946C	GTATCCAGGGATGCTCGAAA	AAATTGCCATCCTTCCTCCT	55	40	1200		*Taq*I
CC2989C	ATTTGGAACTTCGGAAGCCT	TTGATGCATATCCTGTCCCA	55	40	700	*Hae*III	
CC3098C	ACAATGCCTTCCCATGAAGT	ATCAGGCTGTTGGGAATCAG	60	40	1100	*Ava*I, *Taq*I	*Taq*I
CC3133C	AAGGTTCATCGCCCTATGTG	AGCTCCAACCTCAAAGACCA	60	40	900		*Taq*I
CC3145C	TCCACTTAGCGTCAATTCCC	CACACTTCCATGTTAGGGGC	60	40	2000	*Alu*I, *Hin*fI	
CC3336C	TGGTCATGATGTGCTTGGTT	AGTTGCTACAATGTTCCCGC	60	40	>2000	*Taq*I	
CC3367C	AGAGATGGCGCTCACTCATT	TACTACACCACCGCTTGCAG	60	40	900	*Alu*I	*Alu*I
CC3393C	TCTCCTGAATGGGATGAAGC	CACATGCTTGCCGAAATAAA	60	40	700		*Hin*fI
CC3413C	GGAAAACAGTGTGAGGGTGC	TGGCATGGTCTCGTTTGTTA	60	40	1300	*Bgl*I, *Bgl*II	
CC3416C	CCCTCAACTCCTCCAATGAA	CATGACCTGTCGTGCTTGAT	60	40	1400	*Hae*III	*Hae*III
CC3430C	GACGAGGGACGACCTGTTTA	ACTCAACACCAGCATCTCCC	60	40	2000	*Hin*dIII	
CC3816C	AGTCAGAGCTGCCTGGAAAG	GCCACGAAGGGATTCATTTA	60	40	2000	*Msp*I	*Rsa*I
CC3823C	CCCCACAGGACATCAAAACT	ACGCATTCTCCATCACTTCC	55	40	900	*Hin*fI	
CC3839C	CTGCATTTCCTCTGGAATCG	TTGGGATAAACCTTTTTGCG	60	40	2000	*Taq*I	
CC3872C	AGCGGAAGTACCCTTTGGAT	GGTTCCCAGTGATTTCCTGA	60	40	1600	*Taq*I	

[a] Putative PCR fragment sizes were deduced by agarose gel electrophoresis (ethidium bromide staining).

APPENDIX B

Description of microsatellite markers in sugi

Locus	Forward primer: 3′ to 3′	Reverse primer: 5′ to 3′	Anneal temp.	PCR cycle	Motif	Putative size (bp)[a]
CJS0002M	CTTTTTTCAAATTTAGTGATGT	CCCATGCCCCACTGTCCACC	55	30	$(TC)_{12}(TC)_{17}$	237
CJS0091M	GAGAGATAAGAGGGTAGAGGT	CAATGCCAACTTAGAAGAC	60	30	$(GA)_{43}$	298
CJS0268M	CCTTAGAAAGCTATGCCAC	GCAACGCATCCATAATACC	60	30	$(AC)_{53}$	352
CJS0331M	GGAGAGATAGACGACAAAAGAG	CCATCTTGCTAATCTGTCC	60	30	$(GA)_{6}$	245
CJS0333M	AGGAGATTAGGATGGTGGG	GGTTTGCCTCTTCTATGAG	60	30	$(GA)_{26}$	264
CJS0356M	CTAAAGAATAGATGACTCCAC	TATAACGCTTTTGCCCTCA	60	30	$(GA)_{64}$	337
CJS0401M	GATCTAAACTTGAGCATAAC	CAATCCTGTCTCCATACCC	55	30	$(CG)_{8}(GA)_{54}$	222
CJS0455M	GTTACTTTGAAAAATGAGCC	AACATCAAGATTAAAGGGAC	58	30	$(CT)_{20}$	166
CJS0485M	CATATCTAATATCTAATACCTTG	TCTCCCTATCTAGCCCTCTG	50	35	$(GA)_{9}(GA)_{30}(GA)_{27}$	331
CJS0520M	TCCCTTTTGGTATTTTACAC	ACTCAAATTGCGATAATCTC	55	30	$(TG)_{18}$	196
CJS0584M	TGGTTTGCCTTTGGTTGCTC	GGACTTTCTATTTACCTCTTGG	60	30	$(AG)_{80}$	329
CJS0665M	CCAAGCATAGGGAAAAAGAG	GGGGAGTAAGGATGACATTT	60	30	$(GA)_{45}(GA)_{29}$	367
CJS0686M	CAATGCAAATATAAGTTCACCC	TCCACCTCTTTTTCATTCTC	55	30	$(GA)_{52}$	275
CJS0838M	TATGTAGAAGCGTGTGATGT	GATAATTGCCTTTGTTGTCC	58	30	$(GT)_{23}$	170
CJS0955M	CACACTCCCCGTCTCCGACAG	ACCCTGATTCCCCATACACC	58	30	$(TCT)_{4}(GA)_{29}$	137
CS1226M	CTCTAGTCCTCAATGGTGGT	TATTAAGCATTTTCCCTCTC	60	35	$(CA)_{14}$	139
CS1281M	CCCCCTCTCATTAGTTACCA	CAAAAATCAACAAGCCAACC	60	30	$(CT)_{15}$	233
CS1413M	GGAAAGGATGTTATGGGTGT	CGGTTGATTTTGTCGGCACT	60	35	$(TG)_{11}(GT)_{15}$	285
CS1522M	AAAGTTTGATTAGGGCAGGG	AAACGTGGGTGCTATCCTTC	62	30	$(AC)_{16}$	222
CS1737M	TACCCTCAACCCTTCACCCT	TTACCCACCTCTCTTTCCTC	60	30	$(AG)_{40}$	248
CS1895M	TGAGAGAGGGAGGGAGGGTT	GAGTCCTTGTCCCGTTTTGT	60	30	$(TG)_{10}$	405
CS2024M	AGTAATACAAGATAAGGGAG	TCCACCTCTATACCTCTACA	55	30	$(AG)_{15}(AG)_{4}(AG)_{10}$	314
CS2056M	GAGAGACATGGGGGAAGAGG	GGTTCTAACACATGAATGGC	60	30	$(GA)_{20}(GA)_{7}$	295
CS2169M	GTAGAGGAGGGATATAGAGT	TCCTTGTCCATCTCTCTTTA	55	30	$(GA)_{9}$	141
CS2484M	TGAGAAAGGGAGAGAGGGAT	CCCCCTTCTCTTTTTCACTC	60	30	$(GA)_{13}$	158

[a] Putative PCR fragment sizes were deduced from sequences of genomic clones between forward to reverse primers.