Saturated Molecular Map of the Rice Genome Based on an Interspecific Backcross Population

Mathilde A. Causse,[1] Theresa M. Fulton, Yong Gu Cho, Sang Nag Ahn,[2] Julapark Chunwongse, Kunsheng Wu,[3] Jinhua Xiao, Zhihong Yu,[4] Pamela C. Ronald,[5] Sandra E. Harrington, Gerard Second,[6] Susan R. McCouch and Steven D. Tanksley

Department of Plant Breeding and Biometry, Cornell University, Ithaca, New York 14853

Manuscript received April 25, 1994
Accepted for publication August 26, 1994

Saturated Molecular Map of the Rice Genome Based on an Interspecific Backcross Population

Mathilde A. Causse,[1] Theresa M. Fulton, Yong Gu Cho, Sang Nag Ahn,[2] Julapark Chunwongse, Kunsheng Wu,[3] Jinhua Xiao, Zhihong Yu,[4] Pamela C. Ronald,[5] Sandra E. Harrington, Gerard Second,[6] Susan R. McCouch and Steven D. Tanksley

Department of Plant Breeding and Biometry, Cornell University, Ithaca, New York 14853

Manuscript received April 25, 1994
Accepted for publication August 26, 1994

ABSTRACT

A molecular map has been constructed for the rice genome comprised of 726 markers (mainly restriction fragment length polymorphisms; RFLPs). The mapping population was derived from a backcross between cultivated rice, *Oryza sativa*, and its wild African relative, *Oryza longistaminata*. The very high level of polymorphism between these species, combined with the use of polymerase chain reaction-amplified cDNA libraries, contributed to mapping efficiency. A subset of the probes used in this study was previously used to construct an RFLP map derived from an inter subspecific cross, providing a basis for comparison of the two maps and of the relative mapping efficiencies in the two crosses. In addition to the previously described *PstI* genomic rice library, three cDNA libraries from rice (Oryza), oat (Avena) and barley (Hordeum) were used in this mapping project. Levels of polymorphism detected by each and the frequency of identifying heterologous sequences for use in rice mapping are discussed. Though strong reproductive barriers isolate *O. sativa* from *O. longistaminata*, the percentage of markers showing distorted segregation in this backcross population was not significantly different than that observed in an intraspecific F_2 population previously used for mapping. The map contains 1491 cM with an average interval size of 4.0 cM on the framework map, and 2.0 cM overall. A total of 238 markers from the previously described *PstI* genomic rice library, 250 markers from a cDNA library of rice (*Oryza*), 112 cDNA markers from oat (Avena), and 20 cDNA markers from a barley (Hordeum) library, two genomic clones from maize (Zea), 11 microsatellite markers, three telomere markers, eleven isozymes, 26 cloned genes, six RAPD, and 47 mutant phenotypes were used in this mapping project. Applications of a molecular map for plant improvement are discussed.

RICE is one of the most important food crops in the world. It has also become a model plant among the cereals for molecular genetic studies. It is a diploid with $n = 12$ chromosomes, has the smallest genome of any monocot known (C = 0.45 pg) (ARUMUGANATHAN and EARLE 1991), a large germplasm collection (>120,000 accessions worldwide), can be regenerated from protoplasts [for reviews, see LYNCH *et al.* (1991) and HODGES *et al.* (1991)], and has a relatively high degree of transformation efficiency relative to other cereal species [for review, see HODGES *et al.* (1991) and KOTHARI *et al.* (1993)].

Early versions of a linkage map of rice chromosomes were reported by YAMAGUCHI (1927), CHAO (1927) and JODON (1948). In 1963, NAGAO and TAKAHASHI proposed the first rice map consisting of 12 linkage groups, corresponding to the haploid number of chromosomes. Work in this area of rice genetics progressed steadily, with updated versions of the rice linkage map published by TAKAHASHI and KINOSHITA (1968, 1977) and annually since 1984 (KINOSHITA 1984–1994). Linkage maps were originally established with morphological mutants, but isozyme markers were subsequently located on the chromosomes [for reviews, see ENDO and MORISHIMA (1983), PHAM *et al.* (1990) and ISHIKAWA *et al.* (1991)]. The cytological basis of linkage groups was first reported by IWATA and OMURA (1971a,b) based on a study of relationships of gene loci with the points of interchange of reciprocal translocations. The development of primary trisomic stocks by several groups resulted in complete trisomic series for both *indica* and *japonica* rice (KURATA *et al.* 1981; KHUSH *et al.* 1984; IWATA and OMURA 1984) and allowed assignment of the linkage groups to their respective chromosomes. The use of morphological mutants in genetic studies has remained limited, mainly because of their deleterious effects and the difficulties encountered when attempting to group a number of these markers in the same genotype. Isozyme markers offer a more versatile set of genetic markers to rice breeders. However, the number of detectable isozyme loci

[1] Current address: Station de Genetique Vegetale, Ferme du Moulon, 91190 Gif/Yvette, France.
[2] Current address: Crops Experiment Station, Rural Development Administration, 109 Seodun Dong, Suweon, 441-100, Korea.
[3] Current address: Central Research Development Department, Experimental Station, P.O. Box 80402, E. I. duPont de Nemours Company, Incorporated, Willmington, Delaware 19898.
[4] Current address: Department of Horticultural Sciences, University of Florida, 1143 Fifield Hall, Gainesville, Florida 32611.
[5] Current address: Department of Plant Pathology, 576 Hutchison Hall, University of California at Davis, Davis, California 95616.
[6] Current address: EMBRAPA/CENARGEN, Caixa Postal 0.2372, CEP: 70770900 Brasilia DF, Brazil.

is still limited (Second 1982; GLASZMANN 1987; PHAM *et al.*, 1990).

The analysis of restriction fragment length polymorphism (RFLP) offers plant geneticists and breeders a powerful set of genetic markers which are abundant, codominant, non-deleterious, and reliable. RFLPs have been successfully used to construct genetic maps in numerous crop species [for review, see O'BRIEN (1993)] and to locate genes of interest along the chromosomes (PATERSON *et al.* 1991; TANKSLEY *et al.* 1989). In addition, high density molecular linkage maps can be used to clone target genes (MARTIN *et al.* 1993).

MCCOUCH *et al.* (1988) described the construction of the first RFLP map in rice. This map was constructed from an F_2 population derived from a cross between varieties representing the two major subspecies (*indica* and *japonica*) of cultivated rice, *Oryza sativa* L. Primary trisomic stocks (KHUSH *et al.* 1984) were used to assign the 12 linkage groups to their respective chromosomes. The long term usefulness of this segregating F_2 population was limited because plants were not maintained vegetatively. In addition, the level of polymorphism in this intraspecific cross was limited, and in order to map a sufficient number of markers it was necessary to screen many probes with a large number of restriction enzymes. Even after mapping nearly 200 markers, several significant gaps still remained in the map which could not be explained by chance alone. It was not clear whether these gaps were due to a bias in the genomic library used for mapping, regions in the rice genome comprised of multicopy sequences that could not be mapped using Southern analysis, or the presence of chromosome segments that were common by descent (and thus monomorphic) in the *indica* and *japonica* mapping parents (MCCOUCH *et al.* 1990; our unpublished data). Nonetheless, the map provided the basis for locating a number of agronomically important genes via linkage to RFLP markers, including both single genes and quantitative trait loci (QTL) linked to blast resistance (YU *et al.* 1991; WANG *et al.* 1994), insect resistance (MCCOUCH and TANKSLEY 1991; MOHAN *et al.* 1993), bacterial blight resistance (MCCOUCH *et al.* 1991; RONALD *et al.* 1992), photoperiod sensitivity (MACKILL *et al.* 1993), grain aroma (AHN *et al.* 1992), wide compatibility (LIU *et al.* 1992; YANAGIHARA *et al.* 1994; ZHENG *et al.* 1992), and the semi-dwarf character, *sd-1* (CHO *et al.* 1994), among others.

A second RFLP map of rice based on a different *indica/japonica* cross was reported by SAITO *et al.* (1991). Where the map developed in our laboratory was based on a *PstI* genomic library from the *indica* variety, IR36, the map developed by SAITO *et al.* (1991) was based on a *PstI* library developed from the *japonica* variety, Nipponbare. Efforts to integrate the two maps are underway (XIAO *et al.* 1992). An additional map based on an *indica* × *japonica* cross is currently under development in Japan (NAGAMURA *et al.* 1993).

The objective of the mapping project reported here was to generate a well-saturated map of the rice genome. To accomplish this, we broadened the type of markers used in the mapping effort. In addition, we utilized an interspecific mapping population that embodied a high frequency of polymorphism. Such crosses have proven very useful for developing maps in other species including tomato (TANKSLEY *et al.* 1992b), potato (BONIERBALE *et al.* 1988), sorghum (CHITTENDEN *et al.* 1994), and wheat (GILL *et al.* 1991). Moreover, the population we have chosen is readily amenable to long term vegetative propagation.

Phylogenetic studies of the *Oryza* genus, based on isozymes (SECOND 1985) or RFLPs (WANG *et al.* 1990), have shown that, among species with the AA genome, *Oryza longistaminata* A. Chev et Roehr is one of the most distantly related to *O. sativa*. This perennial species propagates in nature by the development of vigorous rhizomes or by outcrossing, enforced by self-incompatibility (NAYAR 1967). GHESQUIÈRE (1985) has described the main features of its population biology. *O. longistaminata* is isolated from the other AA genome species by a strong reproductive barrier (CHU and OKA 1970a) which can nevertheless be overcome (CHU and OKA 1970b; GHESQUIÈRE 1988). Certain traits of this species may be of interest for rice breeding, such as specific disease resistance genes (VALES 1985), allogamic traits (TAILLEBOIS and GUIMARAES 1987), and plant architecture (CAUSSE and GHESQUIÈRE 1991).

The purpose of this report is to describe the construction of an RFLP map based on an interspecific backcross population involving *O. sativa* and *O. longistaminata*. A comparison with the previous map (MCCOUCH *et al.* 1988) is presented based on a common set of probes mapped in both studies. A total of 238 markers from the previously described *PstI* genomic rice library, 250 markers from a cDNA library of rice (Oryza), 112 cDNA markers from oat (Avena), and 20 cDNA markers from a barley (Hordeum) library, two genomic clones from maize (Zea), 11 microsatellite markers (WU and TANKSLEY 1993a), 3 telomere markers (WU and TANKSLEY 1993b), 11 isozymes, 26 cloned genes, 6 RAPD, and 47 mutant phenotypes were used in this mapping project. Levels of polymorphism detected by different types of molecular markers and the frequency of identifying heterologous sequences for use in rice mapping are discussed.

MATERIALS AND METHODS

Plant material: A backcross population of 113 plants, derived from the cross *O. sativa/O. longistaminata//O. sativa* was used as the mapping population (referred to as the SL population). BS125 (*O. sativa*) was an *indica* land race collected in Guinea Bissau. WL02 (*O. longistaminata*) was a unique plant coming from a seed originally collected from a wild population in Botswana (MIEZAN and SECOND 1979). The F_1 hybrid (BS125/WL02) and the backcross seeds were obtained by controlled pollination in the ORSTOM (Institut Français de Recherche Scientifique pour le Développement en

Coopération) Research Center in Ivory Coast, by GHESQUIÈRE (1988), who kindly provided them. The 113 backcross plants were grown in either a growth chamber or a greenhouse facility at Cornell University, maintained vegetatively, and propagated by shoot cuttings.

Hybridization of the cDNA probes from oat and barley was evaluated in a preliminary screen using genomic DNA of the rice cultivar IR36. Seeds from this cultivar were provided by the International Rice Research Institute.

The results of this study were compared to the data obtained on the intraspecific F_2 population previously used to establish the map (MCCOUCH *et al.* 1988). This population was derived from a cross between an *indica* breeding line, IR34583-19-3-3, and a *japonica* landrace from Indonesia, Bulu Dalam (referred to hereafter as the IB population). We have also compared the percent polymorphism detected between the parents of the SL population to that observed between the cultivars, Apura and IRAT177, the parents of a population of doubled haploid lines obtained through anther culture (hereafter referred to as the AI population) (GUIDERDONI *et al.* 1988, 1990), whose RFLP map is still under construction.

DNA extraction, Southern hybridization: The DNA extraction based on the technique proposed by SHURE *et al.* (1983) has been used in combination with the procedure described by TAI and TANKSLEY (1990), where freshly harvested leaves were dried in a food dryer at 45–50°, for 16–24 hr, before powdering in an electric coffee grinder. The urea-phenol extraction buffer was replaced by a potassium acetate buffer (derived from the procedure of DELLAPORTA *et al.* 1983). Using both techniques, yields of approximately 250–500 micrograms of DNA per 5–10 g of fresh tissue were obtained.

The DNA digestion, Southern blotting and hybridization procedures are similar to those described in MCCOUCH *et al.* (1988). Five to seven micrograms of total DNA were digested at 37° using one of the following enzymes: *Eco*RI, *Eco*RV, *Dra*I, *Hind*III, *Sca*I, *Xba*I, *Bam*HI, and *Bgl*II (BRL and NEBiolab) in the buffer recommended by the manufacturer. After digestion, the DNA was fractionated on 0.9% agarose gels, and run in neutral electrophoresis buffer (1 M Tris, 10 mM EDTA, 125 mM NaAc; pH 8.1). The fractionated DNA was then transferred to nylon membrane filters, Genescreen Plus (DuPont) or Hybond N+ (Amersham Corp.), in either 0.5 M NaOH, 1.5 M NaCl or 0.4 M NaOH buffers, following the procedure described by SOUTHERN (1975). Probes were random hexamer labeled (FEINBERG and VOGELSTEIN 1983), and hybridization was performed at 65°, in 5 × SSC buffer, overnight. The filters were washed at 65°, three times for 20 min each time, with successive stringencies of 2 ×, 1 × and a last wash at 0.5 × SSC (each wash 0.1% SDS) for most probes. When mapping known genes, lower stringency washes (2 ×, 1 ×, 1 × SSC) were occasionally necessary for obtaining clear signal with cloned genes from other species, and higher stringency washes (2 ×, 1×, 0.5 ×, 0.05 × SSC) were sometimes used to obtain clear bands where gene families or repeated sequences were involved. The filters were exposed with an intensifier screen at −80° for 1–5 days.

To survey for polymorphism, clones were hybridized onto filters containing DNA from the recurrent *indica* parent, BS125, and the F_1 hybrid, digested by different restriction enzymes. Initially, we included eight restriction enzymes (*Eco*RV, *Xba*I, *Hind*III, *Sca*I, *Eco*RI, *Dra*I, *Bam*HI and *Bgl*II, those found most efficient by MCCOUCH *et al.* 1988), but due to the high level of polymorphism between the *O. sativa* and *O. longistaminata* parents, only five (*Eco*RV, *Hind*III, *Sca*I, *Xba*I, and *Dra*I) were retained, as they were sufficient to detect polymorphism in most of the clones. For mapping, DNA from the 113 backcross progeny was digested, blotted, and used in Southern hybridization (as described above) for clones showing polymorphism between the parents. After examining the molecular weights of the hybridizing bands in survey filters, two or more clones with non-overlapping banding patterns were frequently hybridized together onto the same set of progeny filters, allowing independent scoring and efficient use of filters.

Mapping of telomere sequences was done using CHEF gels as described in WU and TANKSLEY (1993b). Microsatellites were mapped on 6% denaturing polyacrylamide gels as described in WU and TANKSLEY (1993a).

Clones: A *Pst*I genomic library from rice (probes designated "RG"), whose construction was previously described in MCCOUCH *et al.* (1988), served as one source of clones. Initially, 100 of these markers were selected at regular intervals from a previous version of the map (MCCOUCH *et al.* 1988; FULTON and TANKSLEY 1990) and transferred to the SL population. An additional 138 probes from this genomic library were also used in this study. Of the previously surveyed rice genomic probes, 30 which were previously unmapped due to monomorphism in the IB combination were mapped onto the SL population. We also mapped markers from three cDNA libraries. These libraries were constructed for use in this study and other RFLP studies involving grass species in this laboratory (O'DONOUGHUE *et al.* 1992; HEUN *et al.* 1991; ANDERSON *et al.* 1992). They were derived from mRNA extracted from etiolated leaf sheath from the cultivars IR36 (rice, *O. sativa*), Brooks (oat, *Avena sativa*), and Willis (barley, *Hordeum vulgare*). cDNA clones from rice are designated "RZ," from oat, "CDO," and from barley, "BCD." Inserts averaging more than 1 kb were cloned into the *Eco*RI site of the Lambda ZapII vector (rice and barley cDNA) or the *Eco*RI-*Xho*I site of the Uni-ZAPXR vector (oat cDNA) (Stratagene). The cDNA inserts were amplified by the polymerase chain reaction (primers were the oligonucleotides GTAAAACGACGGCCAGT and AAAAGCTATGACCATG; 34 cycles: 1 min at 92°, 40 sec at 50°, 1 min at 72°; final extension cycle for 10 min) and then purified through G-50 Sephadex spin columns.

More than 1800 cDNA probes from these three libraries were screened for hybridization signal on filters containing lanes of *Eco*RI-digested total genomic DNA from five grass species (barley, rice, oat, wheat and sugarcane). Of those presenting a strong signal and a low copy number with rice variety, IR36, 382 clones were utilized in this study.

Telomere sequences were detected by pulsed-field gel electrophoresis using an Arabidopsis telomere probe as described in WU and TANKSLEY (1993b). Microsatellite markers containing the GA, GT, and AT repeat motifs were identified based on a search of all rice sequences in the GenBank and EMBL databases or subcloned from a 15-kb genomic library from cv IR36 as described in WU and TANKSLEY (1993a).

Clones obtained from other laboratories are listed in Table 1. All of these were mapped using Southern analysis, except that the polymorphism for the large subunit of ribosomal DNA (*r45s*) was read directly from photographs of agarose gels containing *Dra*I-digested genomic DNA stained in ethidium bromide.

Nomenclature: Clones were classified as single copy if >90% of the signal was accounted for by only one or two hybridizing bands for at least one enzyme on a survey filter. When a marker detected more than two bands for all enzymes, a suffix (A, B, or X) was added to the probe number to indicate the specific copy that was mapped. If only one band of a multiple copy probe was studied, an X was used as the suffix. If two loci of a multiple copy clone were mapped, the marker was given an A or a B suffix. There were no cases were more than two copies of a particular sequence were mapped.

Map construction: Linkage analysis was performed using Mapmaker Version 3.0 (LANDER *et al.* 1987) on a Sun II workstation, and using Map Manager (MANLY 1993) on a Macintosh

TABLE 1

Known genes

Gene	Type	Product/Phenotype	Chromosome	Reference
ACC1	RFLP	ACC synthase	*3*	A. THEOLOGIS, Pl. Gen. Exp. Cntr. (personal communication)
ACC3	RFLP	ACC synthase	*5*	A. THEOLOGIS, Pl. Gen. Exp. Cntr. (personal communication)
Amy1B	RFLP	α-Amylase	*1*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy1C	RFLP	α-Amylase	*2*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy1A	RFLP	α-Amylase	*2*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy2A	RFLP	α-Amylase	*6*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy3A	RFLP	α-Amylase	*9*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy3B	RFLP	α-Amylase	*9*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy3C	RFLP	α-Amylase	*9*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy3D	RFLP	α-Amylase	*8*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Amy3E	RFLP	α-Amylase	*8*	HUANG *et al.* (1990); RANJHAN *et al.* (1991)
Acp1	Isozyme	Acid phosphatase	*12*	L. ZHU, Academia Sinica (personal communication)
Acp2	Isozyme	Acid phosphatase	*12*	L. ZHU, Academia Sinica (personal communication)
Acp4	Isozyme	Acid phosphatase	*7*	MCCOUCH (1990)
Actin 1	RFLP	Actin	*3*	MCCOUCH (1990)
Adh-1	Isozyme	Alcohol dehydrogenase	*11*	ABENES *et al.* (1994)
ATP	RFLP	ATPase	*5*	N. CHUA, Rockefeller University (personal communication)
atub	RFLP	α-Tubulin	*9*	MCCOUCH *et al.* (1988)
Bph-10(t)	Morph	Brown planthopper resistance	*12*	T. ISHII and D. BRAR, IRRI (personal communication)
CALa	RFLP	Calmodulin	*5*	Z. CHEN, Peking University (personal communication)
CALb	RFLP	Calmodulin	*7*	Z. CHEN, Peking University (personal communication)
Cl	Morph	Clustered spikelets	*6*	YU (1991)
cyc1	RFLP	Cyclophilin	*9*	W. BUCHHOLZ, Texas A&M (personal communication)
cyc2	RFLP	Cyclophilin	*2*	W. BUCHHOLZ, Texas A&M (personal communication)
d-5	Morph	Bunketsu-waito tillering dwarf	*2*	YU (1991)
d-11	Morph	Norin-28 dwarf	*4*	YU (1991)
d-27	Morph	Bunketsuto tillering dwarf	*11*	ABENES *et al.* (1994)
Dn-1	Morph	Dense panicle	*9*	YU (1991)
ef	Morph	Early flowering	*10*	T. ISHII and D. BRAR, IRRI (personal communication)
ESTI-2	Isozyme	Esterase (isoelectric)	*1*	CHO *et al.* (1994)
EST-2	Isozyme	Esterase	*6*	NAKAGAHARAand HAYASHI (1976)
fgr	Morph	Fragrance	*8*	AHN *et al.* (1992)
Gm-2	Morph	Gall-midge resistance	*4*	MOHAN *et al.* (1993)
gl-1	Morph	Glabrous leaf	*5*	YU (1991)
Glh	Morph	Green leafhopper resistance	*4*	L. SEBASTIAN, IRRI and Cornell University (personal communication)
Hbv	Morph	Hoja blanca resistance	*12*	J. TOHME, CIAT (personal communication)
Hg	Morph	Hairy glume	*3*	YU (1991)
la	Morph	Lazy	*11*	ABENES *et al.* (1994)
lax	Morph	Lax panicle	*1*	YU (1991)
LEC	RFLP	Lectin	*12*	MCCOUCH *et al.* (1988)
Mal 1	Isozyme	Malate dehydrogenase	*1*	MCCOUCH (1990)
ZB8	RFLP	Phenylalanine ammonia lyase	*5*	XHU and LAMB (1991)
Pgd 1	Isozyme	Phosphogluconate dehydrogenase	*11*	MCCOUCH (1990)
Pgi-2	Isozyme	Phosphoglucose isomerase	*6*	MACKILL *et al.* (1993)
Ph	Morph	Phenol staining	*4*	MCCOUCH (1990)
PHY-A	RFLP	Phytochrome-A	*3*	KAY *et al.* (1989)
Pi-1(t)	Morph	Blast resistance	*11*	YU (1991)
Pi-2(t)	Morph	Blast resistance	*6*	YU *et al.* (1991)
Pi-4(t)	Morph	Blast resistance	*12*	YU *et al.* (1991)
Pi-5(t)	Morph	Blast resistance	*4*	WANG *et al.* (1994)
Pi-6(t)	Morph	Blast resistance	*12*	Z. YU, Cornell University (personal communication)
Pi-7(t)	Morph	Blast resistance	*11*	WANG *et al.* (1994)
Pi-9(t)	Morph	Blast resistance	*6*	P. REIMERS and R. NELSON, IRRI (personal communication)
Pi-10(t)	Morph	Blast resistance	*5*	N. NAQVID and B. CHATOO, University of Baroda (personal communication)
Pi-11	Morph	Blast resistance	*8*	L. ZHU, Academia Sinica (personal communication)
Pi-?	Morph	Blast resistance	*4*	J. TOHME *et al.*, CIAT (personal communication)
IPi-(t)	Morph	Blast resistance	*12*	K. L. ZHENG, CNRRI (personal communication)
IPI-3(t)	Morph	Blast resistance	*12*	K. L. ZHENG, CNRRI (personal communication)
Pox-2	Isozyme	Peroxidase	*12*	MCCOUCH (1990)
Pr	Morph	Purple hull	*4*	YU (1991)
R45s	RFLP	45S ribosomal	*9*	MCCOUCH *et al.* (1988)
R5s	RFLP	5S ribosomal DNA	*11*	MCCOUCH *et al.* (1988)
Rf	Morph	Fertility restorer	*10*	YU (1991)
Rc	Morph	Brown pericarp	*7*	YU (1991)
RCH10	RFLP	Rice basic chitinase	*3*	XHU and LAMB (1991)
RTSV	Morph	Rice tungro spherical virus resistance	*4*	L. SEBASTIAN, IRRI and Cornell University (personal communication)
S-5	Morph	Wide compatibility	*6*	ZHENG (1992); LIU *et al.* (1992); YANAGIHARA *et al.* (1994)
SALT	RFLP	Salt tolerance	*1*	CLAES *et al.* (1990)
Se-1	Morph	Photoperiod sensitivity	*6*	MACKILL *et al.* (1993)
sd-1	Morph	Semi-dwarf	*1*	CHO *et al.* (1994)

TABLE 1

Continued

Gene	Type	Product/Phenotype	Chromosome	Reference
Sdh-1	Isozyme	Shikimate dehydrogenase	*12*	McCouch (1990)
Se-3	Morph	Photoperiod sensitivity	*6*	M. Maheswaran, IRRI (personal communication)
Telsa-1	RFLP	Telomeres	*9*	Wu and Tanksley (1993b)
Telsm-3	RFLP	Telomeres	*11*	Wu and Tanksley (1993b)
Telsm-1	RFLP	Telomeres	*8*	Wu and Tanksley (1993b)
TRYP	RFLP	Trypsin-inhibitor	*1*	Z. Chen, Peking University (personal communication)
Wph-1	Morph	Whiteback planthopper resistance	*7*	McCouch (1990)
wx	RFLP	Waxy	*6*	Shure *et al.* (1983); McCouch *et al.* (1988)
Xa-1	Morph	Bacterial blight resistance	*4*	Yoshimura *et al.* (1992)
Xa-2	Morph	Bacterial blight resistance	*4*	Yoshimura *et al.* (1992)
Xa-3	Morph	Bacterial blight resistance	*11*	Yoshimura *et al.* (1992)
Xa-4	Morph	Bacterial blight resistance	*11*	Yoshimura *et al.* (1992)
xa-5	Morph	Bacterial blight resistance	*5*	McCouch *et al.* (1991)
Xa-10	Morph	Bacterial blight resistance	*11*	S. Yoshimura and R. Nelson, IRRI (personal communication)
Xa-21	Morph	Bacterial blight resistance	*11*	Ronald *et al.* (1992)
z-2	Morph	Zebra stripe	*11*	Abenes *et al.* (1994)
OTHERS				
RRH-18	RFLP	Linked to Pi-10 (from Tongil)	*5*	N. Naqvid, University of Baroda (personal communication)
RRO-02	RFLP	Linked to Pi-9 (from O. minuta)	*6*	P. Reimers, IRRI (personal communication)
RRA-19	RFLP	Linked to Se-3	*6*	M. Maheswaran, IRRI (personal communication)
pTA248	RFLP	Linked to Xa-21 (from O. longist.)	*11*	Ronald *et al.* (1992)
UMC44	RFLP	From maize map	*4*	J. Gardiner, University Missouri (personal communication)
BNL8.29	RFLP	From maize map	*3*	B. Burr, Brookhaven National Laboratory (personal communication)

IIci. All pairs of linked markers were first identified using the "group" command with LOD > 4.0, and recombination fraction (θ) = 0.25 on the Mapmaker program. Cosegregating markers (*e.g.*, no recombination among markers within a linkage group) were identified by scanning two-point linkage data. Framework maps were constructed using only one marker from each set of cosegregating markers. The "orders" and the "compare" commands in Mapmaker, were used to identify the most probable marker order within a linkage group. The "ripple" command was used to verify the order. Markers were retained within the framework map only if the LOD value for "ripple" was >2.5 and if more than 70 individuals had been scored at a locus. The command "uninformative loci" in the Map Manager program was used to identify the number of scored data points at each locus. Following the establishment of a framework map, each interval was scanned for double cross-overs using the "double crossovers" command (Map Manager program). If any single-locus double-crossovers were observed, the primary data was re-checked for accuracy of scoring at the markers in question. Markers responsible for more than three double crossover events in any interval were removed from the framework map and placed in parentheses to the right of the interval most likely to contain them. Additional markers were assigned to intervals within the LOD 2.5 framework using the "try" command, followed by the process of submitting marker orders to "compare," pulling out markers responsible for double crossovers, and reconfirming the LOD > 2.5 framework map using the "ripple" command in Mapmaker. Map units (cM) were derived using the Kosambi function (Kosambi 1944).

The software program, Map Manager (Manly 1993) was used to perform a chi-square test to determine if the allele frequency at individual loci deviated from the expected 1:1 segregation for the BC population ($P > 0.05$).

RESULTS

Comparison of copy number and detection efficiency among libraries: When the rice cDNA library was surveyed for copy number, approximately 66% of clones were single copy (1–2 bands), 17% were multiple copy (3–9 bands), and 17% were repeated (smear) when filters were washed at 0.5 × SSC at 65° following Southern hybridization (Table 2). This compared with 58% single copy, 20% multiple copy, and 22% repeated sequences observed for a rice genomic library at the same washing stringency (McCouch *et al.* 1988). When the oat cDNA library was hybridized onto rice, 18% of the clones gave no signal, but of clones with signal, approximately 64% were single copy, 28% were multiple copy (with 3–8 bands), and 8% were repeated (giving a smear). When the barley cDNA library was hybridized onto rice, 16% of clones gave no signal, but of clones with signal, 48% were single copy, 42% were multiple copy, and 10% were repeated (Table 2). Therefore, the most efficient source of single copy sequences for mapping was the rice cDNA library.

Comparison of polymorphism in intraspecific and interspecific crosses: Table 3 shows the percentages of probes detecting polymorphism in three parental combinations for at least one of five restriction enzymes. The parents include the interspecific combination, *O. sativa* and *O. longistaminata* [BS125/WL02//BS125 (SL)], and two intra-*O. sativa* crosses [IR34583-19-3-3/Bulu Dalam (IB) and Apura/IRAT177 (AI)]. The percent polymorphism is compared among parental combinations and for two different libraries of probes: a *Pst*I genomic library from rice and a cDNA library from oat (Avena). Polymorphism in the interspecific parents was higher than for either of the intraspecific combinations for every restriction enzyme tested. In the interspecific cross, 85% of clones from the *Pst*I genomic library detected polymorphism with at least one of the five

TABLE 2

Frequency of single copy, multiple copy or repeated sequences in cDNA libraries derived from rice, oat, and barley based on Southern hybridization with total rice DNA (washing stringency = 0.5× SSC at 65°)

Source of cDNA clones	Single copy (%)	Multiple copy (%)	Repeated (%)	No signal (no.)	*n*
Rice (cv. IR36)	66	17	17	0	576
Oat (cv Brooks)	64	28	8	36	200
Barley (cv Willis)	48	42	10	13	80

enzymes. In comparison, 75% of clones from the same library were polymorphic in the IB combination when the same enzymes were evaluated, and 38% were polymorphic in the AI combination. Statistics are similar with respect to the oat cDNA library, where 87.5% of clones detected polymorphism in the SL combination and 47% detected polymorphism in the AI combination. The IB combination was not tested with cDNA clones, and comparisons of polymorphism with RZ and BCD clones are not available because the IB and AI mapping parents were not surveyed with clones from these libraries.

Genomic marker analysis: To determine the usefulness of a rice genomic library for mapping in other *Gramineae* species, 37 clones with clear, single copy signal in rice were hybridized onto filters containing DNA from rice, wheat, barley and oat. Results from this experiment demonstrated that clear hybridization signal was detected for 65% of clones on wheat, 32% on barley, and 11% on oat. This suggests that the wheat genome is likely to contain a large proportion of DNA with homology to rice genomic sequences.

The level of polymorphism detected by a single enzyme for genomic clones in the interspecific combination in this study was in good agreement with the genetic distance between these species observed in an RFLP study by WANG *et al.* (1990). They found 54% of probes were polymorphic between *O. longistaminata* accessions and *indica* varieties of *O. sativa*, using *Eco*RI digests only. This compares with an average of 34% for a single enzyme for the IB combination and 13% for the AI combination. The significant differences in levels of polymorphism observed between the two intraspecific combinations included in this study support the concept that some putative *indica* × *japonica* combinations are genetically much more distant than others. In addition, this work suggests that for rice, as for other crops such as tomato (BERNATZKY and TANKSLEY 1986), potato (BONIERBALE *et al.* 1988) or sorghum (CHITTENDEN *et al.* 1994), the choice of an interspecific cross is an efficient way of detecting high levels of molecular polymorphism.

Construction of the rice linkage map: The total length of the map based on the interspecific (SL) population is 1491 cM, which corresponds to approximately one marker every 2.1 cM. It is comprised of 250 rice cDNAs, 112 oat cDNAs, 20 barley cDNAs, 238 rice genomic clones, 2 maize genomic clones, 26 cloned genes, 11 isozymes, 11 microsatellite markers, 3 telomeric markers, 6 RAPD and 47 morphological mutant loci. The morphological mutant loci, which are underlined and in bold print in Figure 1, were mapped using a variety of plant material but a common set of molecular markers. All underlined markers in Figure 1 represent loci that were mapped on other populations, and whose map position on the SL map has been estimated based on linkage to common markers. Thirty markers, whose location previously had been unambiguously determined by trisomic analysis (MCCOUCH *et al.* 1988; YU 1991) were used to assign linkage groups to their respective chromosomes. The linkage analysis was performed using successive thresholds of recombination values of 0.20, 0.25 and 0.30, and a LOD score of 2.5. When the order of adjacent markers could not be established with a LOD higher than 2.5, only one marker was used in the mapping framework (Figure 1), the other one being represented in parentheses at its most probable location to the right of the most closely linked clone on the framework. The LOD 2.5 framework of the map is based on 372 markers and the average interval defined by markers on the framework map is 4.0 cM. The markers that are separated from framework markers by a comma are tightly linked (less than 1 cM) to a framework marker. Two hundred twenty-four additional probes that were mapped with LOD < 2.5 onto the same population are represented in parentheses to the right of the framework markers. Chromosomes are comprised of 20–96 markers, with an average of 50 per chromosome.

There was a good correlation between the relative length of chromosomes measured at pro-metaphase by image analysis (FUKUI and IIJIMA 1991) and the number of probes per chromosome on our map (Figure 2A; $R^2 = 0.91$; $P < 0.01$). The relationship between relative length of pro-metaphase chromosomes and the length of the chromosomes in cM on the RFLP map was also significant (Figure 2B; $R^2 = 0.65$; $P < 0.05$), though lower than for number of markers. This is related to the fact that the recombination distance between markers was not uniform along the chromosomes. Indeed, the average distance between markers differed significantly ($F = 3.01$, $P < 0.001$) between chromosomes. The variation in interval size was notable both between and within chromosomes. The average interval size was smallest for chromosomes *1*, *2* and *3* (the largest and most populated chromosomes), and was largest for chromosomes *7*, *11* and *12* ($P < 0.05$). The distribution of markers along several of the chromosomes was markedly uneven. Two densely populated regions can be noticed on chromosome *3*, punctuated by intervals of 21.5 and 14.0 cM. Several markers place to these intervals but cannot be mapped with precision (*i.e.*, LOD < 2.5). This uneven distribution of markers suggested that the frequency of

TABLE 3

Percent of genomic and cDNA clones detecting polymorphism in three cross combinations based on a survey of 200 *PstI* rice genomic clones and 166 oat (Avena) cDNA clones

Library	Cross combination	Percent probes polym					Percent probes polymorphic ≥1 restriction enzyme
		*Eco*RV	*Hin*dIII	*Xba*I	*Sca*I	*Dra*I	
Rice genomic	BS125/WL02//BS125	52	57	57	58	46	85.0
Rice genomic	IR34583-19-3-3 × Bulu Dalam	39	31	40	30	31	75.0
Rice genomic	Apura × IRAT177	16	12	9	18	13	38.0
Oat cDNA	BS125/WL02//BS125	65	64	49	55	—	87.5
Oat cDNA	Apura × IRAT177	17	25	32	25	23	47.0

Significant differences in levels of polymorphism ($P < 0.01$ based on a one-way ANOVA) were detected between parents of the SL, IB and AI populations. Differences in levels of polymorphism between libraries were not significant. "—" signifies no data available.

recombination may vary greatly from one region to another on the same chromosome. A similar pattern was observed on chromosomes *1*, *2* and *7* (Figure 1).

Eleven isozyme loci have also been located via linkage to RFLP markers. Currently located on the map are *Mal-1*, *Acp-1*, *Acp-2*, *Acp-4*, *Pgi-2*, *Adh-1*, *Pgd-1*, *Sdh-1*, *Est-1*, *Est-2* and *Pox-2*. The molecular map developed in this laboratory has been oriented with respect to the classical linkage map of rice (KINOSHITA 1993) based on isozyme and morphological markers (Figure 1) and the rice RFLP map constructed by SAITO *et al.* (1991) and NAGAMURA *et al.* (1993) (XIAO *et al.* 1992) (data not shown). It provides a stable framework for rapidly and efficiently locating new markers on rice chromosomes. The results of this analysis and the RFLP data set associated with the *O. sativa* × *O. longistaminata* BC population are accessible in the Rice Genome Database ("RiceGenes") through the National Agricultural Library in Washington, D.C., or through Gopher (MCCOUCH and PAUL 1994).

Relationship between molecular and classical linkage map in rice: Figure 1 summarizes the current status of both the molecular map developed in this laboratory and the classical linkage map of rice (KINOSHITA 1993). Morphological mutant loci in common between the maps are placed to the left of the chromosomes on the classical map. The classical map is constructed from hundreds of different crosses, each segregating for only a few loci. Though the order of loci is expected to be stable, recombination frequency is highly variable among crosses. For example, *sd-1* and *lax* on chromosome *1* are both linked to markers that map within 10 cM of each other on the molecular map, but are much further apart on the morphological map. Similarly, the distances between *pH*, *Pr* and *d-11* on chromosome *4* of the two maps appear quite different. Thus, the genetic distances between markers represented on the classical map cannot be directly compared to those on the molecular map. In a few cases, the order of markers along a chromosome does not agree when the two maps in Figure 1 are compared. This is the case for *Pgd-1* and *la* on chromosome *11*. Additional marker analysis is required in order to resolve these discrepancies. Two of the chromosomes have no markers in common, while seven others have between one and four markers located on both maps. Chromosome *6* stands out with eight markers in common, followed by chromosomes *11* and *4*, with six and five markers in common, respectively. This reflects interest in specific chromosomal regions containing genes of interest where linkage has been established between target genes and molecular markers.

Colinearity of the intra and interspecific maps: A sample of 100 probes, selected at regular intervals from an updated version of the map described in MCCOUCH *et al.* (1988), were initially mapped onto the interspecific population to compare the order of the probes in the two maps. This served to ensure that a map based on an interspecific cross would provide similar information as one based on an intraspecific cross. With few exceptions, probes were found to be located on the same chromosome and in the same order in both populations.

Two types of modifications could be observed: small modifications in the placement of markers and no change in the chromosome assignment, or change of assigned chromosome for a marker or a group of closely linked markers. Changes in chromosomal assignments for a marker or linked cluster of markers occurred in only five cases. In all five cases, the placement of markers to a specific linkage group in the IB F_2 population had been based on dosage analysis, rather than linkage analysis, using the trisomic stocks described by KHUSH *et al.* (1984).

The apparent contradictions may be explained by the fact that multiple copy clones, having multiple chromosomal locations, were involved in several cases. These sequences were originally mapped in the IB population based on high stringency washes. When they were mapped in the SL population, it was not clear whether the same or a different allele was segregating, providing an explanation for new locations of some markers. Difficulties in locating markers in distal regions of chromosomes are another source of error. Alternatively, irregularities in the trisomic stocks have been reported (OKA and WU 1988; CHUNG and WU 1990) and small structural rearrangements between related species and subspecies of rice (JENA *et al.* 1992; CHANDRARATNA 1964) may explain other discrepancies.

The five cases of chromosomal discrepancies included *RG365*, previously placed on chromosome *8*, now linked internally on chromosome *2*; *RG136*, previously on chromosome *9*, now linked internally on chromosome *8*; *RG375* and *RG396*, previously mapped onto chromosome *12*, but currently linked in the lower portion of chromosome *4*; *RG29*, previously on chromosome *7*, now located at a distance of 10 cM from one of the telomeres on chromosome *8*; and *RG98*, *RG304A* and *RG235*, three linked, single copy probes, previously reported to reside on chromosome *12*, now placed distally on chromosome *11*. The clearest evidence obtained from this study for the occurrence of a small translocation is related to the placement of *RG190*. In the SL and the IB populations, *RG190* maps to chromosome *12* (LOD > 2.5), supported by trisomic data (McCouch *et al.* 1988). However, in other populations, *RG190* is reported to be linked to *RG375* and *RG396* at the bottom of chromosome *4* (M. Champoux, IRRI, Philippines, personal communication; W. Park and Z. Li, Texas A & M University, Texas, personal communication; A. Saito, NIAR, Japan, personal communication). A study of the extra chromosomes in the IR36 trisomic series by Chung and Wu (1990) suggested that the extra chromosome in Triplo 12 contained a translocated section of chromosome *4*. Further study is necessary to resolve the possibility of structural rearrangements in these regions of chromosomes *4* and *12*.

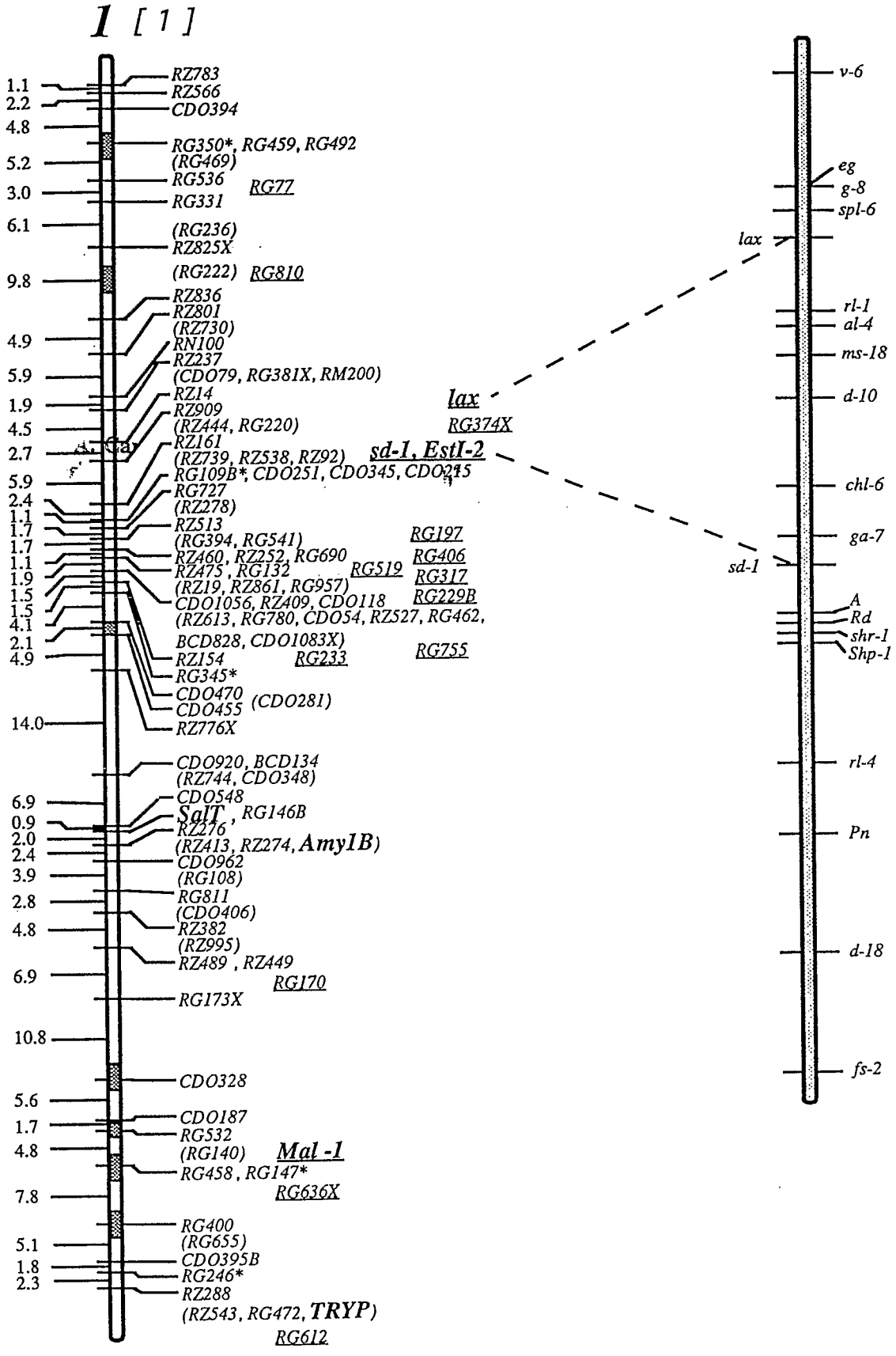
Based on both genetic and physical mapping, Wu and Tanksley (1993b) demonstrated that *RG29* was linked to a telomere on chromosome *8*, and that *RG98* was linked to a telomere on chromosome *11*. The distance between *RG98* and the chromosome *11* telomere was estimated to be 8 cM, corresponding to a physical distance of approximately 270 kb. This suggests an exceptionally high rate of recombination, such that 1 cM is equivalent to approximately 34 kb in the distal portion of this chromosome. Linkage estimation is more dependable when markers are nested among other markers on a genetic map than when they are located at the ends of chromosomes. Though physical mapping of distal markers based on pulsed field gel electrophoresis (PFGE) offers convincing evidence that markers are actually located near telomeres, only a few probes to date (*RG29* on chromosome *8*, *RG98* on chromosome *11*, and *r45S* on chromosome *9*) have been mapped using this approach in rice due to the difficulty of clearly identifying segregation using PFGE techniques (Wu and Tanksley 1993b). The availability of libraries composed of large DNA fragments, in yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or cosmid vectors will facilitate the mapping of distally located markers and provide confirmation of marker order and placement on existing genetic chromosome maps.

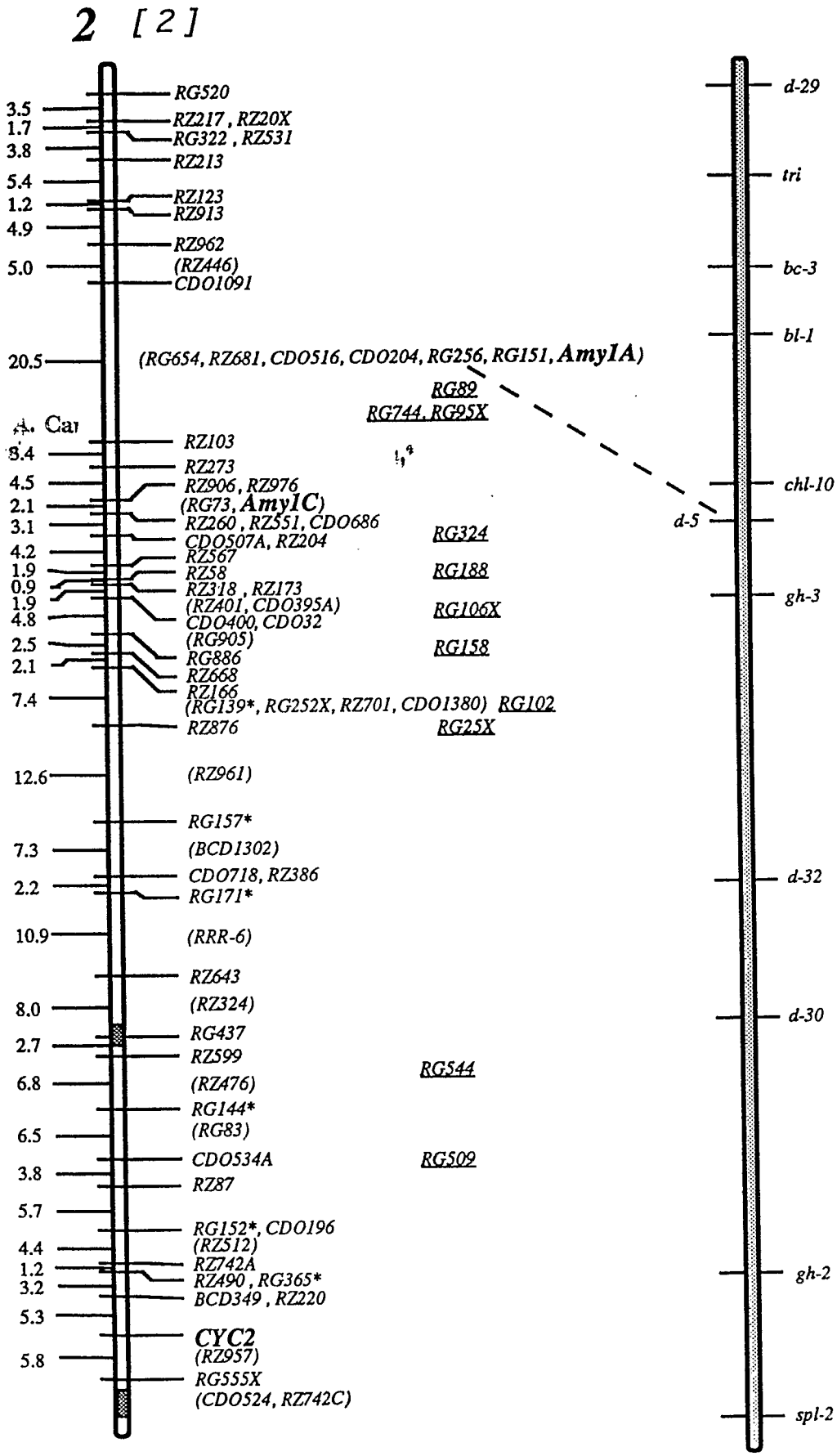
Multiple copy markers: There are a total of 50 multiple copy markers on the existing RFLP map of rice. In most cases, only one copy could be mapped because segregation could be clearly detected for only one locus. In eight cases (*RG146*, *RG369*, *CDO395*, *CDO507*, *CDO534*, *CDO1387*, *BCD454* and *CDO127*) two copies of a single cloned sequence were mapped. In only one instance (*RG369A* and *RG369B*) did both copies of a clone map to the same chromosome (chromosome *3*). Evaluation of the map positions of other duplicate-copy clones reveals no clear pattern of regional duplication in the rice genome. This situation can be contrasted to that of maize, an ancient polyploid, where almost all chromosomal regions contain contiguous sequences that exist in duplicate elsewhere in the genome (Helentjaris *et al.* 1988; Ahn and Tanksley 1993).

A comparison of copy number of clones mapped in different rice populations revealed no markers that were single copy in one population and highly repeated in another. However, variations in the number and location of members of multiple copy clone families (2–8 copies) among rice populations makes it difficult to use them in comparative mapping studies. This problem is confounded if comparative mapping between species is attempted. The emphasis on cDNA libraries as a source of clones for mapping in this interspecific population provided an efficient way to enrich for single copy sequences. We currently impose stringent selection criteria, targeting only clean, single copy clones for mapping in rice, and find that these are useful in comparative mapping efforts across a range of monocot species (Ahn and Tanksley 1993; Ahn *et al.* 1994; Van Deynze *et al.* 1994).

Framework mapping kit: A kit of 96 single copy markers providing good coverage of the rice genome has been assembled for ready distribution to researchers interested in locating genes of agronomic importance in rice. Thirty-five of the clones in this kit are from the rice genomic library, 47 are cDNA clones from rice, and 14

Figure 1.—Rice linkage maps: on right is the molecular map based on the interspecific backcross population (*O. sativa/O. longistaminata//O. sativa*); on left is the classical map based on morphological mutants and isozyme markers (reproduced with permission from Kinoshita 1993). The chromosome numbering, indicated at the top of chromosomes, follows the nomenclature established by the Rice Genetics Cooperative Committee in IRRI, May 1990 (Khush 1990). Between square brackets is presented the nomenclature used in McCouch *et al.* (1988), following Khush *et al.* (1984). Probe designation to the right of the chromosomes: cDNA markers are designated as RZ (from rice), CDO (from oat), BCD (from barley), rice genomic markers are designated as RG, maize genomic markers are UMC and BNL, microsatellite markers are RM or RN (Wu and Tanksley 1993a). Probes hybridizing with two or more major bands are indicated with an X if only one locus has been mapped; with A and B if two loci were mapped. Known genes are listed in Table 1. The probes located by trisomic studies are indicated with a star (*). Map distances are presented in centimorgans (Kosambi function) to the left of the chromosomes. Markers located to intervals with a LOD score < 2.5 are represented in parentheses in the appropriate interval. Stippled regions along the chromosomes represent regions containing markers with skewed allele frequencies ($P < 0.05$).


1 [1]
1.1 RZ783
2.2 RZ566
CDO394
4.8
RG350*, RG459, RG492
5.2 (RG469)
RG536 RG77
3.0 RG331
6.1 (RG236)
RZ825X
9.8 (RG222) RG810
RZ836
RZ801
4.9 (RZ730)
RN100
RZ237
5.9 (CDO79, RG381X, RM200)
1.9 RZ14
RZ909
4.5 (RZ444, RG220)
RZ161
2.7 (RZ739, RZ538, RZ92)
5.9 RG109B*, CDO251, CDO345, CDO215
RG727
2.4 (RZ278)
1.1
1.7 RZ513
(RG394, RG541)
1.7 RZ460, RZ252, RG690
1.1 RZ475, RG132
1.9 (RZ19, RZ861, RG957)
1.5 CDO1056, RZ409, CDO118
1.5 (RZ613, RG780, CDO54, RZ527, RG462,
4.1 BCD828, CDO1083X)
2.1 RZ154
4.9 RG345*
CDO470
CDO455 (CDO281)
14.0 RZ776X
CDO920, BCD134
(RZ744, CDO348)
6.9 CDO548
0.9 SalT, RG146B
2.0 RZ276
2.4 (RZ413, RZ274, Amy1B)
CDO962
3.9 (RG108)
RG811
2.8 (CDO406)
RZ382
4.8 (RZ995)
RZ489, RZ449
6.9 RG170
RG173X
10.8
CDO328
5.6
CDO187
1.7 RG532
4.8 (RG140) Mal-1
RG458, RG147*
7.8 RG636X
RG400
5.1 (RG655)
1.8 CDO395B
2.3 RG246*
RZ288
(RZ543, RG472, TRYP)
RG612
lax
RG374X
sd-1, EstI-2
RG197
RG406
RG519 RG317
RG229B
RG233 RG755
v-6
eg
g-8
spl-6
lax
rl-1
al-4
ms-18
d-10
chl-6
ga-7
sd-1
A
Rd
shr-1
Shp-1
rl-4
Pn
d-18
fs-2


2 [2]
RG520
3.5
1.7
3.8
RZ217, RZ20X
RG322, RZ531
RZ213
5.4
1.2
4.9
RZ123
RZ913
RZ962
5.0
(RZ446)
CDO1091
20.5
(RG654, RZ681, CDO516, CDO204, RG256, RG151, Amy1A)
RG89
RG744, RG95X
RZ103
3.4
RZ273
4.5
RZ906, RZ976
2.1
(RG73, Amy1C)
3.1
RZ260, RZ551, CDO686
CDO507A, RZ204
4.2
RZ567
1.9
RZ58
0.9
RZ318, RZ173
1.9
(RZ401, CDO395A)
4.8
CDO400, CDO32
2.5
(RG905)
2.1
RG886
RZ668
RZ166
7.4
(RG139*, RG252X, RZ701, CDO1380)
RZ876
RG324
RG188
RG106X
RG158
RG102
RG25X
12.6
(RZ961)
RG157*
7.3
(BCD1302)
CDO718, RZ386
2.2
RG171*
10.9
(RRR-6)
RZ643
8.0
(RZ324)
RG437
2.7
RZ599
6.8
(RZA76)
RG144*
6.5
(RG83)
CDO534A
3.8
RZ87
5.7
RG152*, CDO196
4.4
(RZ512)
1.2
RZ742A
3.2
RZ490, RG365*
BCD349, RZ220
5.3
CYC2
5.8
(RZ957)
RG555X
(CDO524, RZ742C)
RG544
RG509
d-29
tri
bc-3
bl-1
chl-10
d-5
gh-3
d-32
d-30
gh-2
spl-2

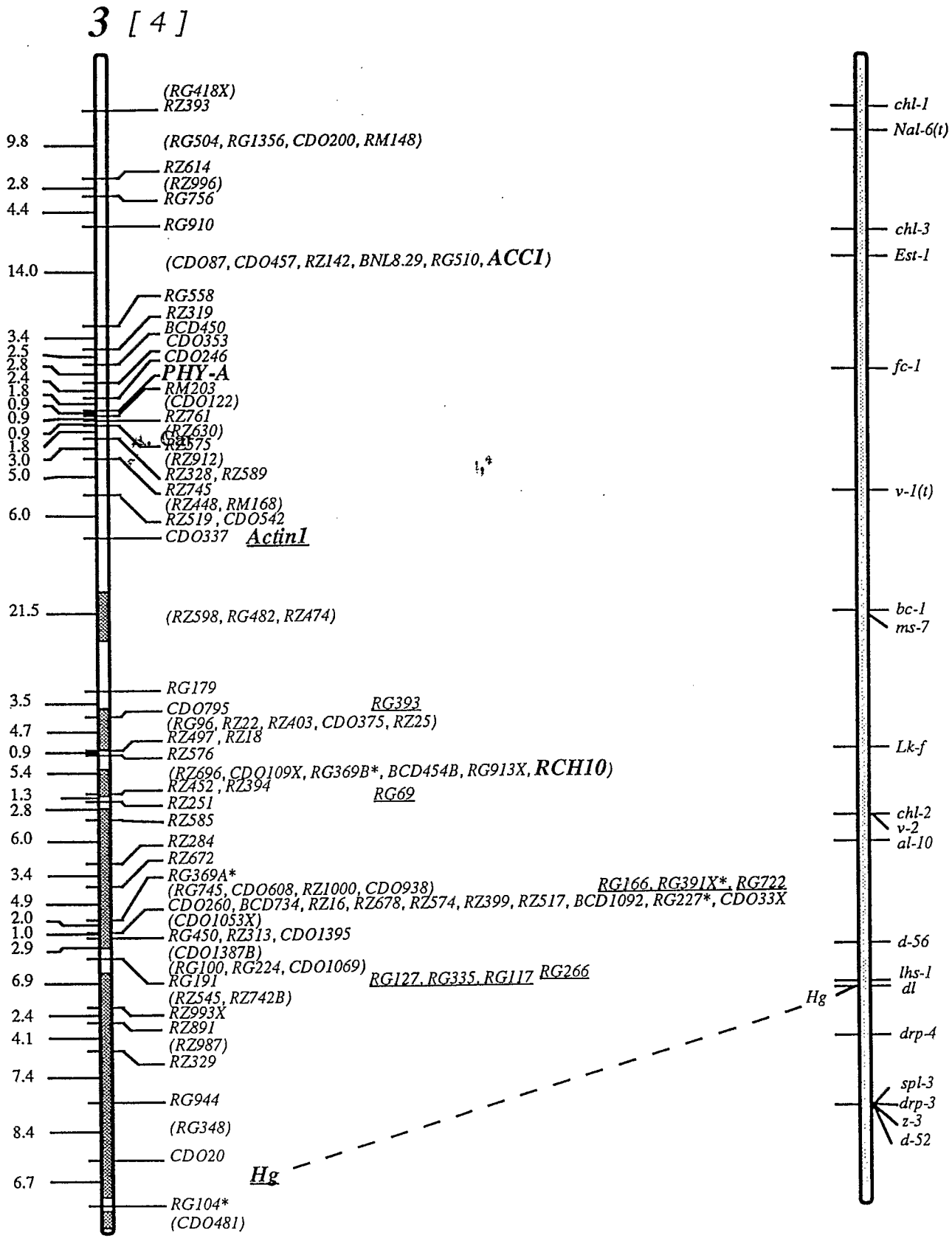


FIGURE 1.—Continued

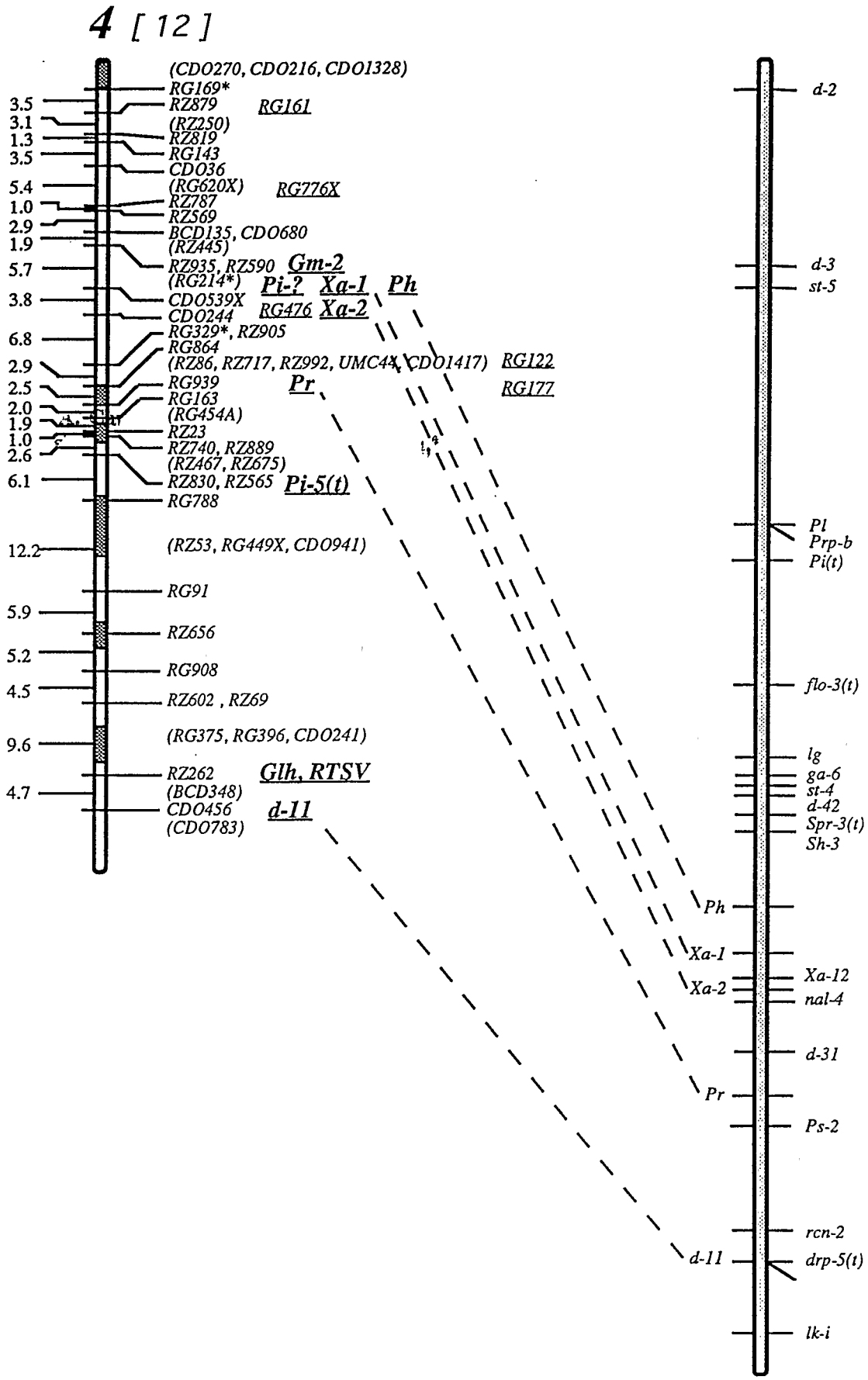


FIGURE 1.—Continued

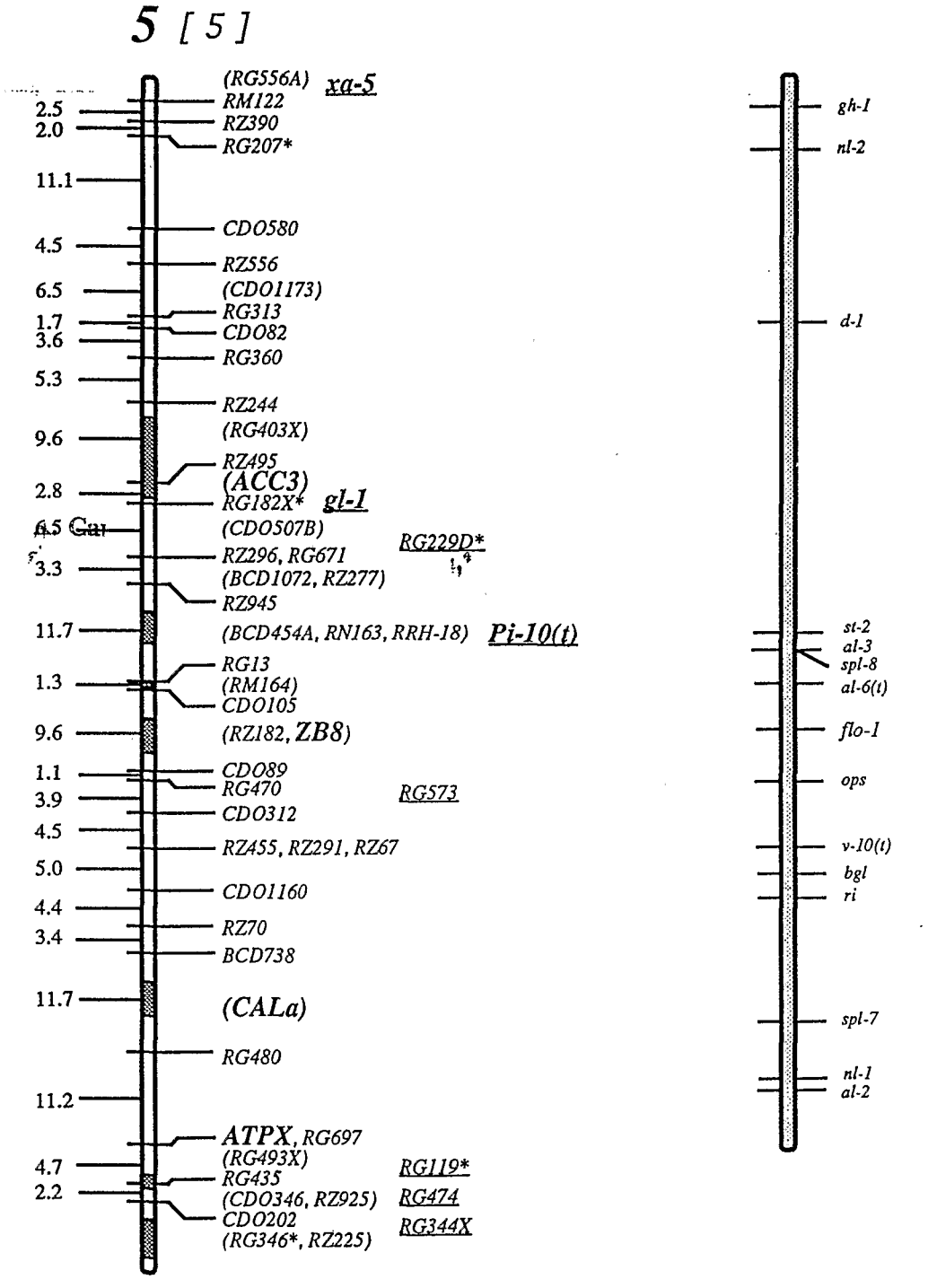


FIGURE 1.—Continued

are cDNA clones from oat. These and other markers have been distributed to over one hundred researchers worldwide and provide the basis for many gene tagging and QTL studies in rice. For studies involving closely related germplasm, where the general level of polymorphism detectable with RFLP markers may be quite low, it is helpful to avail of multiple markers in a given region. Alternatively, microsatellite markers are proving especially useful in these cases because of their high level of allelic diversity (WU and TANKSLEY 1993a). The widespread use of a common set of publicly available clones has provided a basis for comparison and integration of results in rice genome research and comparative mapping efforts in many parts of the world.

Comparison of recombination distances: The recombination fractions between identical pairs of linked markers in the IB population studied by MCCOUCH *et al.* (1988) and in the SL population studied here were compared to determine whether levels of recombination observed in the intra and interspecific mapping populations were simi-

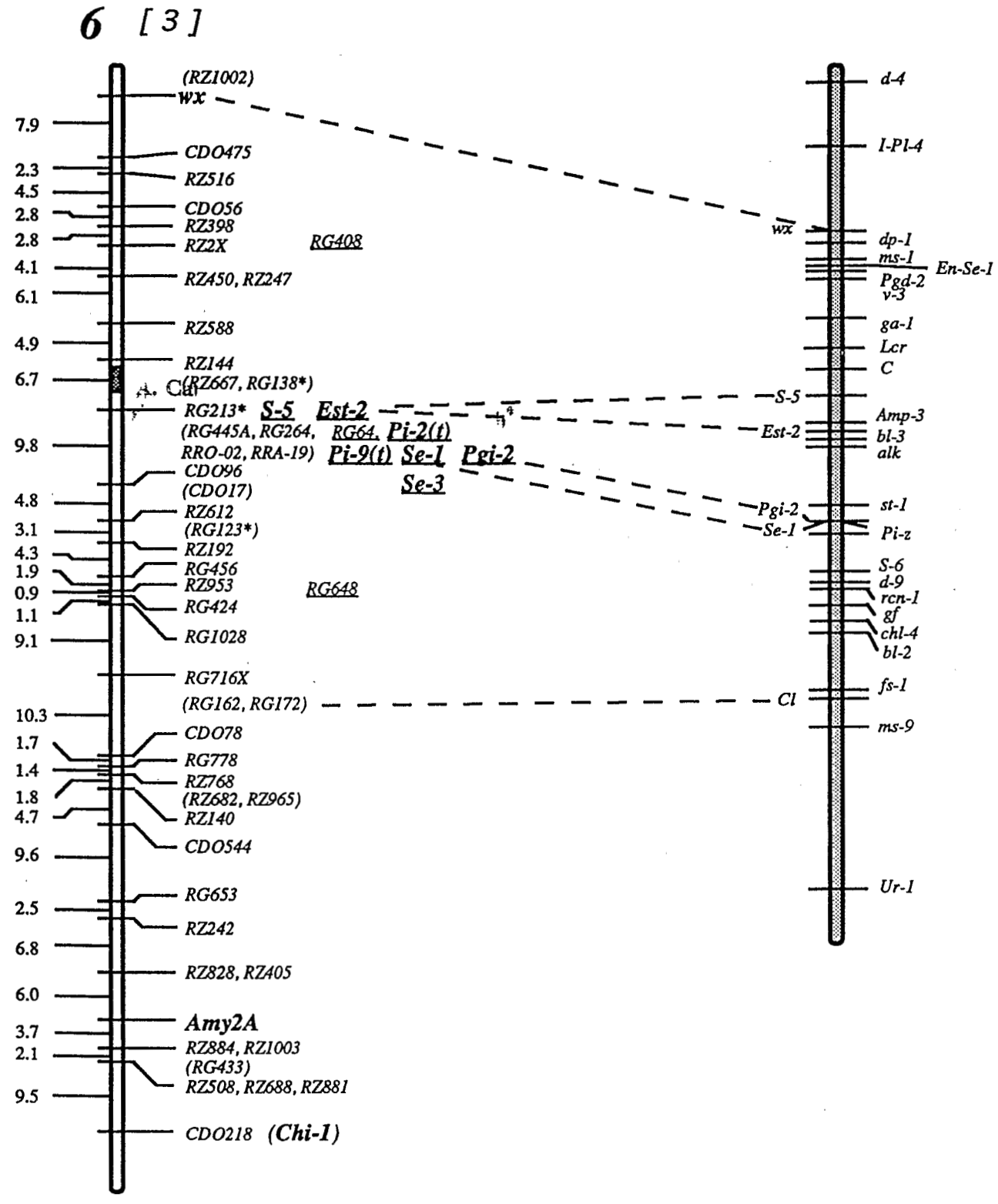


FIGURE 1.—Continued

lar. Based on two-point analysis, 50 intervals located throughout the genome were compared in the two populations. An overall reduction of approximately 25% was observed in the interspecific population. Different selection pressures may act in favor of or against recombination in specific regions. The general reduction of genetic distances between markers observed in this study is in agreement with observations of other interspecific crosses (PATERSON *et al.* 1988; BONIERBALE *et al.* 1988). In interspecific crosses, the homology between DNA strands is reduced, and this is generally related to a reduction in the frequency of chiasmata (GRANT 1958; BORTS and HABER 1987).

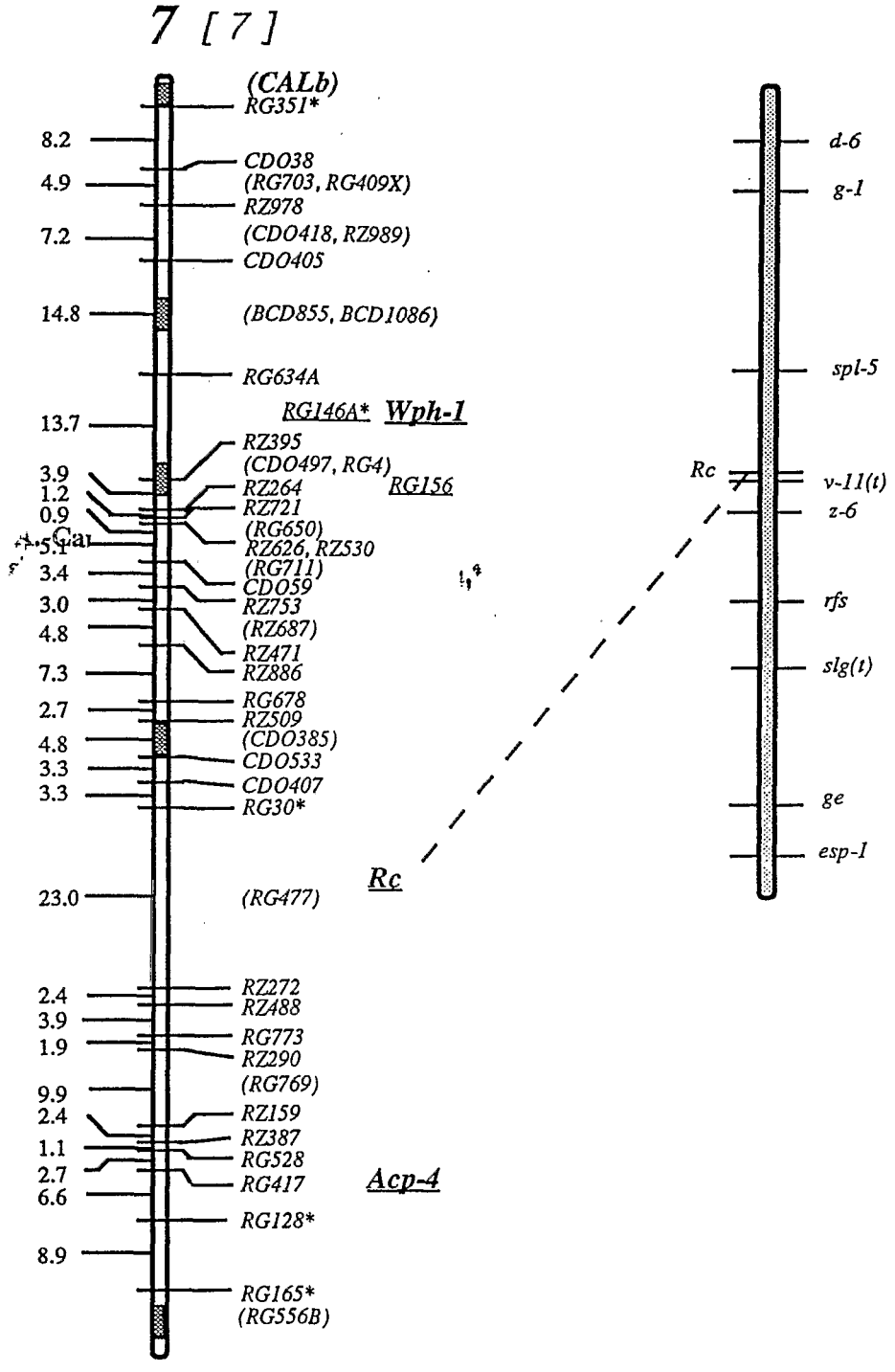


FIGURE 1.—Continued

Occasionally, extreme differences in recombination frequency were detected when the SL and the IB maps were compared using two-point analysis between pairs of markers. At one end of the spectrum, three pairs of markers (*RG458-RG147* on chromosome *1*; *RG224-RG100* on chromosome *3*; *RG125-RG386* on chromosome *9*) showing linkage in the IB map appeared completely linked in the SL population. These discrepancies may be the result of differential rates of recombination, or they may be due to small translocations or inversions along the chromosomes of *O. sativa* and *O. longistaminata*. The observed lack of recombination may cover long distances on the physical map, as these pairs were separated by as much as 12 cM in the IB map.

At the other end of the spectrum, intervals in which the recombination in the SL population was greater than that observed in the IB population were observed on 6 of the 12 chromosomes. These intervals were not consistently located in any particular region of a chromosome. In two cases, they were associated with regions

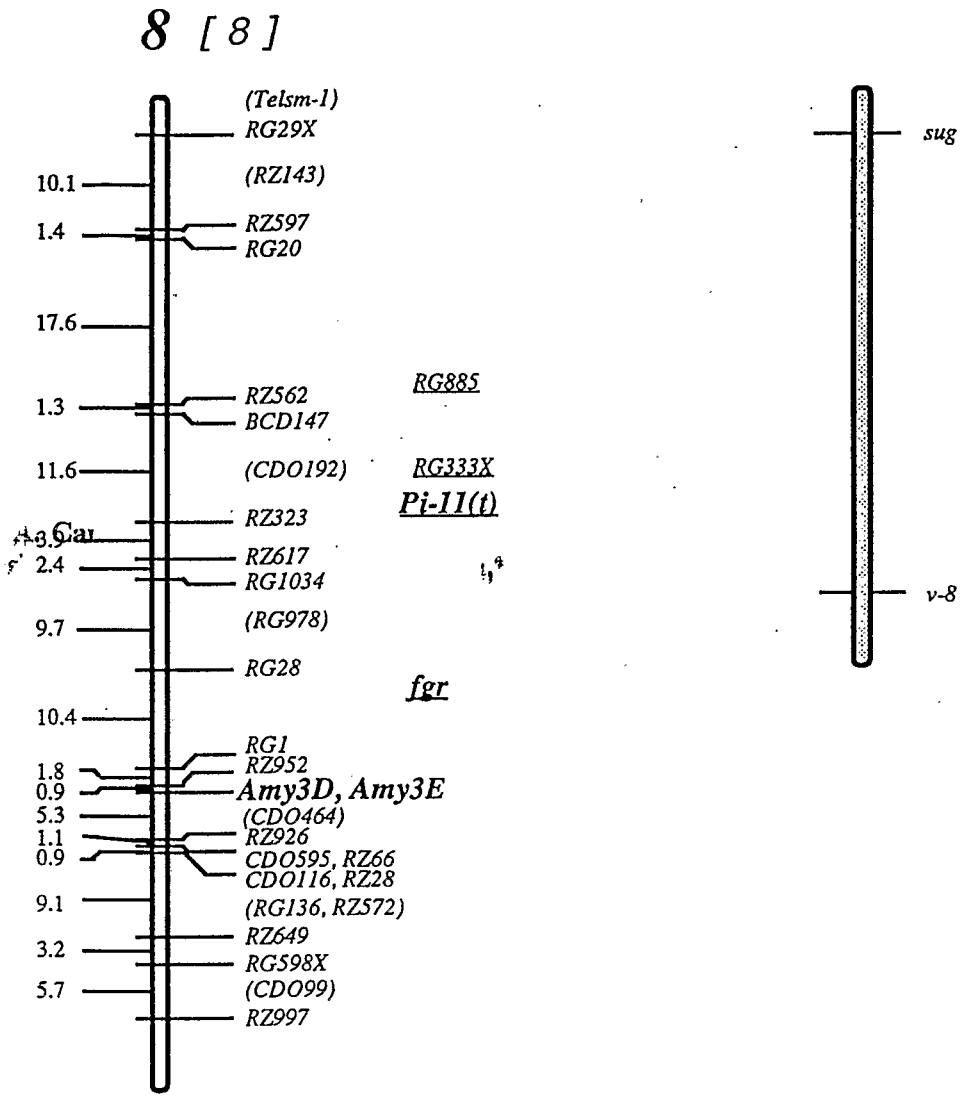


FIGURE 1.—Continued

containing markers that could not be mapped with a LOD > 2.5 (markers in parenthesis on the map), including an interval on chromosome *4* (*RG375-RG396*), and one on chromosome *12* (*RG958-RG323*). In another instance, a higher rate of recombination was observed along a segment of chromosome *6* extending from the *waxy* locus to *RG456*. This region has been reported to have a particularly variable level of recombination. Several workers (YANAGIHARA *et al.* 1992; OKA 1988) have demonstrated drastic differences in estimations of genetic distance between specific markers in this region when crosses between different pairs of *O. sativa* parents are used. Our observations suggest that recombination in the interspecific SL population may exceed that of intraspecific combinations in certain regions.

A consequence of reduced recombination is greater assurance of linkage between markers and ease in recognizing linkage groups in a sparsely populated map. On the other hand, this reduction in map distances limits the ability to precisely order an array of tightly clustered markers when establishing a high density map. For that reason, it is necessary to estimate the precise order of closely linked markers using larger populations and crosses demonstrating a higher rate of recombination in specific regions of interest. Recombinant inbred populations developed via single seed descent offer an alternative way of increasing recombination frequency and simultaneously provide excellent material for mapping quantitative traits of interest (BURR *et al.* 1988; WANG *et al.* 1994). A practical consequence of recombination shrinkage in interspecific crosses is the difficulty in breaking linkage when favorable traits are linked to undesirable ones. However, information about specific regions which are highly recombinagenic in specific cross combinations provides information that can be favorably exploited in a breeding program. Reduction of recombination fractions in interspecific crosses have generally been studied using few morphological markers (RICK 1969; OKA 1988). The use of molecular maps and

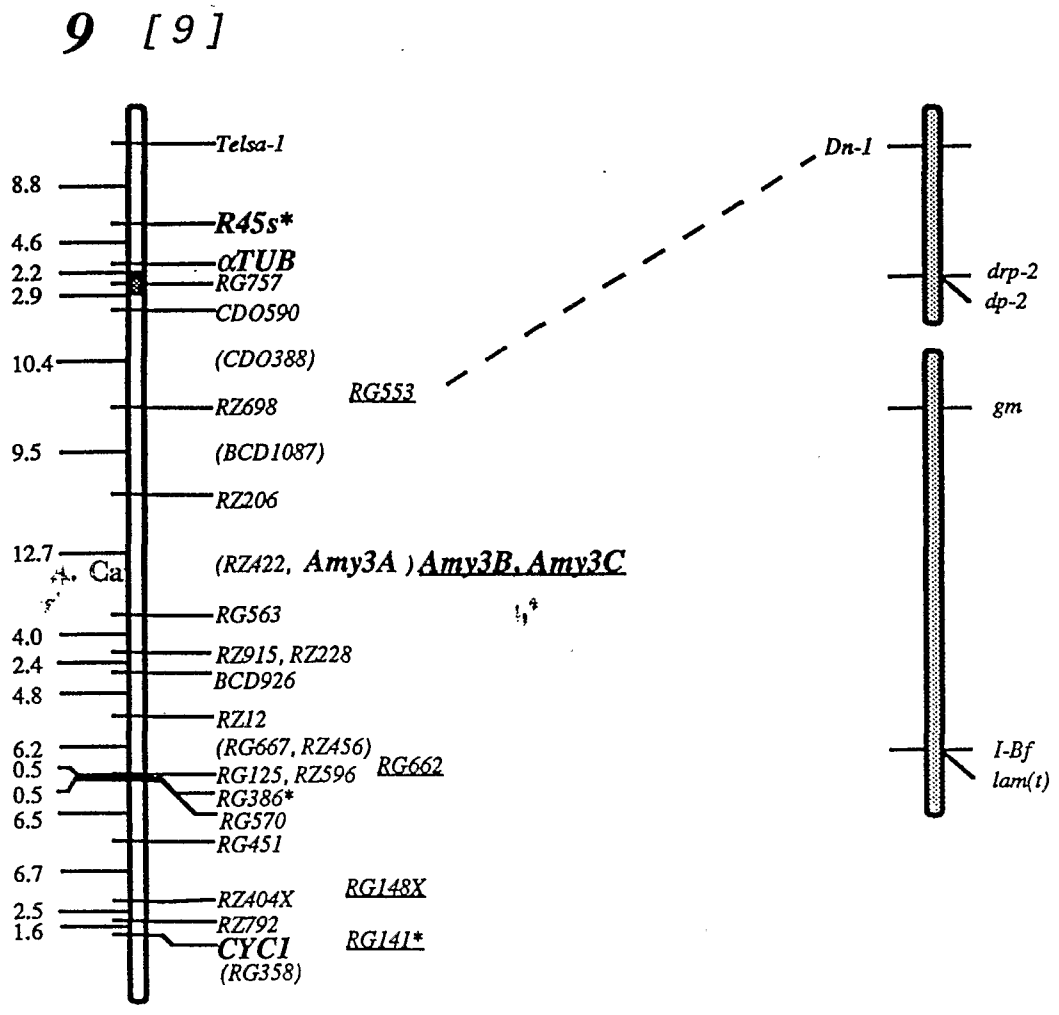


FIGURE 1.—Continued

markers allows us to compare recombination fractions throughout the rice genome for the first time. Fine mapping based on RFLPs or other molecular markers may be helpful in clarifying whether the observed variation is under genetic control and whether it could be exploited to the benefit of plant improvement.

Genome coverage provided by the interspecific map: Despite the continuous addition of new markers to an existing map, once a dependable framework has been established for a population, the addition of new markers is not expected to add substantially to the overall length of the map. Indeed, most markers fall within regions already mapped and tend to map very near or on top of markers already on the framework. This would suggest that a map containing 300 markers for a genome the size of rice would provide fairly complete genome coverage. Based on an exchange of 70 markers, a comparison of the maps prepared by SAITO *et al.* (1991) and that reported by TANKSLEY *et al.* (1992a) suggested that these two independently constructed rice maps provided very similar genome coverage and that the order of markers along the chromosomes was in good agreement (XIAO *et al.* 1992). However, both maps contained several persistent, sparsely populated regions, appearing as large intervals. In some cases, these large intervals fell in comparable regions of the chromosomes, such as the region on chromosome *3* defined by *RG96*, *RG179* and *CDO337*, or that on chromosome *11* defined by *RG1094* and *RG118*.

Our decision to use an interspecific cross provided us with an opportunity to test the hypothesis that regions of chromosomes might be monomorphic in a cross between two cultivated rice species but polymorphic in a cross between a cultivar and a wild species. The efficiency of this approach was demonstrated, as mapping in the interspecific cross allowed us to readily assemble 12 linkage groups without the unlinked segments that remained in our intraspecific map with the same number of markers. It also allowed us to define a region of approximately 80 cM on chromsome *3* which was mapped for the first time using the SL population.

Skewing in the interspecific backcross: Sixteen percent of the markers mapped in the interspecific backcross population exhibited skewed segregation ratios. Deviations from the expected 1:1 allele frequency were

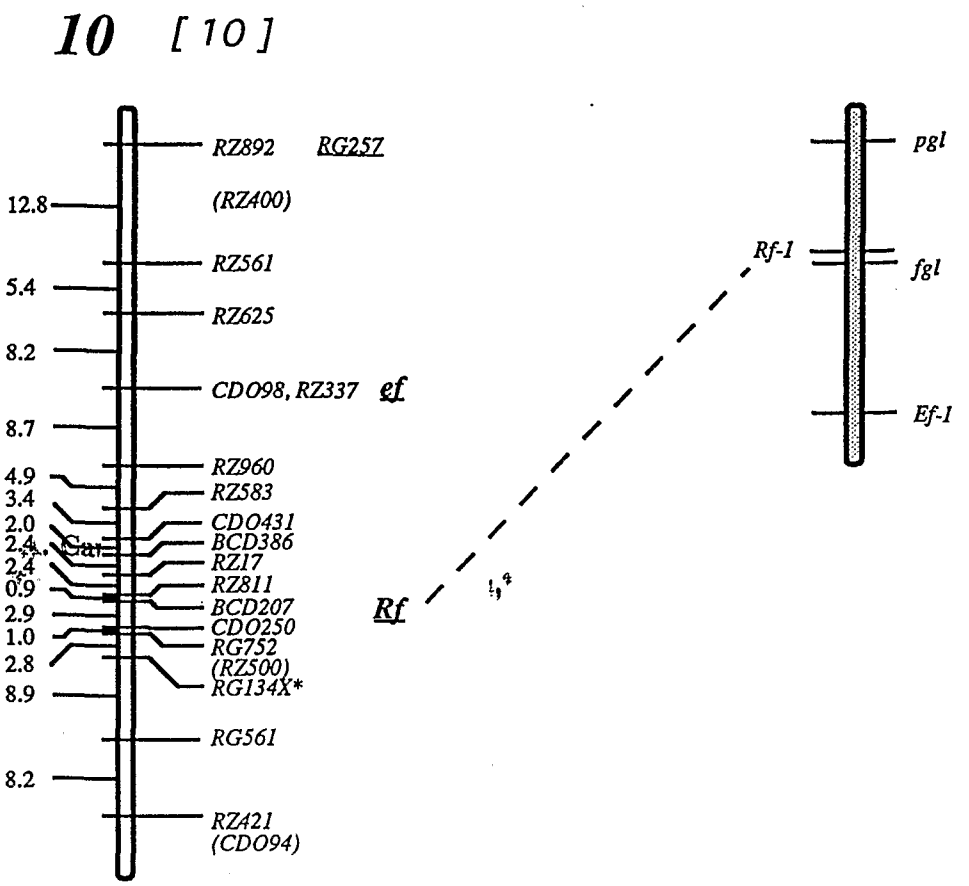


FIGURE 1.—Continued

encountered for markers mapping to nine of the chromosomes (Figure 1). Almost half (46/94) of the skewed markers mapped to chromosome *3*. All of the skewed loci on chromosomes *4* and *5*, and one marker each on chromosomes *2* and *11* (*RZ742C* and *RG98*), deviated in favor of the *O. longistaminata* allele (28/94), while markers on all the other chromosomes deviated in favor of the *O. sativa* allele (66/94). The mean frequency of the *O. longistaminata* alleles in the backcross population, over all the marker loci, was 49%. This statistic was in accordance with observations of allele frequencies at isozyme loci in similar backcross populations (CAUSSE and GHESQUIÈRE 1991, 1992). Distortions are common in interspecific and intersubspecific populations and their origin has been discussed by several authors (IWATA *et al.* 1964; TANKSLEY 1983; ZAMIR and TADMOR 1986; SATO *et al.*, 1990; LIN *et al.* 1992; LIN and IKEHASHI 1993). Because the ability to measure recombination is limited to alleles coming from only one parent in a backcross population, these populations are less sensitive to skewing than are F_2 populations. The frequency of markers detecting skewed segregation was similar in both the interspecific population and the previously mapped *indica* × *japonica* population (MCCOUCH *et al.* 1988).

It is interesting to note that markers in the same region of chromosome *3* were reported as skewed by 1). The segregation distortion in the population studied here can be used to identify the map position of the genetic factor(s) responsible for this phenomenon (Figure 3). NAKAGAHRA (1972) has shown that this region of the genome is involved in the sterility mechanisms which isolate the *indica* and *japonica* subspecies of *O. sativa*. The sterility is due to gametophytic selection, systematically favoring *indica* alleles. Whether an identical mechanism is involved in regulating fertility in this interspecific cross is not clear at this time. The mechanism may be similar, though *O. longistaminata* alleles tended to be favored over *indica* alleles in the population studied here. On the other hand, the sterility may be due to a different mechanism which is controlled by one or several genes residing in the same chromosomal region. The *O. longistaminata* × *O. sativa* F_1 hybrid showed very low male fertility; less than 20% of the pollen grains were stainable by ALEXANDER (1969) solution (GHESQUIÈRE 1988). Though backcross seeds could be obtained, the success rate of the back crosses was incomplete, and specific selection could have taken place at this step. In addition, during germination or later development, some plants were weak or developed poorly and were discarded from the mapping population. The reproductive barrier which isolates *O. longistaminata* from the other *Oryza* species represents a possible cause of deviation in the allele frequencies. It

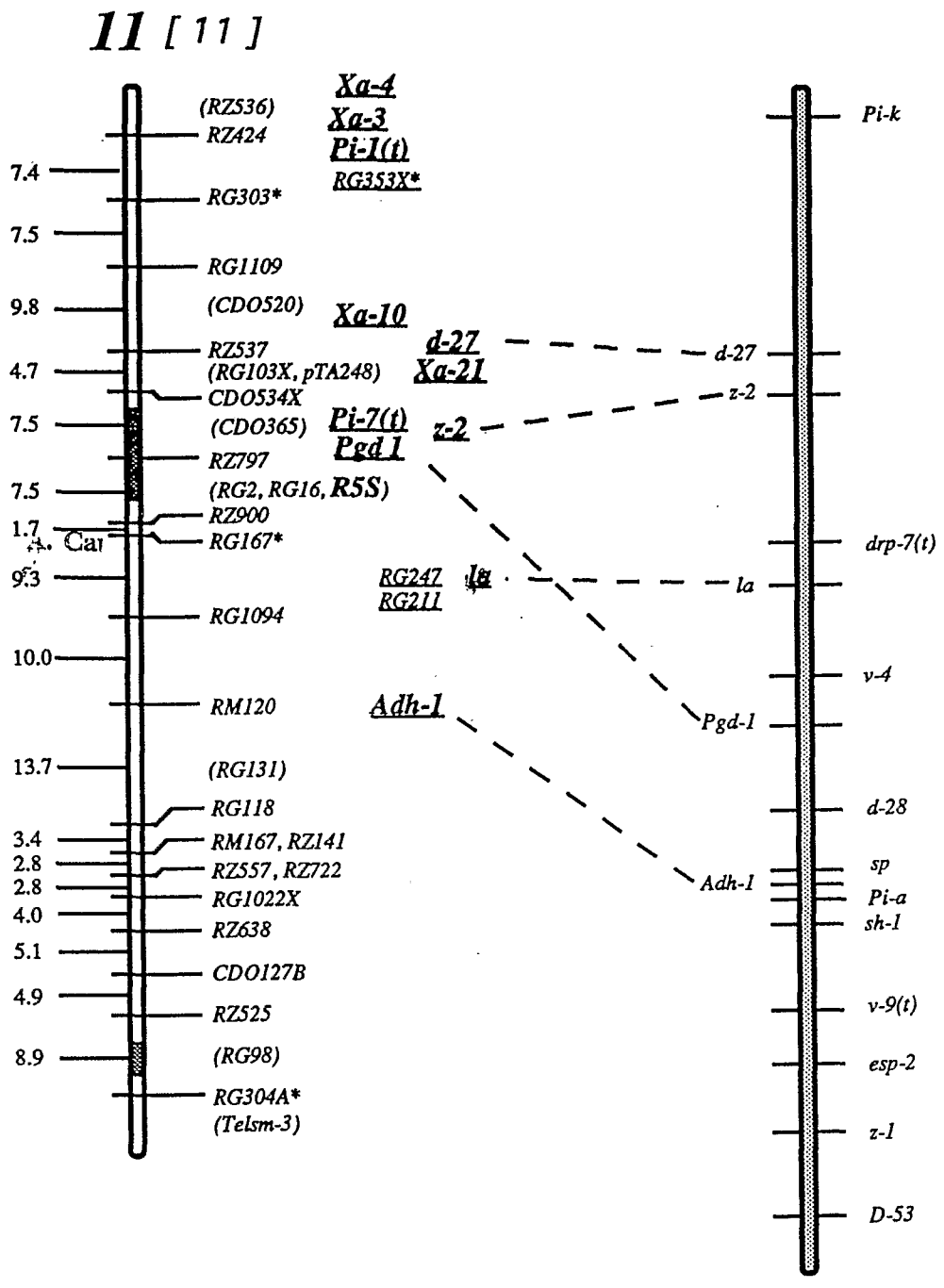


FIGURE 1.—Continued

rility mechanism (outcrossing barrier) is operating in *indica* × *japonica* and in interspecific crosses. PATERSON *et al.* (1990) proposed an approach based on substitution mapping to localize small chromosomal fragments involved in segregation distortions. Studies with subsequent backcross generations from this interspecific cross could be performed to fine map the region associated with the deviations observed here.

DISCUSSION

The use of cDNA libraries to efficiently construct molecular maps provides a basis for comparative mapping in distantly related species, such as rice, oat, barley, wheat, maize, and sugarcane. Maps based on common sets of probes constitute a basis for the comparison of genome organization and evolutionary change, as was first demonstrated with *Solanaceae* species (BONIERBALE *et al.* 1988; TANKSLEY *et al.* 1992b), and later in sorghum and maize (HULBERT *et al.* 1990; WHITKUS *et al.* 1992) and wheat, barley, and rye (DEVOS *et al.* 1992, 1993). While genomic clones work well when the species being compared are very closely related, as with tomato and potato or sorghum and maize, genomic sequences are often not well enough conserved to allow comparative mapping across more distantly related genomes. This was demonstrated in the case of rice genomic *Pst*I

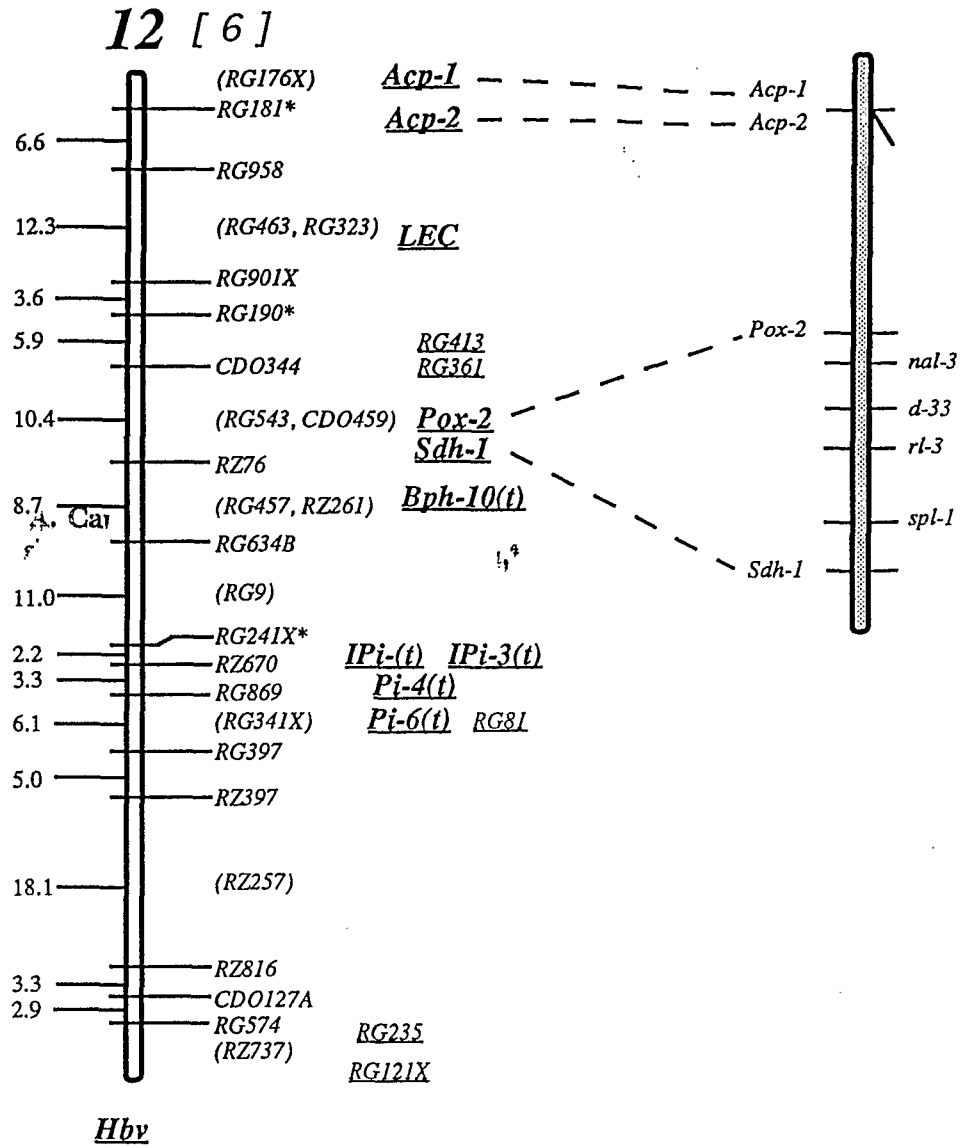


FIGURE 1.—Continued

probes, which frequently gave poor signal on oat and barley. On the other hand, cDNA sequences are sufficiently conserved to cross hybridize with a wide range of species and allowed comparative maps to be developed between rice, wheat, maize and other cereals (AHN and TANKSLEY 1993; AHN *et al.* 1994; KURATA *et al.* 1994; VAN DEYNZE *et al.* 1994). In some instances, cDNAs have been demonstrated to hybridize clearly across much greater evolutionary distance, as is the case with *ATPase* which was cloned out of tobacco (N. CHUA, Rockefeller University, personal communication), or the *Pto* gene which was cloned out of tomato, and gives a clear hybridization signal on a wide range of plants, including rice (MARTIN *et al.* 1993).

The interspecific population has been found to be much more efficient than most intraspecific crosses; on average, one restriction enzyme detected 54% polymorphism, while a single enzyme detected only 34% and 14% in the two intra species crosses examined in this study. The mapping population can be easily multiplied through shoot cuttings, maintained over years, and can be distributed to researchers who would like to map cloned genes. The resolution of our map is limited by the size of the population, but higher resolution mapping in specific regions of interest can be readily accomplished by selecting clones which map to the target region and analyzing them on larger populations, or by pooling individuals for targeted addition of markers (GIOVANNONI *et al.* 1991; MICHELMORE *et al.* 1991).

One of the main uses of the RFLP map of rice is to locate markers linked to genes of interest, both single gene and quantitatively inherited characters. Over 80 genes of agronomic importance have been located on the map (Table 1) and are identified in bold in Figure 1. The SL population has also been used to locate genes related to specific traits of *O. longistaminata*, such as growth habit (T. FULTON, unpublished data) and bacterial blight resistance. This species (though a different accession) was

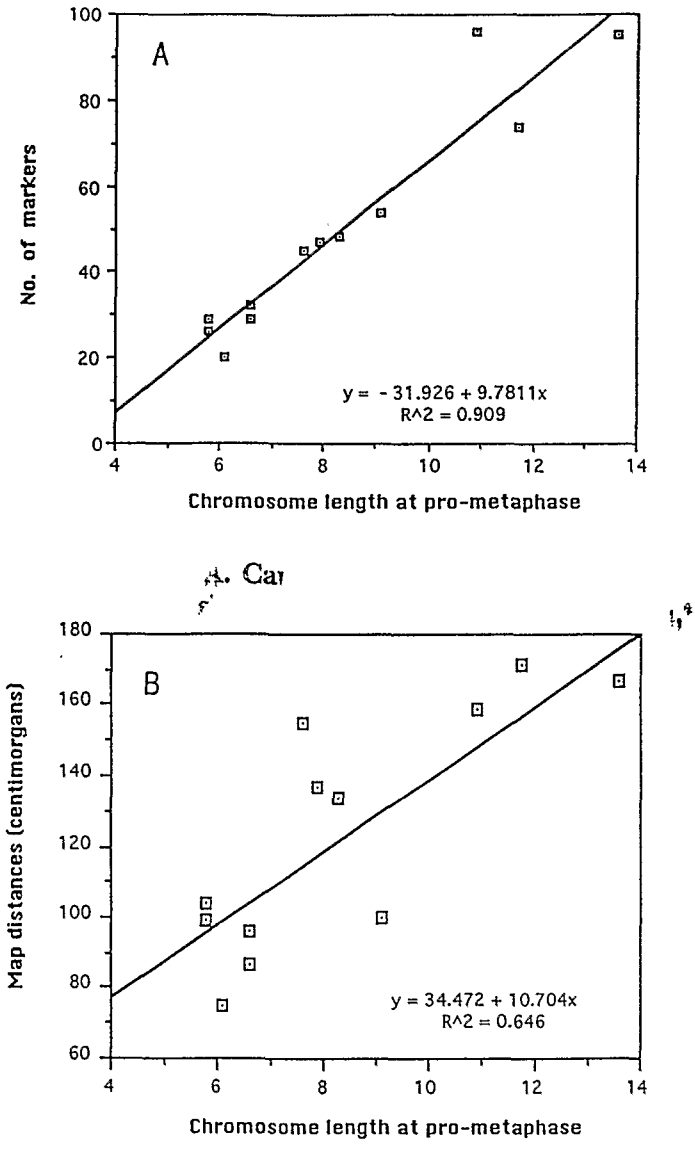


FIGURE 2.—Relation between the relative length (RL) of chromosomes measured at pro-metaphase (FUKUI and IIJIMA 1991) and (A) the number of markers per chromosome; (B) the chromosome length in centimorgans on the RFLP map. The chromosome numbering follows the system established by the Rice Genetics Cooperative Committee, in IRRI, May 1990.

the source of the *Xa-21* gene for broad spectrum resistance to bacterial leaf blight (IKEDA *et al.* 1990; KHUSH *et al.* 1991). VALES (1985) showed that this species also has genes conferring specific resistance to blast. *O. longistaminata* has also interested rice breeders for its allogamy traits: it has the longest anthers and stigmas among the *Oryza* species, and these traits have been found to be correlated with the outcrossing behavior of plants (CAUSSE and GHESQUIÈRE 1991). Production of varieties with long, well exerted stigmas would be valuable in the production of hybrid seeds at a lower cost (VIRMANI *et al.* 1982).

It is generally argued that reduced recombination and skewed segregation impose limits to the ability to introgress traits from wild species into cultivated forms of rice. We have shown that in spite of an overall reduction of recombination, the actual frequency of recombination in this interspecific cross remained high. Though a strong reproductive barrier isolates the wild species from the cultivars, segregation deviation did not constitute a more important problem than within intersubspecific *O. sativa* crosses. Unfavorable genes are numerous in wild species and molecular marker-assisted studies of the inheritance of these traits may help to get rid of such deleterious effects. In addition to *O. longistaminata*, species which are closer to *O. sativa*, such as *O. rufipogon*, represent sources of interesting genes for rice improvement. RFLP maps constitute a powerful tool for following introgressions (YOUNG *et al.* 1988) and separating desirable from unfavorable traits (DE VICENTE and TANKSLEY 1993; PATERSON *et al.* 1990). PANAUD (1992) and P. REIMERS and R. NELSON (IRRI, personal communication) demonstrated the use of RFLP markers to identify which chromosome had been inherited from a wild species in alien addition lines derived from remote crosses.

Finally, our results offer a foundation for the use of rice as a genetic model among grass species. Recent estimations based on flow cytometry suggest that the amount of DNA in a haploid nucleus (C-value) is about 0.45 pg (ARUMUGANATHAN and EARLE 1991). In addition, it has a large proportion of single copy DNA (approximately 85% at high stringency) (MCCOUCH *et al.* 1988). With a map of 1491 cM, 1 cM corresponds to an average of approximately 300 kb. Physical mapping experiments have demonstrated that this estimate is reasonably accurate (RONALD *et al.* 1992) and that in telomeric regions, the recombination frequency may be as high as 1 cM:30–50 kb (WU and TANKSLEY 1993b). The relationship between physical and genetic distance estimated from the interspecific map represents a conservative estimate, as the total number of cM in the rice genome is greater when mapping is done on an intraspecific cross, making the estimated kb:cm ratio nearer 1 cM:250 kb. In either case, the ratio of genetic to physical distance in rice is the smallest for any monocot known, and is only slightly higher than that for Arabidopsis (150–200 kb per cM). This estimate can be compared to tomato, which has an average distance of 700 kb per cM, or to other monocots such as maize, with an average of 2000 kb per cM. The first successful map-based cloning gene experiment in any crop plant was recently reported for the *Pto* gene in tomato (MARTIN *et al.* 1993), providing evidence that saturated molecular maps can be productively employed in the isolation of genes whose protein product is unknown. Because of its small genome, high proportion of single copy DNA, high recombination frequency, densely populated genetic map, and relative ease of transformation, rice represents an ideal candidate as the basis for comparative mapping studies among monocot species, for

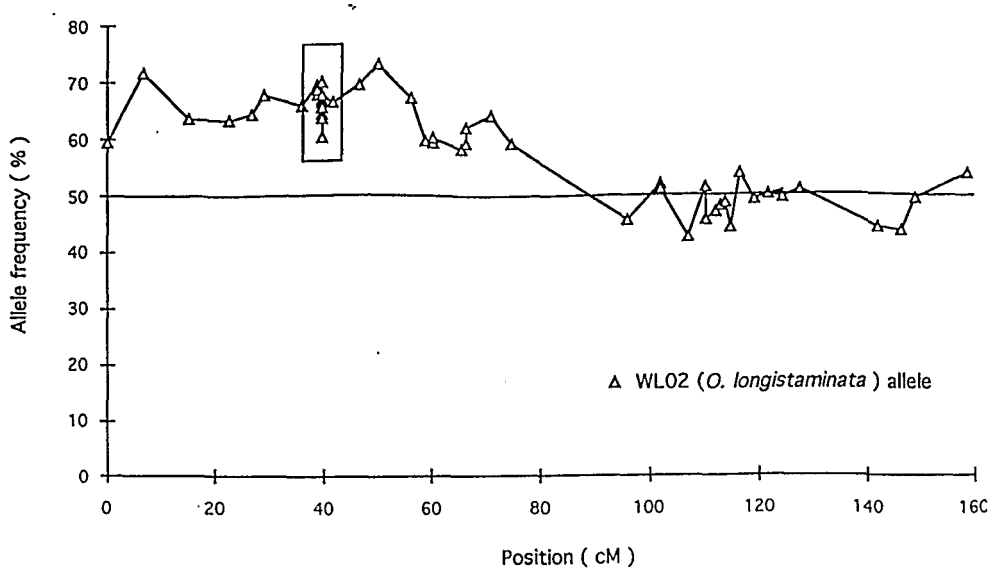


FIGURE 3.—Scatter plot of allele frequencies of markers in highly skewed region of chromosome *3*. Horizontal line represents expected 1:1 ratio in a backcross population. Rectangle at 40 cM region represents cluster of 10 markers showing no recombination in center of highly skewed region where a gene or cluster of genes associated with sterility are putatively located.

map-based gene isolation, and for transformation in crop improvement.

This research was supported by a grant from the Rockefeller Foundation. M.A.C. was sponsored by a post doctoral Lavoisier grant provided by the Foreign Affair Ministry of France. G.S. was supported by ORSTOM for a sabbatical leave to Cornell University. The seeds for the mapping population were kindly provided by A. GHESQUIÈRE, and the plants have been maintained and vegetatively propagated with great care by N. VAN ECK since 1990. Thanks to Z. LI and B. SONTHAYANON for contributions to the mapping effort, and to C. MOREHOUSE for help in preparing the manuscript.

LITERATURE CITED

ABENES, M. L. P., R. E. TABIEN, S. R. MCCOUCH, R. IKEDA, G. S. KHUSH *et al.*, 1994 Orientation of the classical and molecular genetic maps of chromosome 11 in rice. Euphytica (in press).

AHN, S., and S. D. TANKSLEY, 1993 Comparative linkage maps of the rice and maize genomes. Proc. Natl. Acad. Sci. USA 90: 7980–7984.

AHN, S., C. N. BOLLICH and S. D. TANKSLEY, 1992 RFLP tagging of a gene for aroma in rice. Theor. Appl. Genet. 84: 825–828.

AHN, S., J. A. ANDERSON, M. E. SORRELLS and S. D. TANKSLEY, 1994 Homeologous relationships of rice, wheat, and maize chromosomes. Mol. Gen. Genet. 241: 483–490.

ALEXANDER, M. P., 1969 Differential staining of aborted and non aborted pollen. Stain Technol. 44: 117–122.

ANDERSON, J. A., Y. OGIHARA, M. E. SORRELLS and S. D. TANKSLEY, 1992 Development of a chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet. 83: 1035–1043.

ANURATHA, C. S., J. HUANG, A. PINGALI and S. MUTHUKRISHMAN, 1992 Isolation and characterization of a chitimase and its cDNA clone from rice. J. Plant Biochem. Biotechnol. 1: 5–10.

ARUMUGANATHAN, K., and E. D. EARLE, 1991 Nuclear DNA content of some important plant species. Plant Mol. Biol. Reporter 9: 208–218.

BERNATZKY, R., and S. D. TANKSLEY, 1986 Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112: 887–898.

BONIERBALE, M., R. PLAISTED and S. D. TANKSLEY, 1988 RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120: 1095–1103.

BORTS, R. H., and J. E. HABER, 1987 Meiotic recombination in yeast: alteration by multiple heterozygosities. Science 237: 1459–1465.

BURR, B., F. A. BURR, K. H. THOMPSON, M. C. ALBERTSEN and C. W. STUBER, 1988 Gene mapping with recombinant inbreds in maize. Genetics 118: 519–526.

CAUSSE, M., and A. GHESQUIÈRE, 1991 Prospective use of *Oryza longistaminata* for rice breeding, pp. 81–89 in Proceedings of the 2nd International Rice Genetics Symposium. IRRI, Los Baños.

CAUSSE, M., and A. GHESQUIÈRE, 1992 Linkage study between molecular markers and genes controlling the reproductive barrier in an interspecific backcross between *O. sativa* and *O. longistaminata*. Rice Genet. Newsl. 9: 28–31.

CHANDRARATNA, M. F., 1964 Sterility, pp. 97–114 in *Genetics and Breeding of Rice*. Longman, London.

CHAO, F., 1927 Linkage studies in rice. Genetics 13: 133–169.

CHITTENDEN, L. M., K. F. SCHERTZ, Y. R. LIN, R. A. WING and A. H. PATERSON, 1994 A detailed RFLP map of *Sorghum bicolor* × *S. propinquum* suitable for high-density mapping suggests ancestral duplication of Sorghum chromosomes or chromosomal segments. Theor. Appl. Genet. 87: 925–933.

CHO, Y. G., M. Y. EUN, S. R. MCCOUCH and Y. A. CHAE, 1994 Molecular mapping and genotypic selection for the semi-dwarf gene, *sd-1:* in rice (*Oryza sativa* L.). Theor. Appl. Genet. (in press).

CHU, Y. E., and H. I. OKA, 1970a The genetic basis of crossing barriers between *Oryza perennis* subsp. *barthii* and its related taxa. Evolution 24: 135–144.

CHU, Y. E., and H. I. OKA, 1970b Introgression across isolating barriers in wild and cultivated *Oryza* species. Evolution 24: 344–355.

CHUNG, M. C., and H. K. WU, 1990 Scrutiny of trisomics from an *indica* rice, *Oryza sativa*. Proc. Natl. Science Council, Part B (Taiwan) 14: 166–174.

CLAES, B., R. DEKEYSER, R. VILLARROEL, M. VAN DEN BULCKE, G. BAUW *et al.*, 1990 Characterization of a rice gene showing organ specific expression in response to salt stress and drought. Plant Cell 2: 19–27.

DELLAPORTA, S. L., J. WOOD and J. B. HICK, 1983 A plant DNA minipreparation: version II. Plant Mol. Biol. Reporter 1: 19–21.

DE VICENTE, M. L., and S. D. TANKSLEY, 1993 QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585–596.

DEVOS, K. M., M. D. ATKINSON, C. N. CHINOY, C. J. LIU and M. D. GALE, 1992 RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor. Appl. Genet. 83: 931–939.

DEVOS, K. M., T. MILLAN and M. D. GALE, 1993 Comparative RFLP maps of homeologous group 2 chromosomes of wheat, rye and barley. Theor. Appl. Genet. 85: 784–792.

ENDO, T., and H. MORISHMA, 1983 Rice, pp. 129–146 in *Isozymes in Plant Genetics and Breeding*, Part B, edited by S. D. TANKSLEY and T. J. ORTON. Elsevier Science Publishers B.V., Amsterdam.

FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132: 6–13.

FUKUI, K., and K. IIJIMA, 1991 Somatic chromosome map of rice by imaging methods. Theor. Appl. Genet. 81: 589–596.

FULTON, T. M., and S. D. TANKSLEY, 1990 Rice RFLP framework map. Rice Biotechnol. Q. 1: 20–21.

GHESQUIÈRE, A., 1985 EVOLUTION OF *Oryza longistaminata*, pp. 15–25 in *Rice Genetics* (Proceedings of the International Rice Genetics Symposium). IRRI, Los Baños.

GHESQUIÈRE, A., 1988 DIVERSITÉ GÉNÉTIQUE DE L'ESPÈCE SAUVAGE DE RIZ, *Oryza longistaminata* A. Chev. et Roehr et dynamique des flux géniques au sein du groupe *Sativa* en Afrique. Thèse d'Etat Université Paris XI, Orsay.

GILL, K. S., E. L. LUBBERS, B. S. GILL, W. J. RAUPP and T. S. COX, 1991 A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome **34:** 362–374.

GIOVANNONI, J. J., R. A. WING, M. W. GANAL and S. D. TANKSLEY, 1991 Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res. **19:** 6553–6558.

GLASZMANN, J. C., 1987 Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. **74:** 21–30.

GRANT, V., 1958 The regulation of recombination in plants. Cold Spring Harbor Symp. Quant. Biol. **23:** 395–400.

GUIDERDONI, E. J., J. C. GLASZMANN and B. COURTOIS, 1988 Segregation of 12 isozyme genes among doubled haploid lines derived from a *japonica* × *indica* cross of rice (*Oryza sativa* L.). Euphytica **42:** 45–53.

GUIDERDONI, E., B. COURTOIS and J. C. GLASZMANN, 1990 Use of isozyme markers to monitor recombination and assess gametic selection among anther culture derivatives of remote crosses of rice (*Oryza sativa* L.), pp. 43–55 in *Review of Advances in Plant Biotechnology 1985–1988* edited by A. MUZEEB-KAZI and L. A. SITCH. (Proceedings of the 2nd International Symposium on Genetic Manipulation in Crops. Mexico D.F., Mexico and Manila, Philippines, August 29–31, 1988, Joint Publ. CIMMYT-IRRI.

HELENTJARIS, T., D. WEBBER and S. WRIGHT, 1988 Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics **118:** 353–363.

HEUN, M., A. E. KENNEDY, J. A. ANDERSON, N. L. V. LAPITAN, M. E. SORRELLS *et al.*, 1991 Construction of restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome **34:** 437–447.

HODGES, T. K., J. PENG, L. A. LYZNIK and D. S. KOETJE, 1991 Transformation and regeneration of rice protoplasts, pp. 157–174 in *Rice Biotechnology*, edited by G. TOENNIESSEN and G. KHUSH. CAB International, Tucson, Ariz.

HUANG, N., T. D. SUTLIFF, J. C. LITTS and R. L. RODRIGUEZ, 1990 Classification and characterization of the rice a-amylase family. Plant Mol. Biol. **14:** 655–668.

HULBERT, S. H., T. E. RICHTER, J. D. AXTELL and J. L. BENNETZEN, 1990 Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc. Natl. Acad. Sci. USA **87:** 4251–4255.

IKEDA, R., G. S. KHUSH and R. E. TABIEN, 1990 A new resistance gene to bacterial blight derived from *O. longistaminata*. Jpn. J. Breed. **40** (Suppl. 1): 280–281.

ISHIKAWA, R., H. MORISHIMA, T. KINOSHITA, T. HARADA, M. NUZEKI *et al.*, 1991 Linkage analysis of nine isozyme genes on the conventional linkage map in rice. Jpn. J. Breed. **41:** 265–272.

IWATA, N., and T. OMURA, 1971a Linkage analysis by reciprocal translocation method in rice plants (*Oryza sativa* L.). I. Linkage groups corresponding to the chromosome 1, 2, 3, and 4 (Japanese/English). Jpn. J. Breed. **21:** 19–28.

IWATA, N., and T. OMURA, 1971b Linkage analysis by reciprocal translocation method in rice plants (*Oryza sativa* L.). II. Linkage groups corresponding to the chromosomes 5, 6, 8, 9, 10, and 11 (Japanese/English). Sci. Bull. Fac. Agr. Kyushu Univ. **25:** 137–153.

IWATA, N., and T. OMURA, 1984 Studies on the trisomics in rice plants, VI. An accomplishment of a trisomic series in *Japonica* rice plants. Jpn. J. Genet. **59:** 199–204.

IWATA, N., T. NAGAMATSU and T. OMURA, 1964 Abnormal segregation of waxy and apiculus coloration by a gametophyte gene belonging to the first linkage group in rice (in Japanese with English summary). Jpn. J. Breed. **14:** 33–39.

JENA, K. K., G. S. KHUSH and G. KOCHERT, 1992 RFLP analysis of rice (*Oryza sativa* L.) introgression lines. Theor. Appl. Genet. **84:** 608–616.

JODON, N. E., 1948 Summary of rice linkage data. Bur. Plant Ind. Soils and Agr. Eng. Agr. Res. Adm. USDA: **34.**

KAY, S. A., B. KEITH, K. SHINOZAKI, M. L. CHYE and N. H. CHUA, 1989 The rice phytochrome gene: Structure, autoregulated expression, and binding of GT-1 to a conserved site in the 5′ upstream region. Plant Cell **1:** 351–360.

KHUSH, G. S., 1990 Report of meeting to discuss chromosome numbering system in rice. Rice Genet. Newsl. **7:** 12–15.

KHUSH, G. S., R. J. SINGH, S. C. SUR and A. L. LIBROJO, 1984 Primary trisomic of rice: origin, morphology cytology and use in linkage mapping. Genetics **107:** 141–163.

KHUSH, G. S., E. BACALANGCO and T. OGAWA, 1991 A new gene for resistance to bacterial blight from *O. longistaminata*. Rice Genet. Newsl. **7:** 121–122.

KINOSHITA, T., 1984–1994 Report of the committee on gene symbolization and linkage map. Rice Genet. Newsl. **1–11.**

KOSAMBI, D. D., 1944 The estimation of map distances from recombination values. Ann. Eugenet. **12:** 172–175.

KOTHARI, S. L., M. R. DAVEY, P. T. LYNCH, R. P. FINCH, and E. C. COCKING, 1993 Transgenic Rice, pp. 3–20 in *Transgenic Plants*, Vol. 2, edited by S. D. KUNG and R. WU. Academic Press, New York.

KURATA, N., N. IWATA and T. OMURA, 1981 Karyotype analysis in rice. II. Identification of extra chromosomes in trisomic plants and banding structure on some chromosomes. Jpn. J. Genet. **56:** 41–50.

KURATA, N., G. MOORE, Y. NAGUMARA, T. FOOTE, M. YANO *et al.*, 1994 Conservation of genome structure between rice and wheat. Bio/Technology **12:** 276–278.

LANDER, E. S., P. GREEN, J. ABRAHAMSON, A. BARLOW, M. J. DALY *et al.*, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

LIN, S. Y., and H. IKEHASHI, 1993 A gamete abortion locus detected by segregation distortion of isozyme locus *Est-9* in wide crosses of rice (*Oryza sativa* L.). Euphytica **67:** 35–40.

LIN, S. Y., H. IKEHASHI, S. YANAGIHARA and K. KAWASHIMA, 1992 Segregation distortion via male gametes in hybrids between *Indica* and *Japonica* or wide-compatibility varieties of rice (*Oryza sativa* L.). Theor. Appl. Genet. **84:** 812–818.

LIU, A., H. LI, Q. ZHANG, X. JIANG, S. SHI *et al.*, 1992 Mapping a wide compatibility gene of rice in relation to RFLP markers. J. Huazhong Agric. Univ. **11:** 213–219.

LYNCH, P. T., R. P. FINCH, M. R. DAVEY and E. C. COCKING, 1991 Rice tissue culture and its application, pp. 135–155 in *Rice Biotechnology*, edited by G. TOENNIESSEN and G. KHUSH. CAB International, Tucson, Ariz.

MACKILL, D. J., M. A. SALAM, Z. Y. WANG and S. D. TANKSLEY, 1993 A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice. Theor. Appl. Genet. **85:** 536–540.

MANLY, K. F., 1993 A Macintosh program for storage and analysis of experimental genetic mapping data. Mamm. Genome **4:** 303–313.

MARTIN, G. B., S. H. BROMMONSCHENKEL, J. CHUNWONGSE, A. FRARY, M. W. GANAL *et al.*, 1993 Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science **262:** 1345–1484.

MCCOUCH, S. R., 1990 Construction and applications of a molecular linkage map of rice based on restriction fragment length polymorphism (RFLP). Ph.D. Thesis, Cornell University, Ithaca, N.Y.

MCCOUCH, S. R., and E. PAUL, 1993 RiceGenes, an international genome database and bulletin board for rice. DNA Link **3:** 40–41.

MCCOUCH, S. R., and S. D. TANKSLEY, 1991 Development and use of restriction fragment length polymorphism in rice breeding and genetics, pp. 109–133 in *Rice Biotechnology*, edited by G. TOENNIESSEN, and G. KHUSH. CAB International, Tucson, Ariz.

MCCOUCH, S. R., G. KOCHERT, Z. H. YU, Z. Y. WANG, G. S. KHUSH *et al.*, 1988 Molecular mapping of rice chromosomes. Theor. Appl. Genet. **76:** 815–829.

MCCOUCH, S. R., M. CAUSSE, T. M. FULTON and S. D. TANKSLEY, 1990 Recent advances in molecular mapping of the rice genome. *Proceedings of the International Symposium on Rice Research: New Frontiers*, Nov. 15–18, Hyderabad, India.

MCCOUCH, S. R., M. L. ABENES, R. ANGELES, G. S. KHUSH and S. D. TANKSLEY, 1991 Molecular tagging of a recessive gene, *xa-5*: for resistance to bacterial blight of rice. Rice Genet. Newsl. **8:** 143–145.

MICHELMORE, R. W., I. PARAN and R. V. KESSELI, 1991 Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88:** 9828–9832.

MIEZAN, K., and G. A. SECOND, 1979 Prospection des variétes traditionnelles et des espèces sauvages des riz en Tanzanie (French with English translation). Multigraph, ORSTOM, Paris.

MOHAN, M., S. NAIR, and J. BENNETT, 1993 Mapping of a rice gene for resistance to biotype-1 of gall midge (*Orseolia oryzae*) by RFLP and RAPD anlayses, pp. 13 in *Proceedings of the Sixth Annual Meeting of the International Program on Rice Biotechnology*, Feb. 1–5, 1993, Chiang Mai, Thailand.

NAGAMURA, Y., B. A. ANTONIO, A. FUKUDA, Y. HARUSHIMA, T. INOUE *et al.*, 1993 A high density STS and EST linkage map of rice. Rice Genome **2:** 3.

NAGAO, S., and T. TAKAHASHI, 1963 Trial construction of twelve linkage groups in Japanese rice. Genetical studies on rice plant, XXVII. J. Fac. Agric. Hokkaido Univ. **53:** 72–130.

NAKAGAHRA, M., 1972 Genetic mechanism of the distorted segregation of marker genes belonging to the eleventh linkage group in cultivated rice. Jpn. J. Breed. **22:** 232–238.

NAKAGAHRA, M., and K. HAYASHI, 1976 Detection of esterase isozyme loci of *Oryza sativa* L. (in Japanese). Jpn. J. Breed. **26** (Suppl. 1): 114–115.

NAYAR, N. M., 1967 Prevalence of self-incompatibility in *O. barthii* Chev.; its bearing on the evolution of rice and related taxa. Genetica **38:** 521–527.

O'BRIEN, S. J. (Editor), 1993 *Genetic Maps*, pp. 6.61–6.79. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

O'DONOUGHUE, L. S., Z. WANG, M. RODER, B. KNEEN, M. LEGGETT *et al.*, 1992 An RFLP-based map of oats on a cross between two diploid taxa (*Avena atlantica* × *A. hirtula*). Genome **35:** 765–771.

OKA, H. I., 1988 *Origin of Cultivated Rice.* Elsevier, Amsterdam.

OKA, H. I., and H. K. WU, 1988 Comparison of data on rice chromosomes presented by different authors. Rice Genet. Newsl. **5:** 34–41.

PANAUD, O., 1992 Mise en oeure d'une methode de marguage non-radioactif de l'ADN pour l'etude des RFLP chez le Riz: cartographie du genome et suivi des introgressions entre *Oryza sativa* et *O. branchyantha.* Ph.D. Thesis, Université de Paris, SUD. Centre, D'Orsay.

PATERSON, A. H., E. S. LANDER, J. D. HEWITT, S. PETERSON, S. E. LINCOLN *et al.*, 1988 Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. Nature **335:** 721–726.

PATERSON, A. H., J. W. DEVERNA, B. LANINI and S. D. TANKSLEY, 1990 Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics **124:** 735–742.

PATERSON, A. H., S. D. TANKSLEY and M. E. SORRELLS, 1991 DNA markers in plant improvement. Advances Agron. **46:** 39–90.

PHAM, J. L., J. C. GLASZMANN, R. SANO, P. BARBIER, A. GHESQUIÈRE *et al.*, 1990 Isozyme markers in rice: genetic analysis and linkage relationships. Genome **33:** 348–359.

RANJHAN, S., J. C. LITTS, M. R. FOOLAD and R. L. RODRIGUEZ, 1991 Chromosomal localization and genomic organization of α-amylase genes in rice (*Oryza sativa* L.). Theor. Appl. Genet. **82:** 481–488.

RICK, C. M., 1969 Controlled introgression of chromosomes of *Solanum penellii* into *Lycopersicon esculentum*: segregation and recombination. Genetics **62:** 753–768.

RONALD, P. C., B. ALBANO, R. TABIEN, L. ABENES, K. WU *et al.*, 1992 Genetic and physical analysis of the rice bacterial blight resistance locus, *Xa-21.* Mol. Gen. Genet. **236:** 113–120.

SAITO, A., M. YANO, N. KISHIMOTO, M. NAKAGAHRA, A. YOSHIMURA *et al.*, 1991 Linkage map of restriction fragment length polymorphism loci in rice. Jpn. J. Breed. **41:** 665–670.

SATO Y. I., R. ISHIKAWA and H. MORISHIMA, 1990 Nonrandom association of genes and characters found in *indica* × *japonica* hybrids of rice. Heredity **65:** 75–79.

SECOND, G., 1982 Origin of the genetic diversity of cultivated rice (*Oryza* spp.), study of the polymorphism scored at 40 isozyme loci. Jpn. J. Genet. **57:** 25–5.

SECOND, G., 1985 Evolutionnary relationships in the *Sativa* group of *Oryza* based on isozyme data. Genet. Sel Evol. **17:** 89–114.

SHURE, M., S. WESSLER and N. FEDEROFF, 1983 Molecular identification of the *waxy* locus in maize. Cell **35:** 225–233.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–505.

TAI, H. T., and S. D. TANKSLEY, 1990 A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol. Biol. Report. **8:** 297–303.

TAILLEBOIS, J., and E. GUIMARAES, 1987 Obtention chez le riz de lignées femelles permettant une production économique de semences hybrides. Agron. Trop. **42:** 121–125.

TAKAHASHI, M., and T. KINOSHITA, 1968 Present status of rice linkage map. Genetical studies on rice plant. XXXI (in Japanese with English summary). Res. Bull. Univ. Farm Hokkaido Univ. **16:** 33–41.

TAKAHASHI, M., and T. KINOSHITA, 1977 List of genes and chromosome map of rice, pp. 416–441 in *Plant Genetics IV. Morphogenesis and Mutation* (in Japanese). Syokabo, Tokyo.

TANKSLEY, S. D., 1983 Introgression of genes from wild species. pp. 331–337 in *Isozymes in Plant Genetics and Breeding*, edited by S. D. TANKSLEY and T. J. ORTON. Elsevier, New York.

TANKSLEY, S. D., N. D. YOUNG, A. H. PATERSON and M. W. BONIERBALE, 1989 RFLP mapping in plant breeding: new tools for an old science. Biotechnology **7:** 257–264.

TANKSLEY, S., M. CAUSSE, T. FULTON, N. AHN, Z. WANG *et al.*, 1992a A high density molecular map of the rice genome. Rice Genet. Newsl. **9:** 111–115.

TANKSLEY, S. D., M. W. GANAL, J. P. PRINCE, M. C. DE VICENTE, M. W. BONIERBALE *et al.*, 1992b High density molecular linkage maps of the tomato and potato genomes. Genetics **132:** 1141–1160.

VALES, M., 1985 Etude de la résistance complète à *Pyricularia oryzae* Cav. d'hybrides *Oryza sativa* × *Oryza longistaminata* et de leurs parents *O. longistaminata.* Agron. Trop. **40:** 148–155.

VAN DEYNZE, A. E., J. DUBCOVSKY, K. S. GILL, J. C. NELSON, M. E. SORRELLS *et al.*, 1994 Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oats. Genome (in press).

VIRMANI, S. S., R. C. AQUINO and G. S. KHUSH, 1982 Heterosis breeding in rice (*Oryza sativa* L.). Theor. Appl. Genet. **63:** 373–380.

WANG, G., D. J. MACKILL, J. M. BONMAN, S. R. MCCOUCH, M. C. CHAMPOUX *et al.*, 1994 RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics **136:** 1421–1434.

WANG, Z. W., G. SECOND and S. D. TANKSLEY, 1990 Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor. Appl. Genet. **83:** 565–581.

WHITKUS, R., J. DOEBLY and M. LEE, 1992 Comparative genome mapping of sorghum and maize. Genetics **132:** 1119–1130.

WU, K. S., and S. D. TANKSLEY, 1993a Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. **241:** 225–235.

WU, K. S., and S. D. TANKSLEY, 1993b Genetic and physical mapping of telomeres and macrosatellites of rice. Plant Mol. Biol. **22:** 861–872.

XHU, Q., and C. J. LAMB, 1991 Isolation and characterization of a rice gene encoding a basic chitinase. Mol. Gen. Genet. **226:** 289–296.

XIAO, J., T. FULTON, S. MCCOUCH, S. TANKSLEY, N. KISHIMOTO *et al.*, 1992 Progress in integration of the molecular maps of rice. Rice Genet. Newsl. **9:** 124–128.

YAMAGUCHI, Y., 1927 Neuere Genetische Untersuchungen uber die Reispflanze (in German). Z. Indukt. Vererbungsl. **45:** 105–122.

YANAGIHARA, S., H. IKEHASHI, H. ARAKI and K. MARUYAMA, 1992 Difference in recombination frequency between Chronogen (*C*) and Waxy (*WX*) genes in *Japonica* and *Indica* rice (*Oryza sativa* L.) by using *Japonica* lines with a wide compatibility gene (S-5n). Jpn. J. Breed. **42:** 15–21).

YANAGIHARA, S., S. R. MCCOUCH, K. ISHIKAWA, Y. OGI, K. MARUYAMA *et al.*, 1994 Molecular analysis of the inheritance of the S-5 locus conferring wide compatibility in *Indica/Japonica* hybrids of rice (*Oryza sativa* L.). Theor. Appl. Genet. (in press).

YOSHIMURA, S., A. YOSHIMURA, A. SAITO, N. KISHIMOTO, M. KAWASE *et al.*, 1992 RFLP analysis of introgressed chromosomal segments in three near-isogenic lines of rice for bacterial blight resistance genes, *Xa-1: Xa-3,* and *Xa-4.* Jpn. J. Genet. **67:** 29–37.

YOUNG, N. D., D. LAMIR, M. W. GANAL and S. D. TANKSLEY, 1988 Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. Genetics **120:** 579–589.

YU, Z. H., 1991 Molecular mapping of rice (*Oryza sativa* L.) genes via linkage to restriction fragment length polymorphism (RFLP) markers. Ph.D. Thesis, Cornell University, Ithaca, N.Y.

YU, Z. H., D. J. MACKILL, J. M. BONMAN and S. D. TANKSLEY, 1991 Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor. Appl. Genet. **81:** 471–476.

ZAMIR, D., and Y. TADMOR, 1986 Unequal segregation of nuclear genes in plants. Bot. Gaz. **147:** 355–358.

ZHENG, K., P. SHEN, H. QUIAN and J. WANG, 1992 Tagging genes for wide compatibility in rice via linkage to RFLP markers. Chinese Rice Sci. **6:** 145–150.

Communicating editor: B. BURR

A. Car