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Analysis of the barley and rice genomes by comparative RFLP linkage mapping

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Abstract Comparative genetic mapping of rice and barley, both major crop species with extensive genetic resources, offers the possibility of uniting two well-established and characterized genetic systems. In the present study, we screened 229 molecular markers and utilized 110 polymorphic orthologous loci to construct comparative maps of the rice and barley genomes. While extensive chromosomal rearrangements, including inversions and intrachromosomal translocations, differentiate the rice and barley genomes, several syntenous chromosomes are evident. Indeed, several chromosomes and chromosome arms appear to share nearly identical gene content and gene order. Seventeen regions of conserved organization were detected, spanning 287 cM (24%) and 321 cM (31%) of the rice and barley genomes, respectively. The results also indicate that most (72%) of the single-copy sequences in barley are also single copy in rice, suggesting that the large barley genome arose by unequal crossing over and amplification of repetitive DNA sequences and not by the duplication of single-copy sequences. Combining these results with those previously reported for comparative analyses of rice and wheat identified nine putatively syntenous chromosomes among barley, wheat and rice. The high degree of gene-order conservation as detected by comparative mapping has astonishing implications for interpreting genetic information among species and for elucidating chromosome evolution and speciation.

Key words Synteny · Orthologous evolution · Genetic maps · Triticeae

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Introduction

Comparative genome analysis involves the use of a common set of molecular markers to map the genomes of different species. The usefulness of comparative mapping is evident by the extensive conservation of linkage relationships reported between tomato and potato (Bonierbale et al. 1988), sorghum and maize (Whitkus et al. 1992), pea and lentil (Weeden et al. 1992), rice and maize (Ahn and Tanksley 1993), cowpea and mung bean (Menancio-Hautea 1993), rice and wheat (Ahn et al. 1993; Kurata et al. 1994), *Arabidopsis thaliana* and *Brassica oleracea* (Kowalski et al. 1994), and most recently among the Triticeae species (Van Deynze et al. 1995).

The conservation in genome structure of related plant species may provide a basis for interpreting genetic information among species. If linkage relationships are conserved among species, genetic information and molecular markers produced in one species may be exploited by related species with less characterized genetic maps. Nowhere is this more advantageous than in the plant family Gramineae, which contains some 10 000 species including most of the world's grain crops (e.g., rice, barley, wheat, rye, and maize). Taken as a group, the family Gramineae contains all the characteristics of an ideal genetic model, including excellent cytogenetic stock collections (wheat and barley), extensive classical and molecular maps (maize, rice and barley), small genome sizes (rice and sorghum), well-characterized mutagenesis and transposon-tagging techniques (maize), and acceptable transformation procedures (rice) (Bennetzen and Freeling 1993). In such an ideal group, genetic information defined as important in one species could be studied in a related and well-characterized species, and then extrapolated to many other species within the group based upon genomic comparisons.

Rice (*Oryza sativa*) and barley (*Hordeum vulgare*) belong to different tribes of the family Gramineae and differ in basic chromosome number (2n=2x=24 and 2n=2x=14, respectively) and in genome size (C=0.45 pg and C=6.18 pg, respectively) (Arumuganathan and Earle

1991). Molecular maps for the rice (Tanksley et al. 1993; Causse et al. 1994; Kurata et al. 1994) and barley (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Kasha et al. 1995) genomes are among the best developed of the grass genetic maps and when combined contain more than 2500 markers. Although some comparative analyses of barley chromosomes with members within the tribe Triticeae are available (wheat, rye and barley: Devos et al. 1993; Van Deynze et al. 1995), global comparisons of chromosome structure and genomic organization between barley and more distantly related Gramineae species (e.g., *O. sativa*) have not been reported.

The North American Barley Genome Mapping Project (NABGMP) has constucted several molecular linkage maps of barley with the ultimate goal of isolating genes associated with disease resistance and agromonic performance. Comparative genetic mapping of barley and rice offers the possiblility of uniting the genetic resources of two well-established and characterized genetic systems. The objective of the present study was to relate the genetic maps of barley with those of rice based on a set of orthologous loci detected with a common set of genomic and cDNA clones.

Materials and methods

Rice genetic materials

The construction of the backcross mapping population utilized in this study has been previously described by Causse et al. (1994). Briefly, a set of 112 BC_1 plants was developed from an interspecific cross of *O. sativa* (BS125)//*O. longistaminata* (WL102)/*O. sativa* (BS125). Tissue samples from the *O. sativa* parent (BS125), the F_1, and each of the 112 BC_1 plants were generously provided by G. Kochert (University of Georgia).

Comparative DNA clones

Two sets of DNA clones were screened for polymorphism between the recurrent parent (BS125) and the F_1 hybrid of the rice population used in this study. The first, or "comparative mapping", set consisted mostly of single- or low-copy cDNA clones which have been previously placed on the molecular maps of barley by Graner et al. (1991), Heun et al. (1991), Kleinhofs et al. (1993) and Kasha et al. (1995). One-hundred and eighty comparative mapping clones were selected to provide complete coverage of the barley genome, including 109 barley clones (designated as BCD, ABC, ABG and MWG), 31 wheat clones (PSR, WG, pTA71 and ksu), 22 oat cDNA clones (CDO), five maize clones (UMC), and 13 clones for barley known-function genes (see Table 1).

Of the 180 comparative clones screened for polymorphism between the *O. sativa* recurrent parent (BS125) and the F_1 hybrid, 120 were polymorphic, and 93 were mapped (column 10, Table 1). Because several comparative clones detected multiple loci in rice, a total of 108 comparative loci were scored for segregation in the progeny of this cross (see Table 1). Seven of the comparative clones (BCD828, CDO105, CDO36, CDO475, CDO680, CDO99, and rDNA) have been previously mapped in the rice genome (Ahn and Tanksley 1993; Ahn et al. 1993; Tanksley et al. 1993).

Framework DNA clones

Due to the possibility that some regions of the rice genome would not be detected by comparative clones, a second set of DNA clones, consisting of 49 rice cDNA and genomic DNA clones (designated as RZ and RG), were selected from the current rice linkage map (Tanksley et al. 1993) to provide a framework over the entire length of the rice genome. Of the 49 "framework clones" screened, 46 were polymorphic, 44 were mapped, and 49 loci were detected (Column 13, Table 1). The linkage framework provided by these loci identified regions of the rice genome not detected by the barley comparative loci and allowed for the correct identification of rice linkage groups. Framework clones were kindly provided by S. D. Tanksley (Cornell University).

Thus, of the total 229 comparative and framework clones screened for polymorphism between the recurrent parent, [BS125 (*O. sativa*)] and the F_1 hybrid of this rice backcross population, 166 were polymorphic, 137 were mapped, and 157 loci were detected (column 14, Table 1).

Molecular marker assay and segregation analysis

DNA isolation and RFLP procedures were as described by Saghai Maroof et al. (1984) and Zhang et al. (1992). Briefly, 5 μg of rice DNA were digested with each of four restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII and *Dra*I) and electrophoresed on 0.8% agarose gels, followed by standard DNA transfer to nylon membranes via Southern blotting. Blots were hybridized overnight with randomly primed ^{32}P-dCTP-labelled probes. Following hybridization, blots were washed three times, twice for 5 min at room temperature with 1 × SSC and 0.1% SDS and once for 15 min at 65°C with 1 × SSC

Table 1 The total number of screened and polymorphic clones, and the number of loci detected in this rice population

Total no. of clones	Comparative mapping clones									Framework clones			
	Barley				Wheat		Oat	Other[b]	Sub total[c]	Rice		Sub total[d]	Grand total[e]
	ABC	BCD	ABG	MWG	PSR	WG[a]	CDO			RZ	RG		
Screened	54	12	32	11	3	28	22	18	**180**	17	32	**49**	**229**
Polymorphic	39	6	11	9	3	20	18	14	**120**	17	29	**46**	**166**
Mapped	33	3	9	6	2	17	16	7	**93**	17	27	**44**	**137**
Loci detected[f]	39	3	11	8	2	18	20	7	**108**	19	30	**49**	**157**

[a] Includes three KSU genomic DNA clones and one rDNA clone (pTA71)
[b] Includes five maize genomic DNA clones (UMC and NPI) and 13 known-function clones
[c] Sub-total for comparative clones
[d] Sub-total for framework clones
[e] Grand total for comparative and framework clones
[f] Total number of loci detected from mapped clones

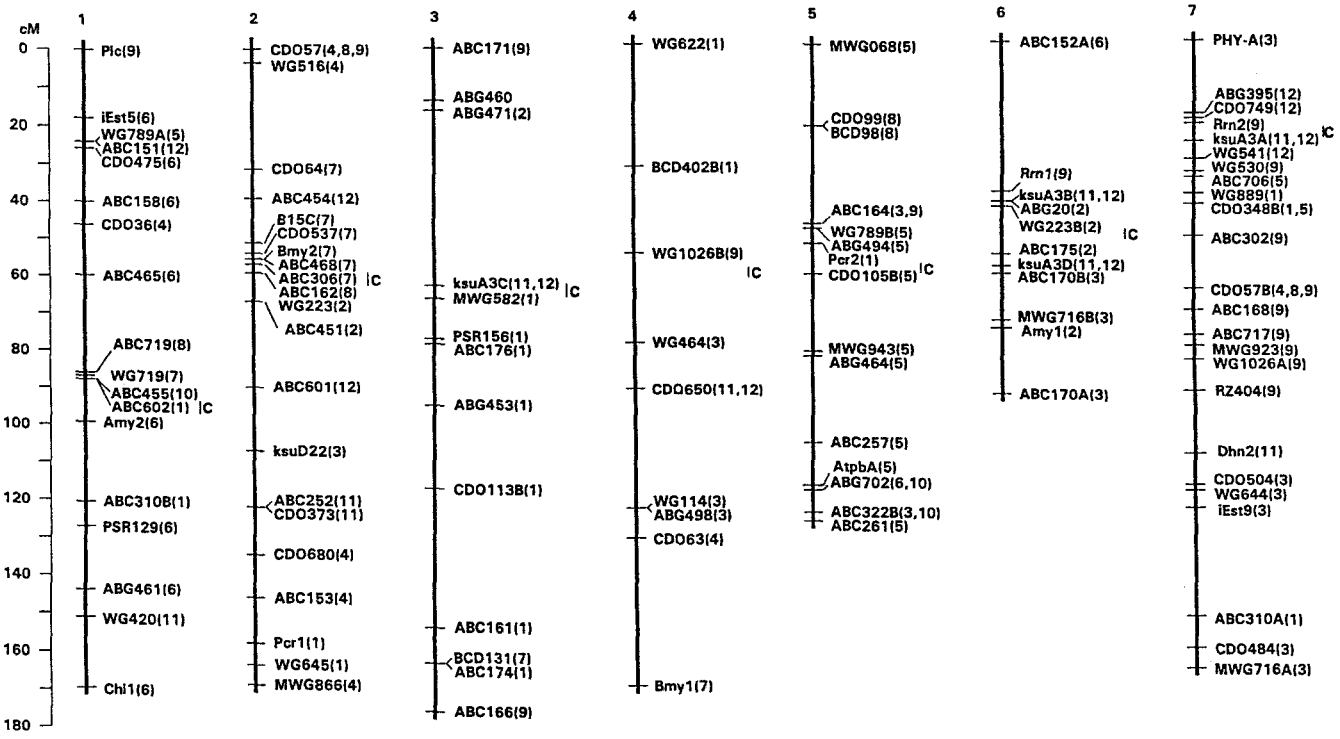


Fig. 1 A barley comparative map consisting of 110 isozyme and RFLP comparative loci spanning 1029 cM. The scale is shown at the left in centiMorgans. Clone names are listed to the right of the chromosomes. Chromosomal locations of the comparative loci in rice are indicated *in parenthesis* following the clone names. The approximate locations of centromere regions are indicated by the symbol "|C"

and 0.1% SDS. After washing, blots were covered with plastic wrap and exposed to X-ray film for 5–7 days.

Data analysis

Segregation ratios for each marker in the rice BC_1 population were tested for goodness of fit to an expected 1:1 genotypic ratio using the computer program Linkage-1 (Suiter et al. 1983). Linkage analysis was performed using the computer program MapMaker 3.0 (Lander et al. 1987). Two-point linkage analysis, using the "group" command (LOD=3.0) were used to assign markers to independent linkage groups. The most probable linkage order and map distances within linkage groups were determined by "three-point", "compare", "order" (LOD=3.0) and the "try" commands of MapMaker. The best order for each linkage group was confirmed by permutating all adjacent triplets using the "ripple" command.

Results and discussion

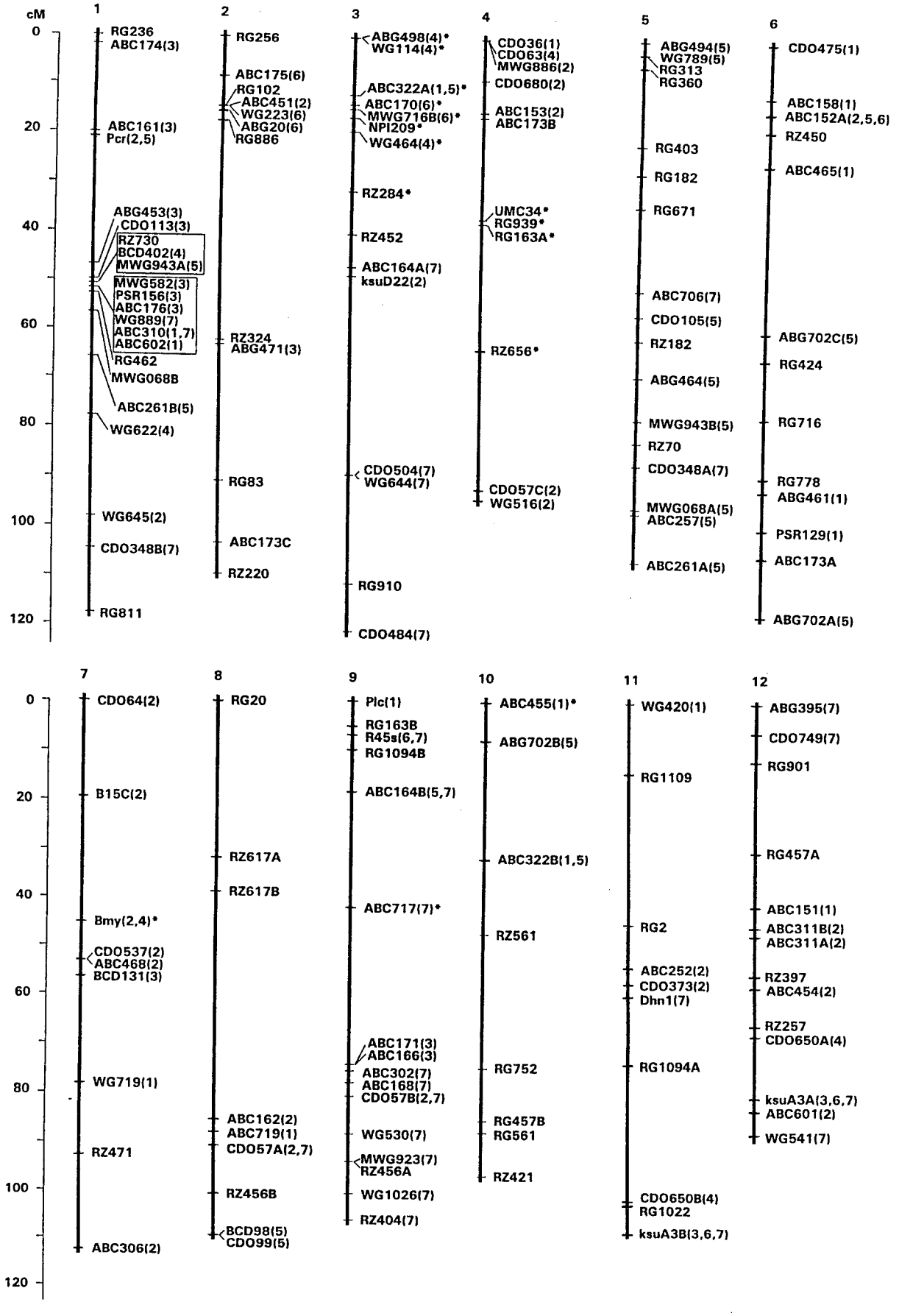
Barley comparative map

The majority (90%) of the 110 comparative loci used in this study have been previously mapped in the barley double-haploid (DH) population developed from the cross of Steptoe × Morex (S/M) (Kleinhofs et al. 1993). The remaining comparative loci were integrated into the S/M map based on the marker loci held in common among the barley mapping populations (Graner et al. 1991; Huen et al. 1991; Kasha et al. 1995). This barley comparative map consists of 104 RFLP and six isozyme loci, spanning nearly 1030 cM or 82% of the total barley genome with an average distance between loci of approximately 9 cM (Fig. 1).

Rice comparative map

The comparative rice map consisted of 108 comparative and 49 framework loci (see Materials and methods; Table 1) on 12 linkage groups, spanning 1 243 cM at an average distance between loci of 8.3 cM (Fig. 2). Only one comparative locus (ABG460) was not linked to any linkage group. The total length of our comparative rice map represents approximately 83% (1 243 cM) of the total 1 491 cM detected on the current rice linkage map (Causse et al. 1994).

Significant segregation distortion ($P<0.05$) was observed for approximately 10% (15/158) of the loci mapped in the rice backcross population. Deviations from the expected 1:1 segregation ratios were detected for loci mapping to 5 of the 12 rice chromosomes (chromosomes 3, 4, 7, 9, and 10). All of the skewed loci on rice chromosomes 3 and 7 had excess *O. sativa* (BS125) alleles, while the remaining skewed loci deviated in favor of the *O. longistaminata* (WL02) alleles. Slightly greater than half (53%) of the loci with distorted segregation ratios mapped to a single chromosomal region on rice chromosome 3. McCouch et al. (1988) and Causse et al. (1994) have previously reported segregation distortion of several loci mapping to the same genomic region of rice chromosome 3 (Fig. 2). Interestingly, Nakagahra (1972) and Causse et al. (1994) suggest that the segregation distortion observed in this region of rice chromosome 3 may be associated with the presence of a closely linked genetic factor(s) regulating fertility in


cM
1
RG236
ABC174(3)
ABC161(3)
Pcr(2,5)
ABG453(3)
CDO113(3)
RZ730
BCD402(4)
MWG943A(5)
MWG582(3)
PSR156(3)
ABC176(3)
WG889(7)
ABC310(1,7)
ABC602(1)
RG462
MWG068B
ABC261B(5)
WG622(4)
WG645(2)
CDO348B(7)
RG811
2
RG256
ABC175(6)
RG102
ABC451(2)
WG223(6)
ABG20(6)
RG886
RZ324
ABG471(3)
RG83
ABC173C
RZ220
3
ABG498(4)*
WG114(4)*
ABC322A(1,5)*
ABC170(6)*
MWG716B(6)*
NPI209*
WG464(4)*
RZ284*
RZ452
ABC164A(7)
ksuD22(2)
CDO504(7)
WG644(7)
RG910
CDO484(7)
4
CDO36(1)
CDO63(4)
MWG886(2)
CDO680(2)
ABC153(2)
ABC173B
UMC34*
RG939*
RG163A*
RZ656*
CDO57C(2)
WG516(2)
5
ABG494(5)
WG789(5)
RG313
RG360
RG403
RG182
RG671
ABC706(7)
CDO105(5)
RZ182
ABG464(5)
MWG943B(5)
RZ70
CDO348A(7)
MWG068A(5)
ABC257(5)
ABC261A(5)
6
CDO475(1)
ABC158(1)
ABC152A(2,5,6)
RZ450
ABC465(1)
ABG702C(5)
RG424
RG716
RG778
ABG461(1)
PSR129(1)
ABC173A
ABG702A(5)
7
CDO64(2)
B15C(2)
Bmy(2,4)*
CDO537(2)
ABC468(2)
BCD131(3)
WG719(1)
RZ471
ABC306(2)
8
RG20
RZ617A
RZ617B
ABC162(2)
ABC719(1)
CDO57A(2,7)
RZ456B
BCD98(5)
CDO99(5)
9
Plc(1)
RG163B
R45s(6,7)
RG1094B
ABC164B(5,7)
ABC717(7)*
ABC171(3)
ABC166(3)
ABC302(7)
ABC168(7)
CDO57B(2,7)
WG530(7)
MWG923(7)
RZ456A
WG1026(7)
RZ404(7)
10
ABC455(1)*
ABG702B(5)
ABC322B(1,5)
RZ561
RG752
RG457B
RG561
RZ421
11
WG420(1)
RG1109
RG2
ABC252(2)
CDO373(2)
Dhn1(7)
RG1094A
CDO650B(4)
RG1022
ksuA3B(3,6,7)
12
ABG395(7)
CDO749(7)
RG901
RG457A
ABC151(1)
ABC311B(2)
ABC311A(2)
RZ397
ABC454(2)
RZ257
CDO650A(4)
ksuA3A(3,6,7)
ABC601(2)
WG541(7)
0
20
40
60
80
100
120

interspecific crosses of rice. The comparative genetic information for this region of rice chromosome 3 may enhance the identification of additional markers for this genomic region and the eventual identification of the genetic factors associated with this phenomeon.

Comparison of the rice and barley genetic maps

Examination of Figs. 1 and 2 reveals extensive chromosomal rearrangement during the evolution of the rice and barley genomes. However, a gobal comparison of the location of comparative loci within the two genomes identifies several striking features of genome conservation between rice and barley. The most interesting of these are:

(1) Many rice chromosomes consist primarily of comparative loci from a single barley chromosome. For example, chromosome 9 of rice consists of 13 comparative loci (loci mapped in both barley and rice), of which nine map to chromosome 7 of barley (Fig. 3 A). Based on the number of comparative loci shared between rice and barley chromosomes a number of potential syntenous chromosomes exist between rice and barley, including rice chromosome 1 and barley chromosome 3, rice chromosomes 4 and 7 and barley chromosome 2, rice chromosome 5 and barley chromosome 5, rice chromosome 6 and barley chromosome 1, and rice chromosome 9 and barley chromosome 7 (Fig. 2; and see Table 4).

(2) Although several large linkage blocks of comparative loci were conserved in both the rice and barley genomes, the linkage distance spanned by these loci differed dramatically between the two genomes. For example, rice chromosome 7 is comprised of five contiguous loci from chromosome 2 of barley (CDO64-B15C-βmy-CDO537-ABC468) (Fig. 3 B). In rice these five loci span 53.6 cM, while in barley they span only 24.3 cM. Dramatic differences in linkage distance between pairs of comparative loci were also observed. For example, marker loci WG114 and WG464 are separated by 18.7 cM in rice (chromosome 3), while in barley they are separated by greater than 44 cM (chromosome 4) (Table 2; Fig. 3). A total of 17 conserved linkage segments, each consisting of two or more loci, were identified between the rice and barley genomes. These conserved linkage segments range in size from 0.0 to 53.6 cM in rice and from 0.0 to 46.3 cM in barley (Table 2; Figs. 1 and 2). Together, these conserved linkage blocks account for 24% (287 cM) and 31% (321 cM) of the rice and barley genetic maps, respectively.

(3) The linearity of comparative loci within conserved linkage blocks was often identical between the rice and barley genomes. Linkage blocks with conserved colinearity of comparative marker loci are of particular interest since orthologous loci which span genes of importance in barley chromosomes may predict the location of orthologous loci in homoeologous rice chromosomes and vice versa. Rice chromosome 5 consists of 11 comparative loci, of which nine map to barley chromosome 5. Except for a small inversion (1.5 cM) involving two loci (MWG943 and ABG464) eight of these loci are colinear in both genomes (Fig. 3 C). One locus (MWG068), although mapped to rice chromosome 5, does not maintain colinearity with the other comparative markers on this chromosome and appears to have undergone an interchromosomal rearrangement (Fig. 3 C). This region of colinearity between the these two species is of particular interest since it spans the centromere in barley and may represent orthologous loci which may predict the location of the centromere on the syntenous chromosome (chromosome 5) of rice. Other linkage blocks which span centromere regions on barley chromosomes 2, 5, and 6 may also span centromeres on the corresponding rice chromosomes, 7, 5, and 2, respectively (Fig. 2). Additional regions of largely conserved gene order include linkage blocks of rice chromosomes 1, 2, 3, 6, 7, and 9 (Fig. 2).

(4) Some rice chromosomes appeared as mosaics of two (or more) different barley chromosomes. For example, rice chromosome 3 is represented by comparative loci from barley chromosomes 4 (44.8 cM) and 7 (44.7 cM) (Fig. 3 D). Such rice chromosomes may represent interchromosomal translocation events during the speciation of rice and bar-

Table 2 A comparison of linkage distances of conserved chromosomal segments between the genomes of rice and barley

Rice chr.	Genomic[a] segment	No. of loci[b]	Rice cM[c]	Barley cM[c]	Barley chr.
1	ABC174–CDO113	4	48.3	46.3	3
	MWG582–ABC176	3	0	12.2	3
2	WG223–ABG20	2	0.9	1.4	6
3	ABC170B–MWG716B	2	0.9	12.4	6
	WG114–WG464	2	19.6	45.5	4
	CDO504–CDO484	3	32.2	43.2	7
4	CDO680–ABC153	2	6.6	11.4	2
	CDO57C–WG516	2	2.1	3.6	2
5	ABG494–WG789	2	2.8	0	5
	CDO105–MWG943B	3	21.6	20.7	5
6	CDO475–ABC465	3	25.1	34.3	1
	ABG461–PSR129	2	7.8	16.8	1
7	CDO64–ABC468	5	53.6	24.3	2
9	ABC302–RZ404[d]	7	31.0	41.9	7
10	ABG702B–ABC322B	2	24.2	6.0	5
11	CDO373–ABC252	2	3.7	0	5
12	ABG395–CDO749	2	6.6	1.3	7
Total		48	287.0	321.3	

[a] Only rice genomic regions without inter- or intra-chromosomal rearrangements are reported
[b] Number of markers spanning the length of the syntenic region
[c] centiMorgan distance spanned by conserved regions in rice or barley
[d] RZ404 is a rice cDNA clone which was mapped in barley by Kasha et al. (1995)

Fig. 2 A rice RFLP comparative map consisting of 108 orthologous and 49 framework (see Material and methods section) rice loci. The scale is shown at the left in centiMorgans. Markers were placed on the map using the computer program MapMaker 2.0 with LOD=3.0 (Lander et al. 1987). Corresponding chromosomal locations in barley are indicated *in parenthesis* following the clone names. RZ and RG clones are rice clones used to identify genomic regions in rice not detected by comparative loci and to anchor specific rice linkage groups

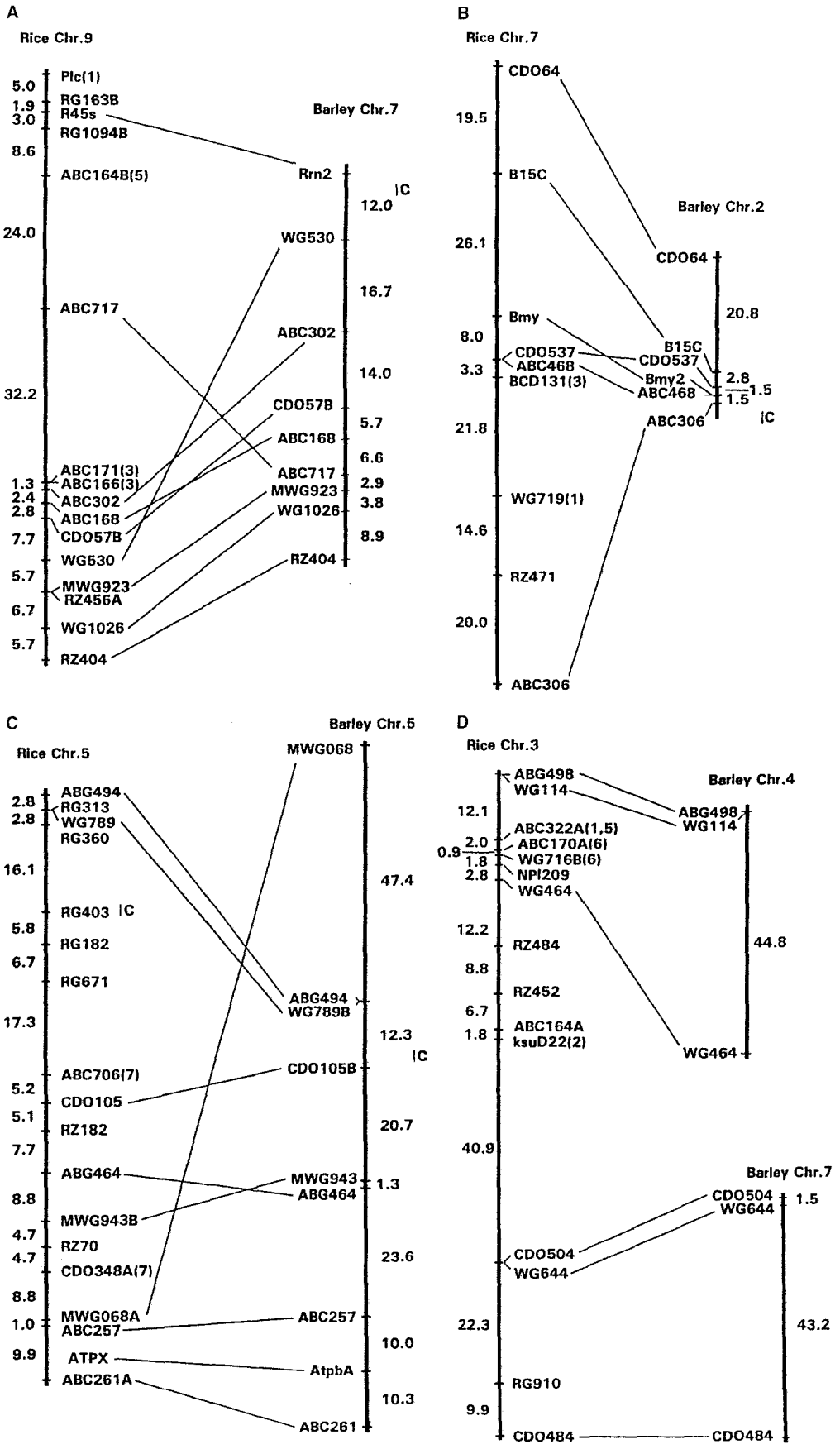


Fig. 3 A–D Comparative chromosomes of rice and barley. Map distances are shown on the right of chromosomes, and clone names on left. Loci connected by a *line* are detected by the same hybridization clone and represent syntenous markers. Rice loci without connecting lines are not syntenous for this specific chromosome and are followed by parentheses indicating their mapping position in barley. **A** Syntenous relationship between rice chromosome 9 and barley chromosome 7. **B** Linkage block conservation between rice chromosome 7 and barley chromosome 2. **C** Conservation of linearity of orthologous loci within linkage blocks between rice chromosome 5 and barley chromosome 5. **D** Mosaic rice chromosome 3 and barley chromosomes 4 and 7

ley. A similar case is apparent in barley chromosome 7 which is composed of discrete segments of rice chromosomes 3 (38.5 cM) and 9 (61.7 cM) (Fig. 1). Similar interchromosomal translocations have been reported for comparative analyses of rice and wheat (Ahn et al. 1993; Kurata et al. 1994).

(5) While the majority of rice chromosomes (1–7, and 9) showed synteny to specific barley chromosomes, some rice chromosomes (8, 10–12) displayed no obvious homoeology to any barley chromosome or chromosomal segments. This inability to relate the genomic structure of these rice chromosomes to specific barley chromosomes (or chromosomal regions) may be due to a lack of evenly spaced comparative markers on these rice chromosomes (e.g., rice chromosome 8; Fig. 2). A more detailed analysis of these chromosomes will be necessary to reveal their evolution and to resolve their relationship to specific barley chromosomes.

Comparison of chromosome homoeologies among rice, wheat and barley

Since it is well established that barley and wheat share homoeologous genomes, it should be possible to combine our comparative mapping results with those reported between wheat and rice. Table 3 summarizes the comparative mapping data from the present study of rice and barley and two comparative analyses of rice and wheat (Ahn et al. 1993; Kurata et al. 1994). Integrating the results from these three investigations provides 248 comparative marker loci spanning all 12 rice chromosomes, with a total number of comparative loci per individual rice chromosome ranging from a low of seven on rice chromosome 10 to a high of 46 on rice chromosome 1 (Table 3). Integration of these studies provides complementary evidence supporting our comparative mapping results between rice and barley, especially when only a few markers supported such conclusions. This is the case for rice chromosome 10 which consists of only two single-copy comparative loci in our study. Combining our results with those of Ahn et al. (1993) and Kurata et al. (1994), however, provides good evidence that rice chromosome 10 is syntenous to barley chromosome 5 (Table 3). Such an observation, while not definitive, supports our conclusion concerning the syntenous relationships between barley and rice.

As seen in Table 3, syntenous relationships between rice and wheat/barley homoeologous groups are apparent for 11 of the 12 rice chromosomes. Each of five rice chromosomes, 4, 5, 6, 9 and 10, consist of greater than 85% of their combined comparative loci from a single homoeologous group (Table 3). For example, rice chromosome 6 consists of 23 comparative loci of which 91% map to homoeologous group 7 (barley chromosome 1). Three additional rice chromosomes (1, 2, and 7) contain greater than 65% of their comparative loci from a single homoeologous group. Interestingly, three rice chromosomes (3, 11 and 12) seemingly consist of a mosaic of several homoeologous groups (Table 3). While these rice chromosomes are not syntenous to a single homoeologous chromosome, they are predominantly represented by a large precentage of two or three homoeologous groups. For example, rice chromosome 3, represented by 27 comparative loci, consists predominantly of loci from homoeologous groups 4 (68%) and 5 (28%) (Table 3). Similar results are evident for rice chromosomes 11 and 12. The remaining rice chromosome (chromosome 8) showed no obvious syntenous relationship with any homoeologous group. Similarly complex results for rice chromosome 8 have been reported by Ahn and Tanksley (1993) for a rice/maize comparison. A more detailed study will be necessary to resolve the organization and evolution of this chromosome. A summary of rice, wheat, and barley chromosomal homoeologies, as determined from this analysis, is presented in Table 4.

Chromosomal organization of barley and rice

The barley genome is approximately 14 times larger than that of rice (Arumuganathan and Earle 1991). Despite this massive difference in genome size, we have detected 17 conserved chromosomal segments, spanning 287 cM (24%) and 321 cM (31%) of the rice and barley genomes, respectively. For some chromosomes, the evidence for conservation includes not only comparative marker content and linkage conservation, but also colinearity of comparative markers along the homoeologous chromosomes. Wheat, another Triticeae species, which has a genome nearly 40-times larger than that of rice, also shows a striking conservation of gene homoeology and colinearity with rice chromosomes (Ahn et al. 1993; Kurata et al. 1994). Some researchers have suggested that the dramatic changes in genome size observed among these closely related species may have resulted from changes in repetitive DNA sequences (Ahn et al. 1993; Bennetzen and Freeling 1993; Moore et al. 1993). Repetitive DNA evolves rapidly by unequal crossing-over and mismatch during DNA replication and diverges substantially during speciation (Flavell et al. 1977). Such repetitive DNA is often localized to centromere and telomere regions, and therefore could change without affecting gene order or content (Ahn et al. 1993).

Gene duplication

The majority (>80%) of clones selected for comparative mapping were single-copy in the barley genome. Single-copy clones were defined as those clones that detected a single genetic locus (appearing as single or several co-segregating bands) using standard wash conditions (see Materials and methods). Based on this criterion, we estimate that approximately 72% of the single-copy clones observed in barley are also single-copy in rice. Similarly, Moore et al. (1993) estimate that approximately 60% of rice single-copy sequences detect single-copy sequences in wheat and barley. The conservation of single-copy loci between rice and barley indicates that the evolution of the large barley genome did not involve the duplication of single-copy se-

Table 3 Integrated comparative mapping data[a] from comparative analyses of rice with barley[b] and wheat[cd]. The number of single-copy loci identified in each rice chromosome for specific wheat/barley homoeologous groups are combined from three comparative mapping analyses

Rice chr.		Wheat/barley homoeologous groups[e]							Total
		1 (5)	2 (2)	3 (3)	4 (4)	5 (7)	6 (6)	7 (1)	
1	W1[c]			6				1	7
	W2[d]	1		19	1	1		1	23
	B[b]	3	1	7	2	2		1	16
	f[f]	0.09	0.02	**0.70**	0.06	0.06		0.06	(46)[g]
2	W1			1			2	1	4
	W2	1					6		7
	B		1	1			3		5
	f	0.06	0.06	0.13			**0.69**	0.06	(16)
3	W1				8	2			10
	W2				6	2			8
	B		1		3	3	2		9
	f		0.04		**0.68**	**0.28**	0.08		(27)
4	W1		7						7
	W2						1		9
	B		5		1			1	7
	f		**0.87**		0.04		0.04	0.04	(23)
5	W1	8							8
	W2	8		1			1		10
	B	9				2			11
	f	**0.86**		0.03		0.07	0.03		(29)
6	W1							2	2
	W2							14	14
	B	2						5	7
	f	0.09						**0.91**	(23)
7	W1		4			1	1		6
	W2		3						3
	B		6	1				1	8
	f		**0.76**	0.06		0.06	0.06	0.06	(17)
8	W1			1				2	3
	W2				1		1		2
	B	2	2					1	5
	f	0.20	0.20	0.10	0.10		0.10	0.30	(10)
9	W1					4			4
	W2					6			6
	B	1		2		8			11
	f	0.05		0.10		**0.85**			(21)
10	W1	3							3
	W2	2							2
	B	1						1	2
	f	**0.89**						0.11	(7)
11	W1					1		1	2
	W2		1			3		1	5
	B		2		1	1		1	5
	f		**0.25**		0.08	**0.42**		**0.25**	(12)
12	W1					1			1
	W2			2	1	4			7
	B		4		1	3		1	9
	f		**0.23**	0.12	0.12	**0.47**		0.06	(17)
	Total	41	45	41	25	44	17	35	(248)

[a] Only single-copy loci with unambiguous mapping locations are included in the table
[b] The barley/rice comparison data as determined in this study
[c] The wheat/rice comparison data taken from Ahn et al. (1993)
[d] The wheat/rice comparison data taken from Kurata et al. (1994)
[e] Barley homoeologous chromosome numbers are shown in parentheses
[f] Frequency of the comparative loci found in each homoeologous-group chromosome for each rice chromosome
[g] Total number of clones mapped per rice chromosome

Table 4 Homoeologous chromosomes between rice, wheat, and barley as concluded from comparative analyses of wheat/rice[a] and barley/rice[b]

Rice	Wheat[a]	Barley[b]
1	3* (4, 5, 7)	3* (5, 2, 4, 7, 1)
2	6* (1, 3, 7)	6* (2, 3)
3	4* (5)	4*, 7* (2,6)
4	2* (6)	2* (4, 1)
5	1* (3, 6)	5* (7)
6	7*	1* (5)
7	2* (5, 6)	2* (3, 1)
8	[1, 2, 3, 4, 6, 7]	[1, 2, 5]
9	5*	7* (3, 5)
10	1*	[5, 1]
11	[5, 2, 7]	[2, 4, 7, 1]
12	[3, 4, 5]	[2, 4, 7, 1]

[a] Data taken from the comparative mapping analyses of wheat/rice from Ahn et al. (1993) and Kurata et al. (1994)
[b] Data taken from the present study
* Most likely an orthologous chromosome, based on the number of syntenic loci. Chromosomes enclosed in backets indicate that no obvious syntenous chromosomes were evident for that specific rice chromosome. Chromosomes enclosed in parantheses identify additional chromosomes containing comparative loci from specific rice chromosomes

quences and suggests that the additional DNA observed in the large barley genome arose by unequal crossing over and amplification of repetitive DNA sequences (Moore et al. 1993).

Potentially orthologous loci in rice and barley

Classical genetic maps, consisting of isozyme and morphological markers, are well developed for both rice and barley (Tanksley et al. 1993; Wettstein-Knowles 1993). Several researchers have placed many of these biochemical and morphological markers on genetic maps and on specific chromosomes, yet few have investigated the potential orthology of specific isozyme loci among related Gramineae species. While the RFLP-based comparative genetic maps reported here provide only basic information concerning potentially syntenic chromosomes, the positioning of isozyme and morphological markers with similar phenotypes in conserved regions of both the rice and barley genomes provides evidence of potentially orthologous relationships (related by descent) and further supports conclusions as to syntenous relationships between barley

Table 5 Potentially orthologous isozyme and morphological loci in rice and barley, based on conserved linkage groups

Description	Locus	Rice chr.	Ref[a]	Locus	Barley chr.	Ref[a]
Esterase	*Est 1-2*	1	1	*Est 1, 2, 4, 10*	3	5
Semidwarf	*sd-1*	1	1	*sdw-b*	3	5
Malic enzyme	*Mal 1*	1	1	*Mal 1*	3	5
Trypsin-inhibitor	*TRYP*	1	1	*TE-1*	3	5
Aspartate aminotransferase	*Got 3*	2	2	*Aat 2*	6	5
Aminopeptidase	*Amp 1*	2	3	*Amp 1*	6	5
alpha-Amylase	*amy 1A, 1C*	2	1	*Amy 1*	6	6
Phytochrome-A	*PHY-A*	3	1	*PHY-A, D*	7	8
Esterase	*Est 1*	3	3	*Est 9*	7	6
Ubiquitin	*Ubi 1*	3	2	*Ubi 2*	7	6
alpha-Tublin	*Tub 2*	3	2	*TubA1*	4	5
Histone	*His 1 (H1)*	4	2	*His3c*	2	6
Malate dehydrogenase	*Mdh 3*	5	3	*Mdh 1*	5	5
Peroxidase	*Pox 1*	5	3	*Prx 1*	5	5
ATPase	*ATPX*	5	1	*AtpbA*	5	7
Low/high amylopectin	*wx*	6	3	*wx*	1	5
Eterase	*Est 2*	6	4	*Est 5*	1	6
alpha-Amylase	*Amy2A*	6	1	*Amy 2*	1	6
Chitinase	*Chi-1*	6	1	*Chi 1*	1	6
Aminopeptidase	*Amp 3*	6	3	*Amp 3*	1	5
Endopeptidase	*Enp 1*	6	3	*Enp 1*	1	5
Peroxidase	*Pox 5*	6	3	*Prx 4*	1	5
Phophogluconate dehydrogenase	*Pgd*	6	2	*Pgd 1*	1	7

[a] Chromosomal locations of isozymes and morphological markers were derived from previously published linkage studies: 1, Causse et al. (1994); 2, Kurata et al. (1994); 3, Kinoshita (1993); 4, Zhu et al. (1993); 5, Wettstein-Knowles (1993); 6, Kleinhofs et al (1993); 7, Kasha et al. (1995); and 8, Biyashev, unpublished data

and rice chromosomes. Figure 1 and Table 5 identify several isozyme and morphological loci which have similar phenotypic or enzymatic properties in both barley and rice and are found in syntenous chromosomes. Twenty-three potentially orthologous genes were identified for 7 of the 12 rice chromosomes, including isozyme and morphological markers (Table 4).

If related species share orthologous genes for traits of agronomic importance, and linkage is conserved among molecular markers and these orthologous genes, it may be possible to characterize and clone these genes in better developed and less complex genetic systems (e.g., rice) based on conserved flanking markers. Such information could then be applied to all related plant species. Preliminary evidence for the conservation of orthologous seed-weight genes and RFLP markers between related species has been reported for mung bean and cowpea (Fatokun et al. 1992).

Examination of the comparative maps of rice and barley reveals the conservation of several genomic intervals (Figs. 1 and 2) which contain important barley disease-resistance genes (data unpublished). Markers flanking these intervals may provide a starting point for the characterization and positional cloning of these barley genes in the smaller, less complex genome of rice. Undoubtedly, as the resolution of the comparative map of barley and rice increases, additional orthologous loci will be identified based on the conservation of genomic regions between these important crop species.

The development of a "generic" Gramineae map based on genome homoeologies within the family Gramineae would provide researchers with a vast supply of heterologous probes for specific genomic regions. Such a vast new source of region-specific clones will not only allow the saturation of regions surrounding genes of interest with genetic markers, but should also accelerate the genetic-map development of less-characterized grass genomes by providing an abundant supply of evenly distributed DNA clones. Moreover, the colinearity observed among the grass species suggests that genes identified in large-genome grasses (e.g., barley) could be isolated more efficiently by positional cloning in the smaller rice genome. Such "comparative positional cloning" will, however, rely on the conservation of microsynteny among the grass species. Undoubtly, the development of a "generic" Gramineae map will add valuable insight into chromosome evolution and grass speciation and should be a priority research goal for grass genetics.

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