Comparison and integration of four barley genetic maps

Xiaoquan Qi, Piet Stam, and Pim Lindhout

Abstract: Barley (*Hordeum vulgare* L.) is one of the most extensively studied food crops in recent molecular research. More than 1000 molecular markers have been located on the barley genome by using five independent populations. For the present study, four segregation data sets, 'Proctor' × 'Nudinka', 'Igri' × 'Franka', 'Steptoe' × 'Morex', and 'Harrington' × TR306, were downloaded from the publicly available GrainGenes databank. Since 22% of the markers are common to at least two of the independent data sets, we were able to establish an integrated map using the computer package JOINMAP V2.0. The integrated map contains 898 markers, covers 1060 cM, and removes many large gaps present in the individual maps. Comparison of the integrated map with the individual maps revealed that the overall linear order of markers is in good agreement and that the integrated map is consistent with the component maps. No significant reordering of markers was found. This conservative property of the barley genome makes the integrated map reliable and successful. Except for chromosome 7 (5H), marker clustering was observed in the centromeric regions, probably owing to the centromeric suppression of recombination. Based on this integrated map, geneticists and breeders can choose their favourite markers in any region of interest of the barley genome.

Key words: *Hordeum vulgare*, RFLP, integrated map.

Résumé : L'orge (*Hordeum vulgare* L.) est une espèce agricole d'importance qui a fait l'objet récemment d'un très grand nombre de recherches au niveau moléculaire. Plus de 1000 marqueurs moléculaires ont été situés dans le génome de l'orge en employant cinq populations indépendantes. Pour les fins de la présente étude, les données de ségrégation pour quatre populations, 'Proctor' × 'Nudinka', 'Igri' × 'Franka', 'Steptoe' × 'Morex' et 'Harrington' × TR306, ont été obtenues de GrainGenes, une banque de données publique. Puisque 22% des marqueurs étaient présents chez au moins deux ensembles de données, une carte génétique intégrée a pu être produite à l'aide du logiciel JOINMAP V2.0. Cette carte intégrée comprend 898 marqueurs, s'étend sur 1060 cM et permet de combler plusieurs trous présents dans les diverses cartes individuelles. Une comparaison de la carte intégrée avec les cartes individuelles a révélé que l'ordre des marqueurs est bien conservé et que la carte intégrée est en bon accord avec les cartes qui la composent. Aucune modification importante de l'ordre des marqueurs n'a été notée. La bonne conservation du génome de l'orge a permis de réaliser avec succès cette intégration et rend utile la carte qui en résulte. À l'exception du chromosome 7 (5H), une forte densité de marqueurs a été observée dans les régions centromériques vraisemblablement en raison de la suppression de la recombinaison dans ces régions. Grâce à cette carte, généticiens et améliorateurs pourront choisir les marqueurs de leur choix dans toute région du génome de l'orge qui présente un intérêt.

Mots clés : *Hordeum vulgare*, RFLP, carte intégrée.

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Introduction

Barley (*Hordeum vulgare* L.) is extensively studied as a favourite genetic experimental plant species, mainly owing to its diploid nature ($2n = 2x = 14$), self-fertility, large chromosomes (6–8 μm), high degree of natural and easily inducible variation, ease of hybridization, wide adaptability, and relatively limited space requirements, as well as its agricultural importance (Kleinhofs and Kilian 1994). Its large genome size (1C = 5.3×10^9 base pairs (bp)) (Bennett and Smith 1976) has slowed down the development of molecular maps. However, techniques for developing doubled haploid lines and the availability of cytogenetic stocks, such as barley–wheat addition lines, have facilitated genetic mapping.

The first incomplete barley restriction fragment length polymorphism (RFLP) map for chromosome 6 was published in 1988 (Kleinhofs et al. 1988). Recently, five more extensive molecular maps covering the entire genome have been generated by using five independent doubled haploid populations. These are 'Proctor' × 'Nudinka' (Heun et al. 1991), 'Igri' × 'Franka' (Graner et al. 1991), 'Vada' × *Hordeum spontaneum* line 1b-87 (Graner et al. 1991), 'Steptoe' × 'Morex' (Kleinhofs et al. 1993*b*), and 'Harrington' × TR306 (Kasha et al. 1994). Many other segregating populations have also been used to construct partial maps and to determine the location of interesting

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Table 1. Four doubled haploid mapping populations and their characteristics.

Parents	Population size	Number of markers	Length of map (cM)	Reference
Harrington × TR306	150	190	1278	Kasha and Kleinhofs 1994 Kleinhofs 1994*a*
Steptoe × Morex	150	423	1227	Kleinhofs et al. 1993*a* Kleinhofs 1994*b*
Proctor × Nudinka	113	154	1192	Heun et al. 1991 Sorrells 1992
Igri × Franka	73	369	1387	Graner et al. 1994 Graner 1994

genes on the genome: for example, the 'Aramir' × *H. spontaneum* derived population was used to generate a map of chromosome 4 (Hinze et al. 1991); 120 F_2 plants obtained from 'Betzes' × 'Golden Promise' and 120 F_2 plants from 'Captain' × *H. spontaneum* were used to map 5S rDNA genes on chromosome 2 (Leitch and Heslop-Harrison 1993); Laurie et al. (1993) located the *denso* dwarfing gene to the long arm of chromosome 3 by using 113 doubled haploid lines from 'Magnum' × 'Goldmarker'; and a photoperiod response gene (*Ppd-H1*) was mapped by using 94 doubled haploid lines from 'Igri' × 'Triumph' (Laurie et al. 1994). Today, more than 1000 markers have been located on the barley genome by using different populations and more markers will be developed and mapped in the near future. The rapid accumulation of markers and mapping populations is a challenge to the merging of separate lines of information to accumulate more valuable information for further research and a better understanding of barley genetics and genome organization.

Recent good communication between North American and European barley mapping efforts has resulted in the frequent exchange of probes. As a consequence, many common markers have been utilized in independent mapping populations. The availability of the mapping software programme JOINMAP (Stam 1993), which enables the integration of individual maps into one composite map by using common markers, makes the construction of an integrated barley map possible. The present study aims at integrating four individual maps into a single map. The combined map provides an easy and convenient way of comparing the component maps and offers important information about the reliability of marker order and distances between markers.

Materials and methods

Four barley segregation data sets (Table 1) were downloaded from the publicly available GrainGenes databank (Graner 1994; Kleinhofs 1994*a*, 1994*b*; Sorrells 1992). The mapping populations of 'Proctor' × 'Nudinka' (P/N) (Heun et al. 1991; Sorrells 1992) and 'Igri' × 'Franka' (I/F) (Graner et al. 1991; Graner 1994) consisted of 113 and 73 doubled haploid lines, respectively, derived by anther culture. In P/N, 154 markers and in I/F, 369 markers have been located on seven chromosomes. 'Steptoe' × 'Morex' (S/M) (Kleinhofs et al. 1993*b*; Kleinhofs 1994*a*) and 'Harrington' × TR306 (H/T) (Kasha and Kleinhofs 1994; Kleinhofs 1994*b*), used in the North American Barley Genome Mapping Project (NABGMP), both contain 150 doubled haploid lines that were derived by the *Hordeum bulbosum* method. Data for 423 and 190 markers, respectively, were available for the two populations.

The new version of JOINMAP (Stam 1993), which can handle a wide variety of mapping population types including the doubled haploid type, was used to regenerate linkage maps and to merge these into an integrated map. From the segregation data, the pairwise recombination frequencies were estimated and the corresponding LOD values were calculated. If several estimates of the recombination frequency between a certain pair of markers were available (markers shared by at least two populations), they were replaced by a single value after appropriate weighting (Stam 1993). Based on the recombination frequencies and LOD values, the individual or integrated maps were constructed by running the JOINMAP programme. Kosambi's mapping function was adopted for map distance calculation (Kosambi 1944). Since the gene ordering algorithm of JOINMAP does not guarantee the best solution, the "fixed order" option was used in a number of cases where the goodness-of-fit criterion cast doubt on the ordering. (The "fixed order" option allows the user to define fixed orders of (sub)sets of markers; by using various fixed orders a better solution is occasionally obtained, especially with data sets of moderate quality.)

Results and discussion

Source and nomenclature of markers and chromosomes

Probes from several different sources have been used as genetic markers for the barley genome (Kleinhofs and Kilian 1994). In the present study, much attention was paid to ascertaining whether markers with different names in different populations represented the same locus. Alternatively, markers with the same name might represent different loci. Multiple MWG markers in the I/F population were designated with lower case letters, for example, MWG555a and MWG555b (Graner et al. 1991); these were converted to capital letters (MWG555A and MWG555B) for consistency with the other three populations (Table 2; Heun et al. 1991; Kleinhofs et al. 1993*b*; Kasha and Kleinhofs 1994). Similarly, meaningless zeros in marker names of the S/M and H/T populations were omitted (e.g., ABA001 becomes ABA1). The prefixes, "i", "m," and "d" were added to marker names to indicate the isozyme markers, morphology markers, and disease resistance genes, respectively. The rest of the marker names remained unchanged

Table 2. Nomenclature of markers.

Present name	Original name	Chromosome Number	Mapping population
ABA1	ABA001	7(5H)	S/M
ABA2	ABA002	5(1H)	S/M
ABA3	ABA003	4(4H)	S/M
ABA4	ABA004	5(1H)	S/M
ABA5	ABA005	2(2H)	S/M
ABA6	ABA006	6(6H)	S/M
ABC151A	ABC151a	1(7H)	I/F
ABC151A	ABC151	1(7H)	S/M
ABC156D	ABC156	1(7H)	I/F
ABC167A	ABC167a	1(7H)	I/F
ABC310B	ABC310	1(7H)	I/F
ABG10	ABG010	3(3H)	S/M
ABG11	ABG011	1(7H)	S/M
ABG14	ABG014	2(2H)	S/M
ABG19	ABG019	2(2H)	H/T
ABG19	ABG019	2(2H)	S/M
ABG1A	ABG001A	6(6H)	H/T
ABG1A	ABG1	6(6H)	I/F
ABG1A	ABG001	6(6H)	S/M
ABG1B	ABG001B	1(7H)	H/T
ABG1C	ABG001C	6(6H)	H/T
ABG2	ABG002	2(2H)	S/M
ABG22A	ABG022A	1(7H)	S/M
ABG3	ABG003	4(4H)	S/M
ABG387A	ABG387a	5(1H)	I/F
ABG387B	ABG387b	6(6H)	I/F
ABG4	ABG004	3(3H)	S/M
ABG5	ABG005	2(2H)	S/M
ABG500B	ABG500	4(4H)	I/F
ABG53	ABG053	5(1H)	S/M
ABG54	ABG054	4(4H)	S/M
ABG55	ABG055	5(1H)	S/M
ABG57	ABG057	3(3H)	S/M
ABG57B	ABG057B	7(5H)	H/T
ABG58	ABG058	2(2H)	H/T
ABG58	ABG058	2(2H)	S/M
ABG59	ABG059	5(1H)	S/M
ABG65B	ABG065B	1(7H)	H/T
ABG69	ABG069	7(5H)	S/M
ABG703B	ABG703b	2(2H)	I/F
ABG705A	ABG705	7(5H)	S/M
ABG72	ABG072	2(2H)	S/M
ABG74	ABG074	5(1H)	S/M
ABG75	ABG075	1(7H)	S/M
ABG77	ABG077	1(7H)	H/T
ABG8	ABG008	2(2H)	S/M
Act8A	Act8	5(1H)	S/M
BCD351E	BCD351e	7(5H)	I/F
BCD453B	BCD453	2(2H)	P/N
BG123A	BG123a	2(2H)	I/F
BG123A	BG123	2(2H)	P/N
BG123B	BG123b	7(5H)	I/F
CDO348B	CDO348	7(5H)	H/T
CDO474C	CDO474	2(2H)	I/F
Chs1B	Chs1b	2(2H)	I/F

Table 2 (*concluded*).

Present name	Original name	Chromosome Number	Mapping population
Dhn3	XDhn3,4	6(6H)	P/N
Glx(Wx)	Glx	1(7H)	H/T
Glx(Wx)	Wx	1(7H)	I/F
Glx(Wx)	Glx	1(7H)	S/M
His3A	aHis3a	1(7H)	H/T
Hor2	aHor2	5(1H)	H/T
MWG10	MWG010	3(3H)	I/F
MWG10B	MWG010B	1(7H)	S/M
MWG3	MWG003	1(7H)	H/T
MWG3	MWG003	1(7H)	S/M
MWG36A	MWG036A	5(1H)	S/M
MWG36B	MWG036B	1(7H)	H/T
MWG36B	MWG036B	1(7H)	S/M
MWG41	MWG041	3(3H)	H/T
MWG41	MWG041	3(3H)	S/M
MWG520A	MWG520	2(2H)	H/T
MWG520A	MWG520	2(2H)	I/F
MWG555A	MWG555a	1(7H)	I/F
MWG555B	MWG555b	3(3H)	I/F
MWG57	MWG057	4(4H)	I/F
MWG571A	MWG571a	3(3H)	I/F
MWG58	MWG058	4(4H)	I/F
MWG58	MWG058	4(4H)	S/M
MWG635A	MWG635a	4(4H)	I/F
MWG636(HT)	MWG636	2(2H)	H/T
MWG636(IF)	MWG636	2(2H)	I/F
MWG64	MWG064	2(2H)	I/F
MWG65	MWG065	2(2H)	I/F
MWG663-2A	MWG663	6(6H)	H/T
MWG77	MWG077	4(4H)	S/M
MWG798A	MWG798	6(6H)	H/T
MWG798A	MWG798a	6(6H)	I/F
MWG813A	MWG813	7(5H)	H/T
MWG813A	MWG813a	7(5H)	I/F
MWG844A	MWG844	2(2H)	H/T
MWG844A	MWG844a	2(2H)	I/F
MWG85	MWG085	3(3H)	I/F
MWG851A	MWG851a	1(7H)	I/F
MWG851B	MWG851b	7(5H)	I/F
MWG89	MWG089	1(7H)	I/F
MWG89	MWG089	1(7H)	S/M
MWG90	MWG090	2(2H)	I/F
RisBPP161A	RisBPP161	1(7H)	H/T
RisBPP161A	RisBPP161a	1(7H)	I/F
WG789A	WG789	1(7H)	P/N
cMWG652A	cMWG652a	6(6H)	I/F
cMWG706A	cMWG706a	5(1H)	I/F
iEst1	Est1	3(3H)	I/F
iEst5	Est5	1(7H)	I/F

Table 3. The number of common markers between or among populations.

Mapping populations[a]	Chromosome 1	2	3	4	5	6	7	Total
H/T and I/F	4	3	2	0	1	2	1	13
H/T and P/N	1	3	1	1	0	2	2	10
H/T and S/M	15	5	7	2	6	7	11	53
I/F and P/N	0	0	0	0	0	0	0	0
I/F and S/M	7	10	14	4	9	4	9	57
P/N and S/M	6	6	1	2	2	0	7	24
Subtotal	33	27	25	9	18	15	30	157
H/T and I/F and P/N	0	0	0	0	0	0	0	0
H/T and I/F and S/M	9	5	2	1	2	8	4	31
H/T and P/N and S/M	2	2	0	1	0	0	1	6
I/F and P/N and S/M	0	1	1	0	0	0	0	2
Subtotal	11	8	3	2	2	8	5	39
H/T and I/F and P/N and S/M	0	0	0	1	0	0	0	1
Total	44	35	28	12	20	23	35	197

[a]H/T, I/F, P/N, and S/M represent 'Harrington' × TR306, 'Igri' × 'Franka', 'Proctor' × 'Nudinka', and 'Steptoe' × 'Morex', respectively.

and the original datasets were used to generate maps of each population seperately and one integrated map. If markers with the same core name were mapped within a 5-cM distance they were considered to represent only one locus and the name was adjusted accordingly. For example, the markers ABC151 and ABC151a from datasets of S/M and I/F, respectively, were mapped at 23.9 and 18.3 cM on chromosome 1 of the "pre-integrated map" (not shown). Subsequently, the names ABC151 and ABC151a were converted into ABC151A and a new map was generated with only one locus position for ABC151A at 24.1 cM (Fig. 1A). All changed gene symbols and their original symbols are listed in Table 2. As in Kleinhofs and Kilian (1994), the chromosome designations 1, 2, 3, 4, 5, 6, and 7 are used in this paper and correspond to 7H, 2H, 3H, 4H, 1H, 6H, and 5H, respectively.

Individual maps

Four individual maps were generated by running JOINMAP V2.0. The same gene order was obtained for most linkage groups across populations. In some cases the fixed order option had to be used to obtain the most likely gene order for the four individual maps. In population P/N, fixed gene orders were used in generating maps of chromosomes 1, 2, 4, and 5. Fixed orders were also used for the mapping of chromosome 3 in the H/T and I/F crosses. For the S/M map, no improvement was obtained by predefining gene orders, which is not surprising since this is the largest data set.

The individual maps generated by JOINMAP were slightly shorter compared with the original published maps (Tables 1 and 3). The original maps were established using MAPMAKER (Lander et al. 1987). The discrepancy between map lengths obtained with JOINMAP and MAPMAKER results from the different methods of calculating map lengths. MAPMAKER calculates the map length as the sum of adjacent distances, i.e., using adjacent marker pairs only. JOINMAP on the other hand uses all pairwise estimates (above a predefined LOD threshold) for calculating the total map length. Whenever the assumed level of interference does not exactly reflect the true interference, the two methods will produce slightly different total map lengths. The likelihood method applied in MAPMAKER assumes an absence of interference, and recombination frequencies are simply translated into centimorgans, according to the chosen mapping function. The JOINMAP package, however, does take interference into account. Therefore, where there is interference JOINMAP will produce shorter maps than MAPMAKER, even when both programmes use the same Kosambi mapping function (Stam 1993).

Integrated map

In total, 190, 369, 154, and 423 markers were assigned to the seven barley chromosomes by using the mapping populations H/T, I/F, P/N, and S/M, respectively. Having standardized the different gene symbols (Table 1), 157 markers were found to be common to two populations, 39 markers were shared by three populations, and only one marker, WG622 on chromosome 4, was present in four populations. Populations H/T and S/M and I/F and S/M had 53 and 57 markers in common, respectively, and 31 markers were shared by the three populations. Only three markers were common to I/F and P/N. The backbone of the integrated map consists, of course, of the common markers. Together with the markers that are unique to one of the four populations, the combined map contains 898 markers, including four morphological markers and six disease resistance genes. For the contruction of the integrated map, no fixed

orders were required. Gene orders on the integrated map are identical to the orders on the four component maps.

The number of common markers is listed in Table 3 and the map data, i.e., length of chromosomes, number of markers, and number of gaps >10 cM, are summarized in Table 4. The integrated maps of seven barley chromosomes are presented in Figs. 1A–1G.

Chromosome 1

Chromosome 1 contains the largest number of markers (Fig. 1A). One morphological marker, naked caryopsis, *mn*, and two disease resistance genes, *dRpg1* and *dSb1*, were mapped on this chromosome. The integrated map shows a fairly uniform distribution of markers. However, clustering of markers occurs in the 70–80-cM region and there is one gap of 15 cM in the 130–140-cM region and one of 10 cM in 60–70-cM region, whereas the individual maps contained 3–8 gaps.

Chromosome 2

With 27 markers shared by two populations and 8 markers common to three populations, the integrated map of chromosome 2 comprises 163 markers with a total map length of 157 cM (Fig. 1B). One morphological marker, *mhex-v*, conferring six- or two-rowed spike, mapped to this chromosome. MWG636 was present in both the H/T and I/F populations but in H/T it was located on the "long" arm near the centromere, while in I/F it mapped at the distal end of the "short" arm. Therefore, with the probe MWG636, two different loci may be identified in these populations. To distinguish them, the names MWG636(HT) and MWG636(IF) were used to designate the different loci. The integrated map has only one gap larger than 10 cM, in the 95–107-cM region, while the most saturated individual map (S/M) showed two such gaps (Table 4). Clustering of markers was observed in the 60–70-cM region.

Chromosome 3

For this chromosome, 25 and 3 markers were common to two and three populations, respectively. The integrated map, comprising 133 markers and spanning 131 cM, represents the shortest of all chromosomes (Fig. 1C). The "pubescent leaf" gene *mPub* resides on chromosome 3. The recessive gene conferring resistance to barley yellow mosaic virus and barley mild mosaic virus, *dym4*, also mapped on this chromosome (Graner and Bauer 1993). Only one 10-cM gap remains in the integrated map and one cluster of markers occurs in the 40–50-cM region; in other regions the distribution is fairly uniform.

Chromosome 4

A total of 81 markers assigned to chromosome 4 in the four populations were remapped on the integrated map (Fig. 1D). The powdery mildew resistance gene, *dMlg*, is in the centromeric region of the composite map. The only marker shared by all four populations, WG622, is on chromosome 4. The markers are quite evenly scattered over the chromosome except for one clustering region around 50–55 cM. One gap larger than 10 cM remains on the [illegible]ual maps have 4–6 such gaps.

Table 4. Summary of individual and integrated mapping data.

Chromosome	H/T			I/F			P/N			S/M			Integrated		
	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]	Length of map (cM)	No. of markers	No. of gaps[a]
1(7H)	180	49	8	156	76	3	167	32	6	138	74	4	152	176	2
2(2H)	160	31	6	167	63	3	176	38	6	151	74	2	157	163	1
3(3H)	131	18	3	128	70	5	187	14	6	162	62	3	131	133	1
4(4H)	142	14	6	136	24	5	140	16	4	139	43	5	134	81	0
5(1H)	131	16	5	120	38	5	153	20	4	157	56	2	150	90	2
6(6H)	160	30	4	128	43	6	36	9	1	140	47	5	141	98	3
7(5H)	214	32	9	199	55	8	158	25	3	184	67	7	195	139	1
Total	1118	190	41	1034	369	35	1017	154	27	971	423	28	1060	898	10

Note: H/T, I/F, P/N, and S/M represent 'Harrington' × TR306, 'Igri' × 'Franka', 'Proctor' × 'Nudinka', and 'Steptoe' × 'Morex', respectively; integrated indicates the integrated map.

[a]A gap is a distance between two adjacent markers of more than 10 cM.

Fig. 1. (A–G) Barley integrated molecular linkage map. Chromosomes are oriented with the short arm at the top. The genetic distances are expressed in map distances (cM) according to Kosambi (Kosambi 1944). Markers in the box are located at the same position as the marker to which it is connected.

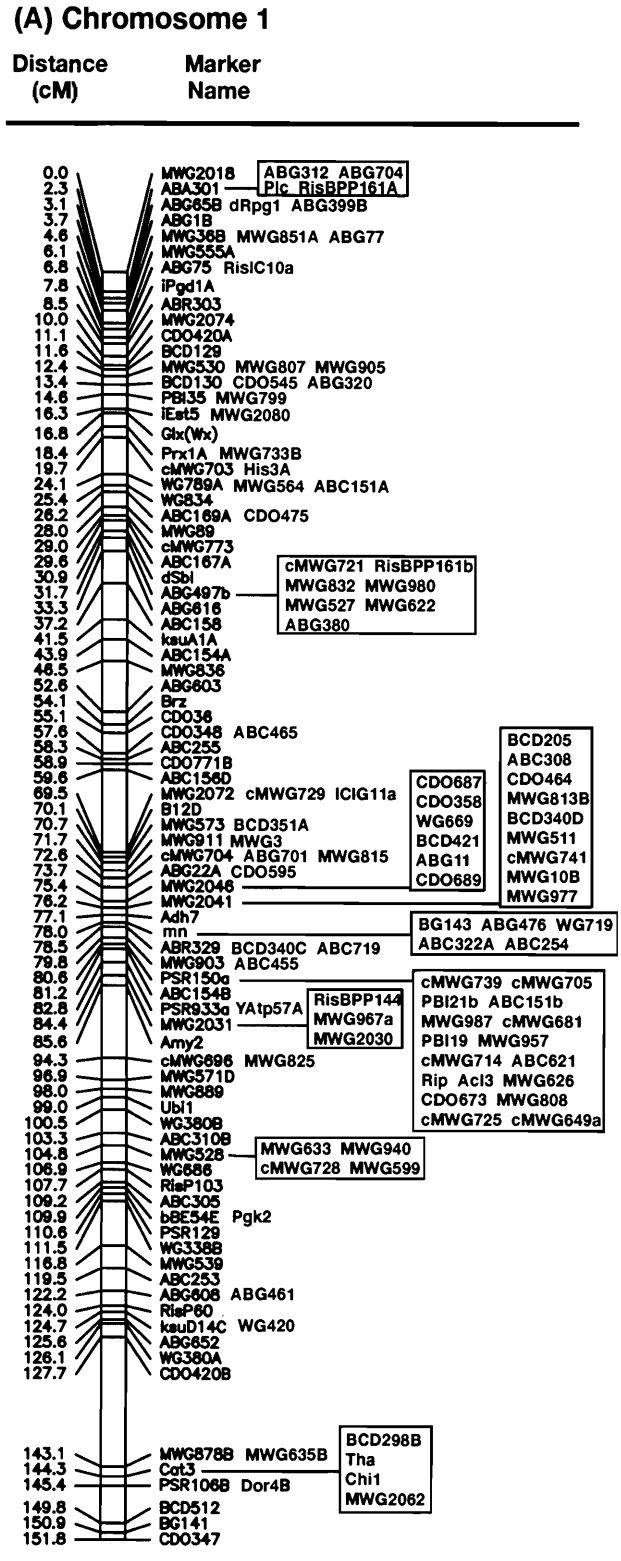


Fig. 1 (*continued*).

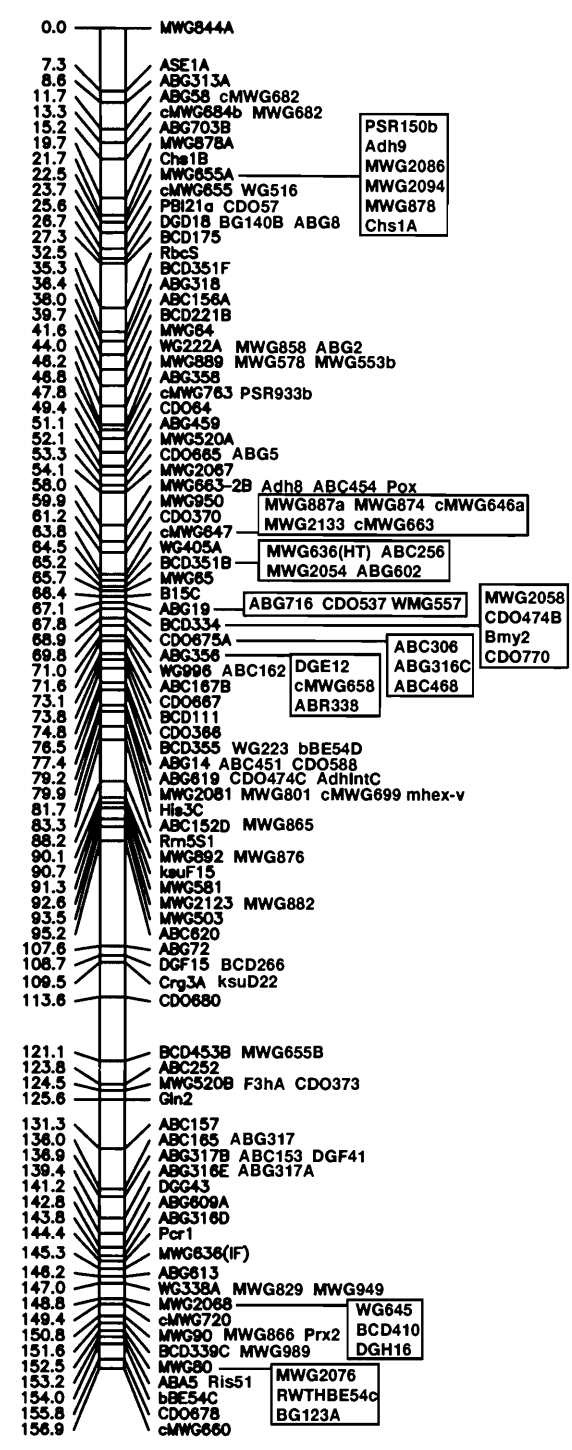


Fig. 1 (*continued*).

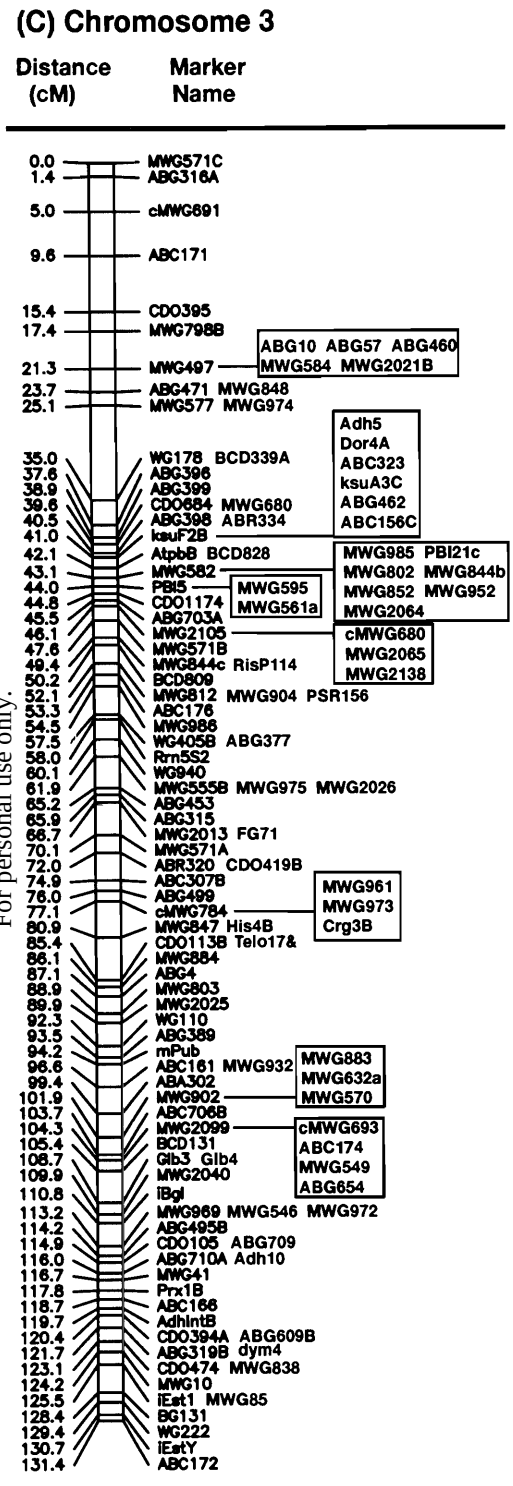
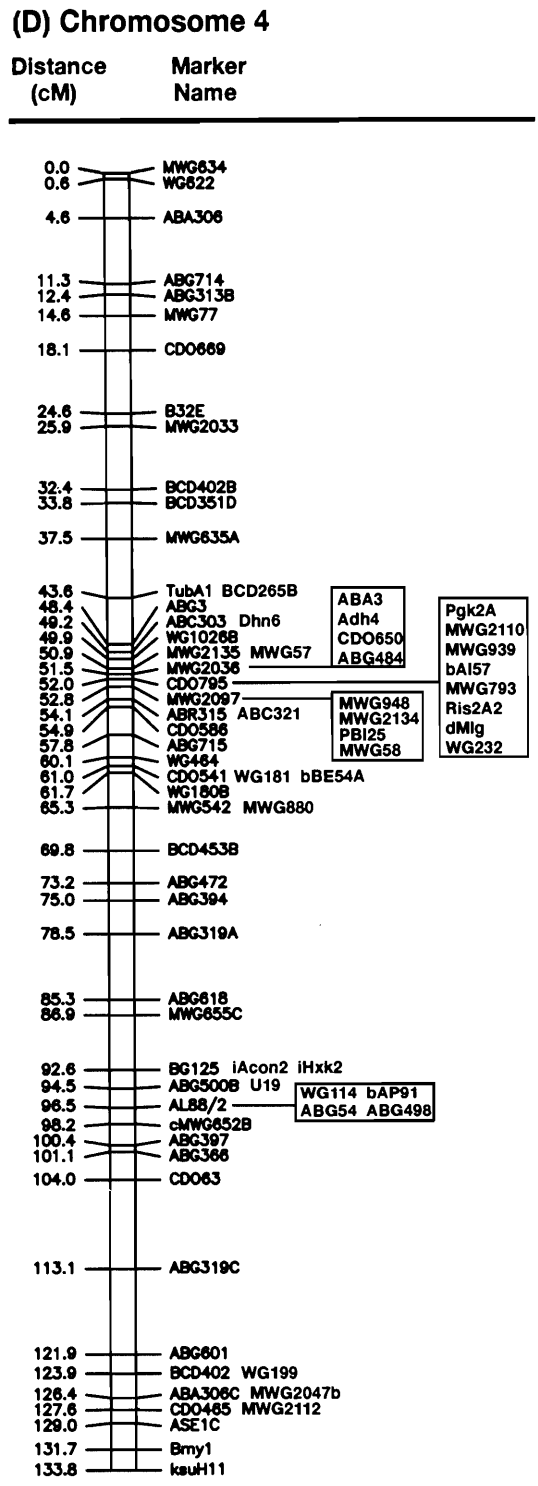


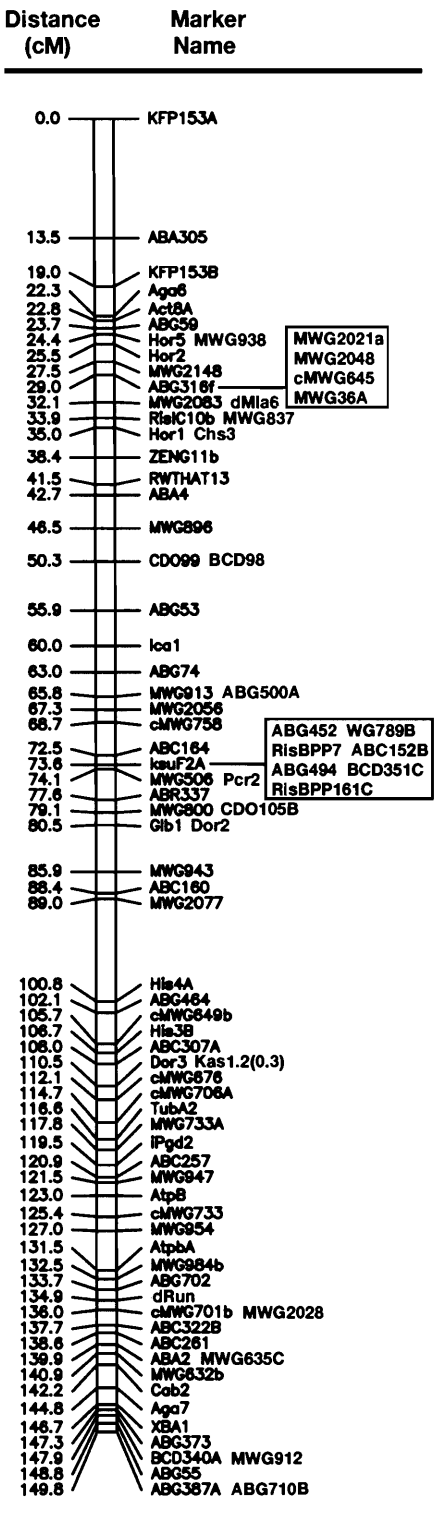
Fig. 1 (*continued*).



Chromosome 5

For this chromosome, a total of 20 markers that are non-unique to any of the four populations are available. The P/N population has only two non-unique markers, CDO99 and BCD98 (shared with S/M). Since these are tightly linked, both in P/N and S/M, there is effectively only a single "anchor point" to which to tie the P/N-specific markers. Since their orientation with respect to the "anchor" cannot be established unambiguously, the markers unique to P/N are not included in the composite map. The integrated map based on the data of the other three populations is shown in Fig. 1E. The genes *dMla6* (resistance to powery mildew) and *dRun* (resistance to *Ustilago nuda*) were located on chromosome 5. Markers were quite uniformly

Fig. 1 (*continued*).

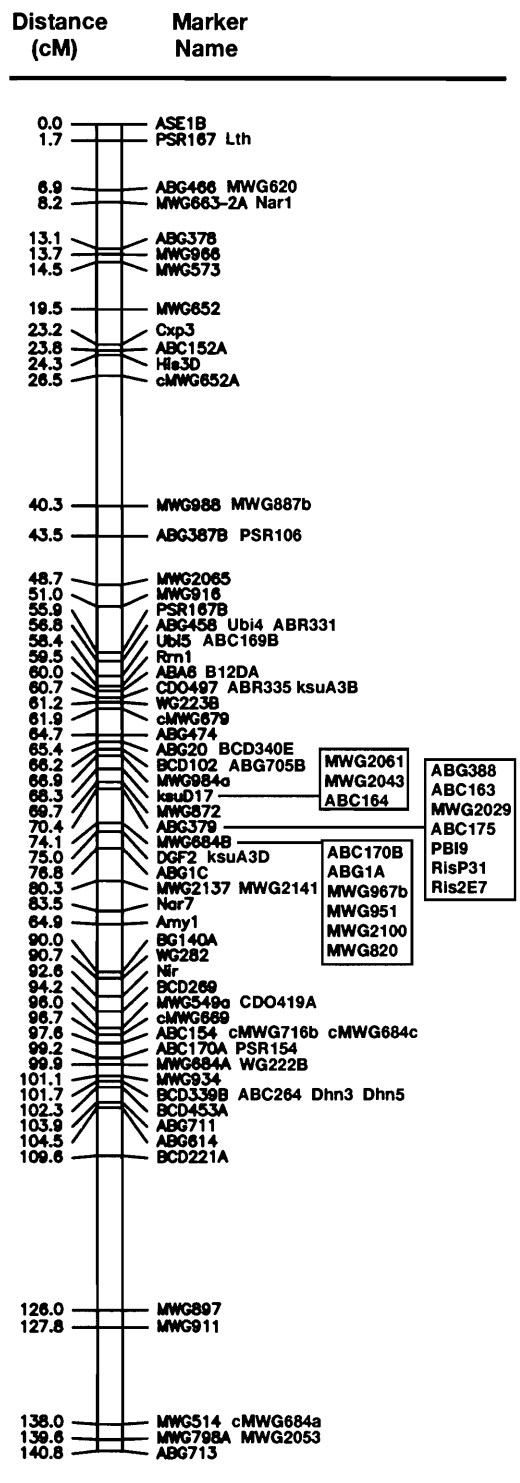


distributed over the integrated map; a small cluster of markers appears around the 74-cM region.

Chromosome 6

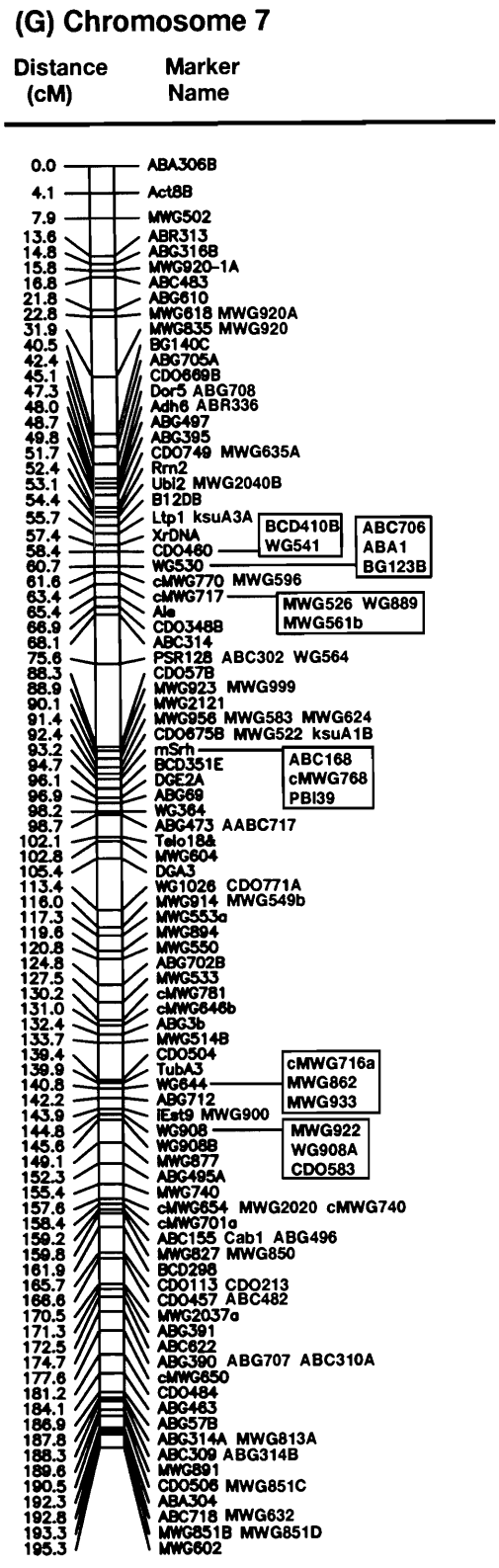
The individual map of chromosome 6 from the P/N population was very short, with only 36 map units and 9 markers.

Fig. 1 (*continued*).



Maps from the other three populations contained more markers and were longer. The composite map still has three large gaps, one of 14 cM at 26–40 cM, one of 16 cM at 110–126 cM, and one of 10 cM in the 128–138-cM region (Fig. 1F). Clustering of markers was found in the 68–75-cM region.

Fig. 1 (*concluded*).



Chromosome 7

Thirty markers were shared by two populations and five were common to three populations. The integrated map of chromosome 7 has 139 markers covering 195 map units, and is the longest map (Fig. 1G). The gene for short rachilla hairs, *mSrh*, resides on this chromosome. A single 10-cM gap (at 76–87 cM) remains on the composite map. There is no obvious clustering for this linkage group.

Comparison of maps

Comparison of the integrated map with the individual maps gives insight into the reliability of the integrated map. For illustrative convenience, only the common markers are shown on the maps in Figs. 2A–2G. In constructing an integrated map, invariably some regions of the component maps will shrink, while other regions will stretch. This is because a weighted average (over component maps) of recombination frequencies is used for calculating the integrated map. This applies to the non-unique markers that represent the reference positions of an integrated map. Markers that are unique to a particular population can, of course, only be positioned on the basis of the information for that single population. Therefore, the ordering of unique markers on a composite map is less reliable than the ordering of common markers, especially in regions where the component maps differ in length. Comparison of the integrated map with the individual maps reveals that the overall linear order of markers is in good agreement and that the integrated map is consistent with the component maps. No obvious reordering of markers was found. This is due to the relatively large number of non-unique markers.

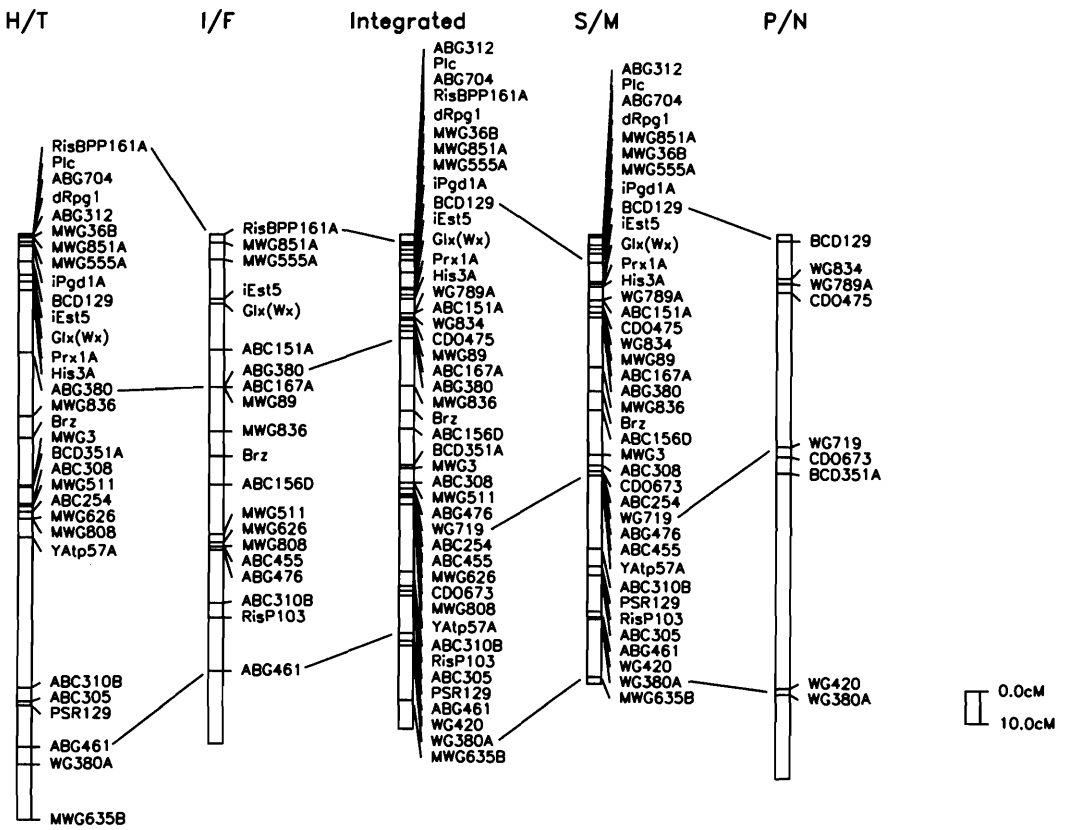
Reliability–accuracy of the integrated map

Theoretically, any calculated map is only as good as the data allow. Integrated maps strongly depend on the number of common markers shared by the individual maps. The seven integrated maps presented in Figs. 1A–1G were established on the basis of 197 common markers. With a total of 898 markers on the integrated map, 22% (197 of 898) of the markers were shared by at least two of the individual mapping populations. The proportions of common markers for each of the seven integrated maps, from chromosome 1 to chromosome 7, were 25, 21, 22, 15, 22, 24, and 25%, respectively.

The integration of maps from different populations is only feasible if common markers are available. The backbone of the integrated map consists of 197 markers that are common to at least two populations. The assumption was made that one probe would recognize the same locus in different populations. So, if a probe was used in different populations, it represented a common marker. Also, some markers were assigned to one locus if the core name was identical and the separate map position was nearly identical. As all markers were mapped with high likelihood (high LOD scores) and the χ^2 value was low (not shown), these assumptions were valid and the maps reliable.

It is also clear that the order of unique markers in regions of the genome containing a low density of common markers will be less accurate than in regions with a high density of common markers (Hauge et al. 1993). The distribution and density of the common markers (Figs. 2A–2G) indicates that common markers from the four populations were relatively uniformly distributed on the maps. The establishment of the integrated map without much difficulty may be partially due to the large number of common markers and the

Fig. 2. (A–G) Individual maps and their integrated map of seven barley chromosomes. Chromosomes are oriented with the short arm at the top. Only the common markers are presented in the figures. The recombination values were converted into map distance (cM) according to Kosambi (Kosambi 1944). The small box on the right represents a 10-cM distance. Lines between maps connect identical markers. "Gap" in some individual maps indicates very loose linkage. H/T, I/F, S/M, P/N, and Integrated refer to the populations of 'Harrington' × TR306, 'Igri' × 'Franka', 'Steptoe' × 'Morex', and 'Proctor' × 'Nudinka' and to the integrated map, respectively.



conservation of gene order in the germplasm represented by the four populations.

Comparative studies of RFLP maps between cereal species have shown an obvious conservation of genome structure (Chao et al. 1989; Devos et al. 1992, 1993; Devos and Gale 1993; Van Deynze et al. 1995; Wang et al. 1992). More extensive analysis of genome organization (Moore et al. 1995*a*) has revealed that the genomes of six major grass species can be aligned by dissecting the individual chromosomes into segments and rearranging these linkage blocks, suggesting that there was a single ancestral cereal chromosome (Moore et al. 1995*b*). In our study, comparison of four barley individual maps and their integrated maps indicates that not only the gene orders are identical within the species but also that the distances between genes are quite similar. Apparently, the recombination frequencies in barley are not dependent upon the populations used.

Distribution of markers and centromere region

The study of tomato high density molecular linkage maps (Tanksley et al. 1992) showed that in some regions higher marker density could be identified in all chromosomes and a comparison with the pachytene karyotype of each chromosome suggested that the regions of high marker density corresponded to centromeric areas and, in some instances, to telomeric regions. In *Arabidopsis* there was no indication of clustering of markers in known centromeric regions (Koornneef et al. 1983; Hauge et al. 1993). In maps of wheat, a high degree of clustering of markers around the centromere was a notable feature (Chao et al. 1989; Devos et al. 1992; Hart 1994). Our barley integrated map analysis indicated a clear nonrandom distribution of markers on the maps. Kleinhofs et al. (1993*a*) identified centromeric regions on each chromosome of barley. An obvious clustering of markers coincided with these chromosome regions (Fig. 3). This result strongly supports the idea of centromeric suppression of recombination (Tanksley et al. 1992).

Use of the integrated map

The conservative feature of the barley genome has provided us with a fairly reliable integrated map from individual

Fig. 2 (*continued*).

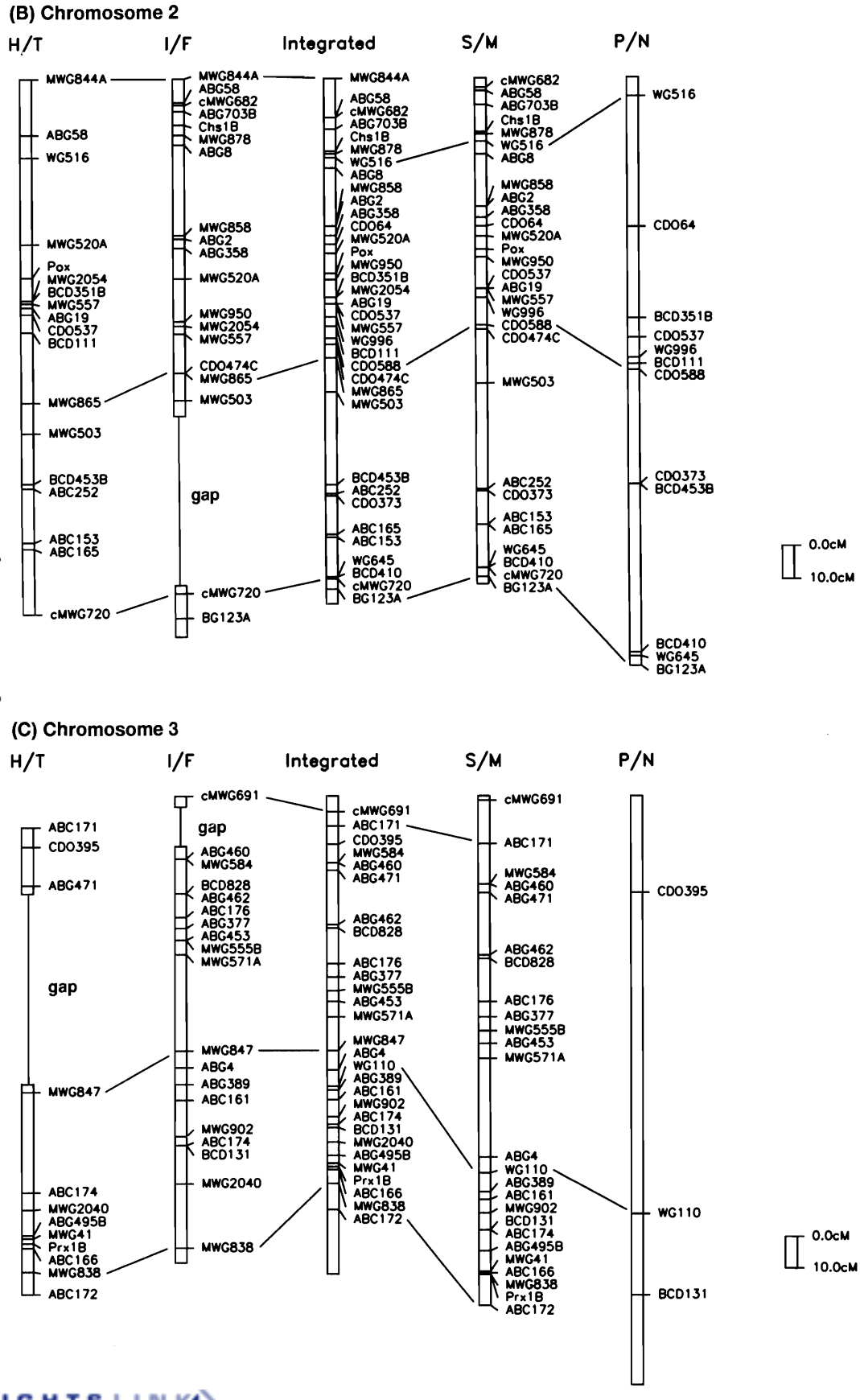
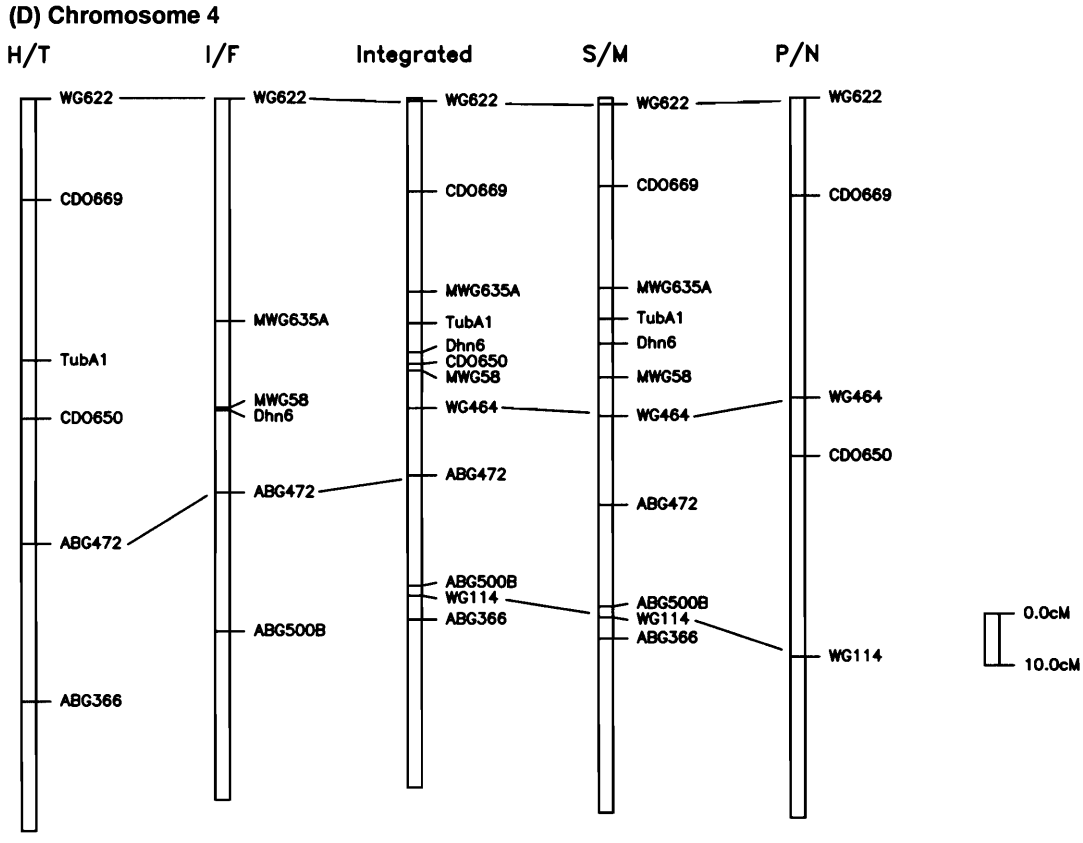


Fig. 2 (*continued*).



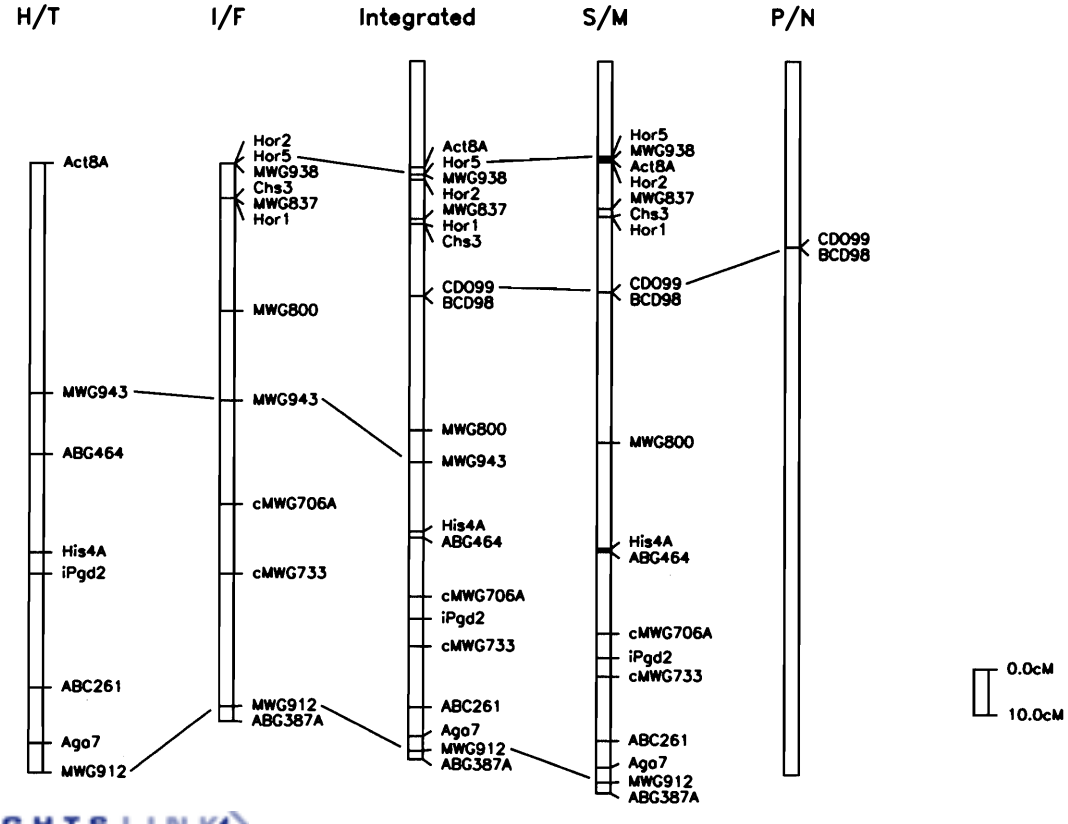


Fig. 2 (*concluded*).

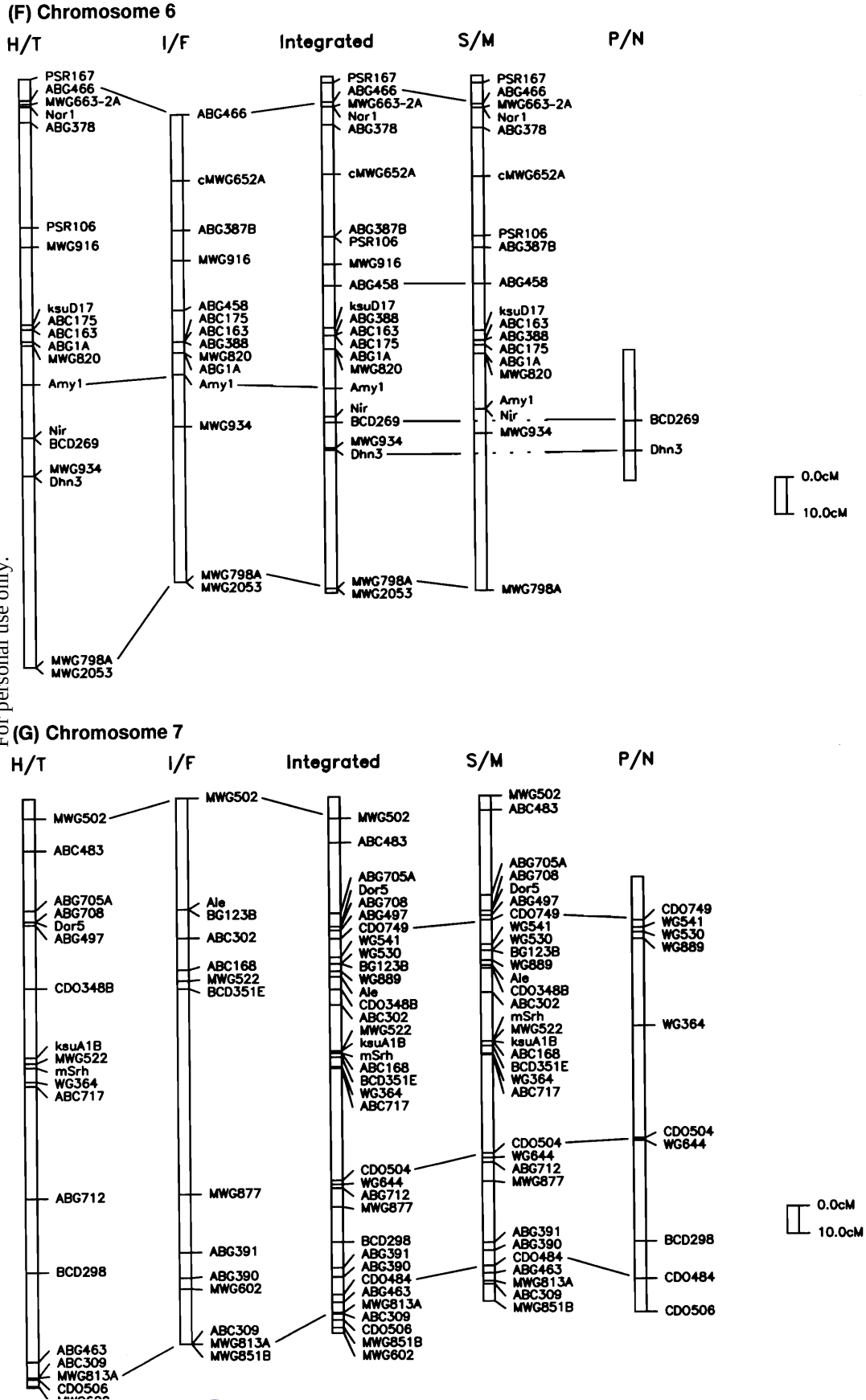
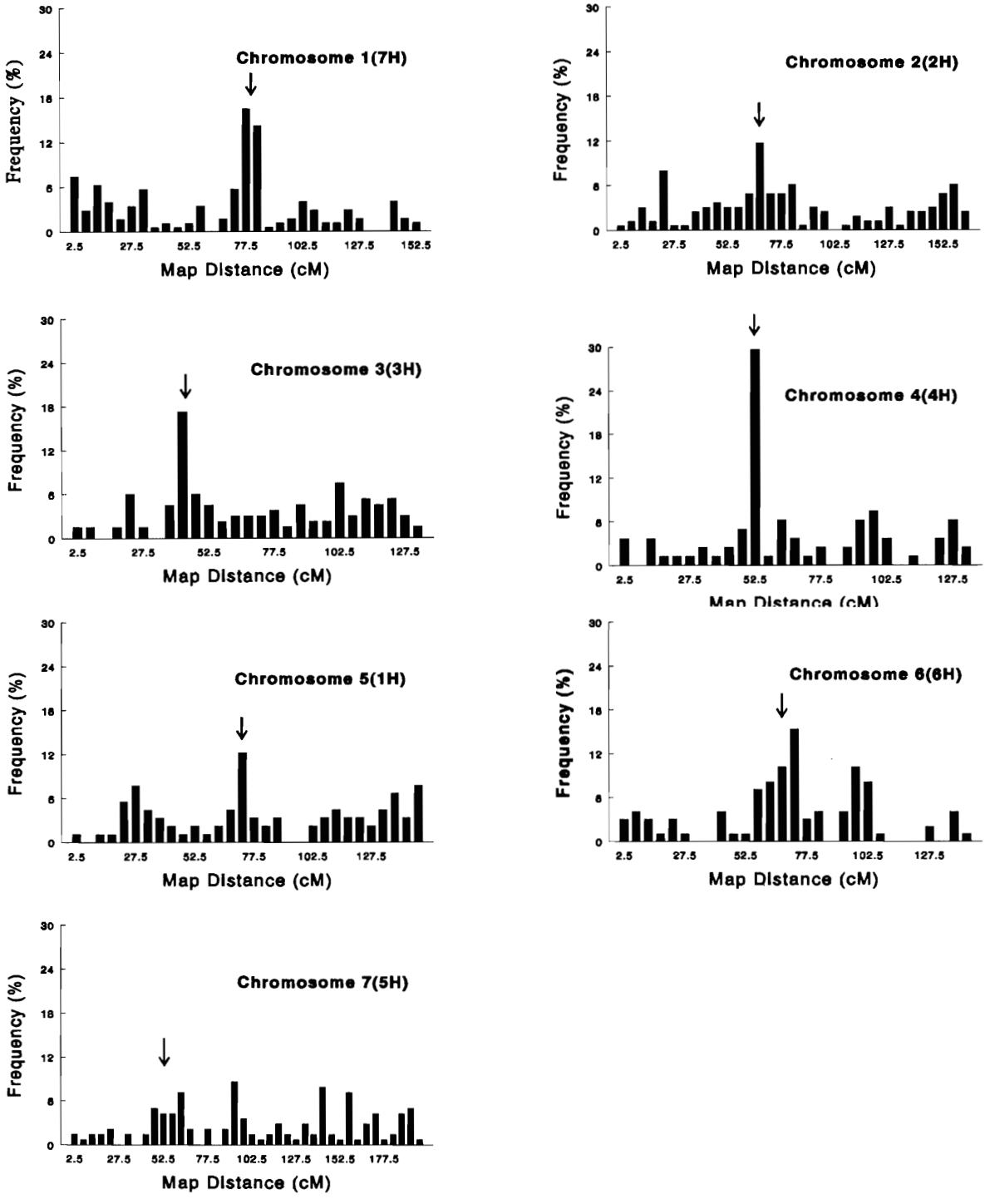


Fig. 3. Frequency distributions showing the density of markers (per 5-cM interval) over the chromosomes of barley. The arrows indicate possible centromeric regions.



maps that have been constructed in different genetic backgrounds. Compared with the individual maps, the density of markers on the integrated map is much higher and the number of gaps (>10 cM) is much lower. Our barley integrated map can serve as a high density map like the tomato high density map (Tanksley et al. 1992), which was based on data from only 67 plants of a single cross.

The integrated map contains about 900 markers and the various kinds and sources of molecular markers provide a good reference map for further research. New molecular markers and genes of economic importance from different genetic backgrounds can now easily be added to the integrated map by the selection of common markers from the integrated map. In our barley mapping project, the AFLP

(amplified fragment length polymorphism) markers will be used to map genes involved in partial resistance to leaf rust on the barley genome. From the integrated map, several RFLP markers, evenly distributed over the genome, have been selected as bridge markers, which will be used for chromosome assignment and the adding of AFLP markers to the integrated map.

In order to have a chance of detecting all quantitative trait loci (QTLs) affecting a character in a particular cross, it is necessary to have molecular markers evenly distributed throughout the genome (Tanksley et al. 1992). The integrated map presented in this paper allows selection of evenly spaced polymorphic markers for the detection and mapping of QTLs.

Some agronomic markers (*mn*, *mhex-v*, *mPub*, and *mSrh*) and disease resistance genes (*dMla6*, *dMlg*, *dym4*, *dRpg1*, and *dRun*) have been mapped on the integrated map. Compared with the individual maps, more molecular markers are now available around economically important genes on the integrated map, allowing marker-assisted selection in breeding programs. Also, the composite high density molecular marker map will be useful for more precise mapping of economically important genes in barley, as well as in other cereals, and thus, possibly provide a basis for map-based cloning of those genes from the small genome of rice (Bennetzen and Freeling 1993; Kilian et al. 1995).

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