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Regions of the Genome That Affect Grain and Malt Quality in a North American Two-Row Barley Cross

D. E. Mather,* N. A. Tinker, D. E. LaBerge, M. Edney, B. L. Jones, B. G. Rossnagel, W. G. Legge, K. G. Briggs, R. B. Irvine, D. E. Falk, and K. J. Kasha

ABSTRACT

Malting is an important end use of barley (*Hordeum vulgare* L.). The suitability of barley for malting depends on numerous quality characteristics, all of which are affected by genetic and environmental variation and many of which are inter-related. Here, our objective was to use genome mapping to improve knowledge about the genetic basis for variation and covariation in grain and malt quality characteristics. Kernel plumpness, kernel weight, grain protein, fine-grind extract, fine-coarse difference, soluble protein, extract β-glucan, extract viscosity, diastatic power, and α-amylase activity were measured on grain produced in six field environments, from parents and doubled-haploid progeny of a two-row barley cross, 'Harrington'/'TR306'. Quantitative trait loci and QTL × environment interactions were detected by means of 127 mapped markers and two methods of QTL analysis: simple interval mapping (SIM) and simplified composite interval mapping (sCIM). Each trait was affected by two to four primary QTL (those detected using both SIM and sCIM) and similar numbers of secondary QTL (those detected by only one of SIM or sCIM). Together, these QTL explained 21 to 67% of the phenotypic variance per trait. The numbers, effects, and relative positions of these QTL were in concordance with the quantitative trait distributions and with correlations among traits. All chromosomes, except chromosome 2, contained regions with at least one important QTL. Several genomic regions affected multiple traits. Most QTL interacted with environment, but many showed effects consistent enough that they might serve as targets for marker-assisted selection. There was little similarity in the QTL positions detected here and those detected previously for the same traits in crosses representing other germplasm groups.

MALTING is an important end use of barley. Barley grain suitable for malting normally commands a premium price. Malted barley (malt) is used predominantly for brewing beer, but some is used for distilling and in food products. The malting process involves steeping the grain in water, followed by germination in a controlled environment. The resulting "green malt" is then dried by kilning at gradually increasing temperatures. During steeping and germination, hydrolytic enzymes are synthesized and/or activated. Some of these enzymes are involved in the breakdown of endosperm cell walls. This breakdown, which opens up the cells to attack by starch- and protein-degrading enzymes, is often referred to as enzyme modification. The resulting malt provides a source of sugars, readily degradable starch, amino acids, and enzymes.

The suitability of barley grain for malting and the quality of the resulting malt are influenced by genetic and environmental factors. In purchasing barley, maltsters seek clean, plump, uniform and viable grain of specific malting barley cultivars. During the development of these cultivars, progeny are subjected to detailed evaluations of their grain and malt quality. The kernel size, plumpness, and proportion of protein are evaluated on the grain. Grain samples are micromalted to produce malt. Solutions of ground malt are filtered to produce malt extracts. These extracts are analyzed to determine the amount of soluble material (malt extract), the concentrations of soluble protein and β-glucan, the viscosity, and the activity of starch degrading enzymes.

The roles of grain and malt characters in malting and brewing have been discussed by Burger and LaBerge (1985) and Bamforth and Barclay (1993). Uniform kernels are desirable because they germinate at a uniform rate. Plump kernels may malt more slowly than thin kernels, but are desirable because they usually produce more malt extract. Excessive grain protein is undesirable because it is associated with lower malt extract levels and because it can cause problems with beer stability and viscosity. However, some grain protein is needed to provide amino acids for yeast nutrition during brewing, and also to provide starch degrading enzymes such as α- and β-amylases. High enzyme levels are needed if adjunct sources of starch are used during brewing. Malt extract is a key quality indicator because it reflects the amount of beer that can be produced from a given quantity of malt. Malt extract is normally measured on two malt samples, one ground to a finer consistency than the other. A small difference between the two measures is one indication that the endosperm has been well modified during malting. Other indicators of thorough endosperm modification are high levels of soluble protein and low levels of extract β-glucan. High levels of β-glucan can increase the viscosity of extract, impeding its filtration.

Specific genes that may affect malt quality traits, including two α-amylase genes and two β-amylase genes, have been cloned and mapped in barley (Kleinhofs et al., 1993). However, grain and malt quality characters are generally considered to be influenced by many genetic loci, and highly dependent on environmental factors (Sparrow, 1971; Briggs, 1978). The recent development of detailed molecular marker maps in barley (Heun et

D.E. Mather and N.A. Tinker, Dep. of Plant Science, McGill Univ., 21111 Lakeshore, Ste-Anne-de-Bellevue, QC H9X 3V9; D.E. LaBerge and M. Edney, Grain Res. Lab., 1404-303 Main St., Winnipeg, MB R3C 3G8; B.L. Jones, USDA-ARS, Madison, WI 53705; B.G. Rossnagel, Crop Development Centre, Univ. of Saskatchewan, Saskatoon, SK S7N 5A8; W.G. Legge, Agriculture and Agri-Food Canada, Brandon, MB R7A 5Y3; K.G. Briggs, Dep. of Agric., Food and Nutritional Sci., Univ. of Alberta, Edmonton, AB T6G 2P5; R.B. Irvine, Saskatchewan Irrigation Development Centre, Outlook, SK SOL 2NO; D.E. Falk and K.J. Kasha, Dep. of Crop Science, Univ. of Guelph, Guelph, ON N1G 2W1. Received 16 Feb. 1996. *Corresponding author (mather@agradm.lan.mcgill.ca).

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Abbreviations: cM, centimorgans; QTL, quantitative trait locus or loci; QTL×E, QTL × environment; DH, doubled haploid; RFLP, restriction fragment length polymorphism; SIM, simple interval mapping; sCIM, simplified composite interval mapping.

al., 1991; Graner et al., 1991; Kleinhofs et al., 1993; Kasha et al., 1995) has provided opportunities to locate specific genomic regions that affect quantitative traits. Such regions are commonly referred to as quantitative trait loci (QTL).

Application of QTL analysis to the study of grain and malt quality traits in barley will improve our understanding of the genetic factors that influence these complex traits. In particular, the ability to detect chromosome regions that affect two or more traits will help us understand the genetic basis for correlations among traits. Information about QTL may also be used in breeding programs to accumulate alleles with desirable effects through marker-assisted selection. This strategy may be of particular value for the improvement of grain and malt quality. For many of these quality characters, measurement is expensive and cannot be performed until late in a breeding program when sufficiently large and uniform grain samples are available. To assure fixation of favorable grain and malt characters, established malting cultivars are often used as parents. This practice may have restricted the amount of genetic diversity among malting barley cultivars (Martin et al., 1991; Saghai Maroof et al., 1994). Marker-assisted selection could facilitate development of malting barley cultivars from more diverse germplasm.

Mapping of QTL that affect grain and malt quality has been performed in a North American six-row barley cross (Hayes et al., 1993; Han et al., 1995) and in a European two-row barley cross (Chalmers et al., 1993; Thomas et al., 1996). Mapping of QTL in other genetic backgrounds will provide additional opportunities for marker-assisted selection, and may reveal loci that consistently affect the quality of barley grain and malt. Here, we report on QTL mapping of grain and malt quality in the cross 'Harrington'/'TR306'. Harrington (Harvey and Rossnagel, 1984) is the dominant two-row malting barley cultivar grown in North America. TR306 is a breeding line developed by the same institution (the University of Saskatchewan), but not selected for grain or malt quality. Recently, Tinker et al. (1996) reported on QTL that affect agronomic performance in the same cross.

MATERIALS AND METHODS

The Population and the Marker Map

A population of 150 F_1-derived doubled-haploid (DH) progeny from the cross Harrington/TR306 was previously used to map over 200 segregating RFLP and other marker loci (Kasha et al., 1995). The DH progeny were produced by the *Hordeum bulbosum* L. method (Kasha and Kao, 1970), resulting in minimal segregation distortion (Tinker et al., 1996). For several of the DH lines, there were suspected errors in data acquisition, so subsets of DH lines (146 for marker mapping; 145 for QTL analysis) were used in subsequent analyses. Analysis of QTL was performed using a base map that consisted of 127 markers (described by Tinker et al., 1996). Of these, 51 "background" markers were chosen for use as cofactors in composite interval mapping. The mean interval length between background markers was 28.8 centimorgans (cM).

Field Experiments

The mapping population was grown in 30 environments (17 locations in 1992 and/or 1993). Field plots were grown using local variety-testing methods. One randomized complete block of parents and DH progeny was grown in 1992, and two such blocks were grown in 1993. It was not possible to conduct detailed grain and malt quality analyses on samples from all 30 environments, so six environments (Table 1) were chosen based on kernel appearance, protein levels, and germination rates of samples. These are characteristics which maltsters consider as indicators of malting quality when they purchase barley. Our objective was to choose environments where Harrington had apparently had the opportunity to express its malting quality potential. Harvested grain from Harrington, TR306, and each of 145 DH progeny was bulked to form one sample per entry for each of the six environments.

Grain and Malt Quality Analyses

Grain samples from five environments (SK92b, SK93c, AB92c, MB92, QC93; see Table 1), were analyzed at the Grain Research Laboratory, Winnipeg, MB, by the standard procedures of that laboratory. Samples from the 1992 trial at Outlook, SK (SK92a; Table 1) were analyzed at the USDA-ARS Cereal Crops Research Unit, Madison, WI, by the standard procedures of that laboratory. Standard procedures refer to those of the American Society of Brewing Chemists (ASBC, 1992) unless otherwise stated.

Grain Quality

Grain samples were cleaned and sized on a Carter Dockage Tester (Simon-Day Limited, Winnipeg, MB). Material retained on a 2.38-mm screen was classified as plump. Subsequent analyses were performed on plump grain (in the Canadian laboratory) or on material retained on a 1.98-mm screen (in the U.S. laboratory). Grain moisture content was determined using a Tag Heppenstall (Brooklyn, NY) moisture meter (Canadian laboratory) or by drying a sample for 3 h at 106°C (U.S. laboratory). The number of kernels in a 20-g sample (dry basis) was counted, and average kernel weight calculated. Grain N concentration was determined using the Kjeldahl method (in the Canadian laboratory) or the Dumas method (in the U.S. laboratory; see Buckee, 1994) and multiplied by 6.25 to estimate grain protein concentration.

Micromalting

In the Canadian laboratory, samples were steeped in water at 13°C for 48 h, with four 4-h air rests, then germinated for 96 h at 15°C and 100% relative humidity. Procedures in the

Table 1. Field environments where grain was produced for grain and malt quality analysis of Harrington, TR306, and 145 doubled haploids from the Harrington/TR306 cross.

Environment†	Location	Latitude	Longitude
SK92a	Outlook, SK	51°30′ N	107°03′ W
SK92b	Saskatoon, SK	52°10′ N	106°40′ W
SK93c	Floral, SK	52°04′ N	106°28′ W
AB92c	Edmonton, AB	53°34′ N	113°25′ W
MB92	Brandon, MB	49°50′ N	99°57′ W
QC93	Ste-Anne-de-Bellevue, QC	45°25′ N	73°56′ W

† For consistency with Tinker et al. (1996), environments are coded as follows: uppercase letters identify the Canadian province, numerals identify the year, and lowercase suffixes distinguish multiple locations in the same province.

U.S. laboratory were similar except that steeping was for 36 h with two 1-h air rests, and germination was at 16°C. In the Canadian laboratory, germinated samples were kilned with the following regime: 6 h from 30°C to 48°C; 16 h at 48°C; 8 h to 66°C; 10 h at 66°C; 2 h to 85°C; 6 h at 85°C; 2 h to 30°C. In the US laboratory, samples were kilned as follows: 10 h at 49°C; 0.5 h to 54°C; 4 h at 54°C; 0.5 h to 60°C; 3 h at 60°C; 0.5 h to 68°C; 2 h at 68°C; 0.5 h to 85°C; 3 h at 85°C; 0.25 h to 25°C.

Grinding of Malt Samples

Fine-grind and coarse-grind subsamples were prepared from each malt sample. The malt was ground with a Buhler-Miag (Braunschweig, Germany) disc mill in the Canadian laboratory and a Miag cone-and-ring mill in the U.S. laboratory. In both laboratories, the fine-grind setting allowed 90% of a standard malt to pass through a 0.06-mm screen while the coarse-grind setting allowed 25% of the malt to pass through the same screen. Moisture contents were determined by drying ground samples for 3 h at 106°C.

Malt Extracts

Standard ASBC (1992) mashing procedures were used to prepare fine-grind extracts in both laboratories, and to prepare coarse-grind extracts in the U.S. laboratory. Coarse-grind extracts in the Canadian laboratory were prepared using a 70°C hot water extract (Meredith, 1959). Malt extracts were filtered and specific gravities determined with Mettler-Parr (Parr, Graz, Austria) density meters. The amount of soluble material in the filtrate was expressed as a percentage of the dry weight of the ground sample. The difference between fine- and coarse-grind extracts was calculated.

Soluble Protein

The N concentration of an aliquot of fine-grind extract was determined using either the Kjeldahl (in the Canadian laboratory) or Dumas (in the U.S. laboratory) methods and multiplied by 6.25 to estimate protein concentration. The soluble protein concentration was expressed relative to the weight of the dry malt from which it was extracted.

Extract β-Glucan and Viscosity

The concentration of soluble, high molecular weight β-glucan in the fine-grind extract was determined by flow injection analysis, measuring the complex formed on addition of Calcofluor (Polysciences Inc., Warrington, PA) (Jorgensen 1988). In the Canadian laboratory, extract viscosity was measured by flow injection analysis combined with a Brookfield (Stoughton, MA) viscometer.

Diastatic Power and α-Amylase Activity

Diastatic power was determined by incubating a salt water extract prepared from fine-grind malt with a starch substrate solution. The amount of reducing sugars released was measured using a ferricyanide assay. In the U.S. laboratory, a sample of the salt water extract was heated to inactivate β-amylase, then the above procedure was used to assess α-amylase activity (Banasik 1971). In the Canadian laboratory, α-amylase activity was measured by using β-limit dextrin as substrate and iodine as an indicator (Briggs, 1961).

QTL Analysis

Analysis of QTL, performed with the software package MQTL (Tinker and Mather, 1995b) consisted of four steps: (i) performing interval mapping to find evidence of QTL, (ii) estimating thresholds for inferring QTL presence, (iii) inferring the presence of QTL and estimating their positions, and (iv) estimating the additive effects at putative QTL.

Interval Mapping

Genome-wide QTL searches were performed by both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) (Tinker and Mather 1995a), each with a test for QTL main effects and a test for QTL×E interaction. Thus, four scans (plots of the test statistic against map position) were produced for each trait. All four scans were display values of a test statistic for linear models described by Haley and Knott (1992). The two interval mapping methods differ in that SIM uses genotype information only for markers in the region being tested, whereas sCIM also includes genotype information for background markers elsewhere in the genome. Thus, sCIM adjusts for the possible effects of QTL elsewhere in the genome. When multiple QTL are segregating, sCIM can improve QTL detection and provide more accurate estimates of QTL position (Tinker and Mather 1995a). One limitation of sCIM is that it is not clear how to control the Type I error rate when sCIM is applied to multiple-environment data (Tinker and Mather 1995a). Here, no significance thresholds were established for sCIM. For SIM, significance thresholds for the test statistic were established by permutation (Churchill and Doerge, 1994), to maintain the genome-wise Type I error rate below 5%, as described by Tinker et al. (1996).

Making Inferences and Estimating QTL Positions

Two levels of QTL inference were made. Primary QTL were declared at positions where both SIM and sCIM gave evidence for the presence of QTL, i.e. where SIM peaks were significant for QTL main effects and/or QTL×E interaction and where sCIM peaks were also strong. Secondary QTL were declared where either SIM or sCIM, but not both, gave evidence for a QTL. The peaks of the sCIM scans were used to estimate the positions of both primary and secondary QTL. When evidence for a QTL main effect and a QTL×E interaction were found near the same position, a single QTL was inferred at the position of the effect that seemed strongest.

Estimating Allelic Effects

Main effects and QTL×E interactions were estimated in multi-locus linear models. Each estimated main effect corresponded to the average difference between homozygous classes for a given QTL. Reduction in variance (R^2) relative to a model that included only the environmental main effects, was estimated for models with four levels of complexity: (i) primary QTL main effects; (ii) primary QTL main effects and primary QTL×E interactions, (iii) main effects and QTL×E interactions for both primary and secondary QTL, and (iv) effects for all background markers estimated separately by environment (but with no terms for specific QTL). In the absence of epistasis, a model containing background markers representing all regions of the genome should explain most of the genetic variance. Thus the percentage of variance explained by such a model can be considered as an estimate of heritability. For agronomic traits in Harrington/TR306, Tinker et al. (1996) found that heritabilities estimated in this manner were similar to those obtained with data from replicated field experiments.

Table 2. Estimated means and phenotypic standard deviations (in parentheses) for grain and malt quality traits measured on parents and 145 doubled haploids of the Harrington/TR306 cross grown in six field environments.

Trait	Environment†					
	SK92a	SK92b	SK93c	AB92c	MB92	QC93
Kernel plumpness (g kg^{-1})‡	940 (20)	790 (40)	910 (30)	940 (20)	750 (80)	740 (110)
Kernel weight (mg)§	43 (2.0)	45 (1.8)	45 (2.0)	47 (2.2)	43 (2.0)	41 (1.8)
Grain protein (g kg^{-1})‡,§	150 (7)	140 (5)	110 (8)	160 (8)	150 (6)	120 (6)
Fine-grind extract (g kg^{-1})‡,¶	780 (8)	780 (9)	800 (8)	760 (11)	770 (8)	800 (8)
Fine-coarse difference (g kg^{-1})‡,¶	21 (7)	88 (15)	22 (8)	54 (11)	86 (17)	38 (10)
Soluble protein (g kg^{-1})‡,¶	55 (6)	41 (4)	43 (4)	56 (6)	52 (5)	54 (4)
Extract β-glucan (mg 1^{-1})#	472 (175)	709 (172)	172 (102)	402 (138)	524 (157)	287 (107)
Extract viscosity (cps)§,††	¶¶	1.7 (0.06)	1.5 (0.05)	1.6 (0.07)	1.6 (0.05)	1.5 (0.05)
Diastatic power (°L)§,‡‡	120 (14)	102 (13)	86 (10)	142 (14)	114 (16)	99 (11)
α-amylase activity (D.U.)§,§§	41 (5)	43 (8)	40 (8)	51 (9)	47 (9)	44 (8)

† Measurements on material grown in SK92a were made at the USDA-ARS Cereal Crops Research Unit, Madison, WI. Measurements on material grown in the other five environments were made at the Grain Research Laboratory, Winnipeg, MB.
‡ Expressed as % by the malting and brewing industries.
§ On a grain dry matter basis.
¶ On a malt dry matter basis.
# On a malt extract basis, expressed as ppm by the malting and brewing industries.
†† Centipoise: international viscosity units used by the malting and brewing industry.
‡‡ Degrees Lintner: a malt has 100°L if 0.1 mL of a 5% infusion, acting on starch substrate under fixed conditions, produces sufficient reducing sugars to reduce completely 5 mL of Fehling's solution.
§§ One dextrinizing unit is the quantity and activity of α-amylase that will dextrinize soluble starch at the rate of 1 g/h at 20°C.
¶¶ Not measured for this environment.

RESULTS

Quantitative Trait Distributions and Correlations

There were substantial differences among environments in mean and standard deviation for some traits (Table 2). Kernel plumpness was lower and more variable in SK92b, MB92, and QC93 than in other environments. Fine-coarse difference was greater and more variable in SK92b and MB92 than in other environments. Extract β-glucan and diastatic power were greater and more variable in 1992 than in 1993. Although they were based on single observations, values for the parents within environments (not shown) generally followed expected trends (i.e., Harrington generally showed better malt quality than TR306, while TR306 had heavier, plumper kernels).

Averaged across environments, levels of kernel plumpness, kernel weight, grain protein, and extract viscosity were at least two standard deviation units higher in TR306 than in Harrington (Table 3). Conversely, levels of fine-grind extract, diastatic power, and α-amylase activity were at least two standard deviation units higher in Harrington than in TR306. Levels of fine-coarse difference, soluble protein, and extract β-glucan differed by less than one standard deviation unit between Harrington and TR306. Based on extreme values of DH progeny, there seemed to be transgressive segregation in both directions for all traits except grain protein, fine-grind extract, extract viscosity, and α-amylase activity. There were no DH progeny with lower extract viscosity or grain protein levels than Harrington. The highest fine-grind extract and α-amylase levels among DH progeny were similar to those of Harrington.

Grain protein was positively correlated with kernel plumpness, and negatively correlated with fine-grind extract (Table 4). Fine-grind extract was strongly and positively correlated with α-amylase activity. Strong positive correlations were also observed among extract β-glucan, extract viscosity, and fine-coarse difference, and among diastatic power, α-amylase activity, and soluble protein.

Table 3. Quantitative trait distributions based on means of parents and 145 doubled haploids from the Harrington/TR306 cross.

Trait	Parents		Doubled-haploid progeny			
	Harrington	TR306	$\hat{\mu}$†	$\hat{\sigma}$‡	Minimum‡	Maximum‡
Kernel plumpness (g kg^{-1})	779	862	846	35	738	910
Kernel weight (mg)	42	46	44	2	40	48
Grain protein (g kg^{-1})	123	141	136	4	127	151
Fine-grind extract (g kg^{-1})	793	772	781	6	767	794
Fine-coarse difference (g kg^{-1})	46	50	52	7	33	70
Soluble protein (g kg^{-1})	50	48	50	4	42	61
Extract β-glucan (mg L^{-1})	333	366	428	87	210	633
Extract viscosity (cps)	1.5	1.6	1.6	0.03	1.5	1.7
Diastatic power (°L)	119	98	111	10	90	144
α-amylase activity (D.U.)	57	36	44	7	32	59

† Unadjusted mean of all observations in all environments.
‡ Expressed as deviations from μ. Observations within each environment were standardized to a mean of zero prior to computing the line means.

Table 4. Pearson phenotypic correlation coefficients among grain and malt quality traits based on means of 145 doubled haploids from the Harrington/TR306 cross.

	Kernel plumpness	Kernel weight	Grain protein	Fine-grind extract	Fine-coarse difference	Soluble protein	Extract β-glucan	Extract viscosity	Diastatic power
Kernel weight	0.35								
Grain protein	0.60	0.20							
Fine-grind extract	−0.31	ns†	−0.53						
Fine-coarse difference	ns	ns	ns	−0.18					
Soluble protein	0.22	ns	0.32	0.34	−0.35				
Extract β-glucan	0.19	0.22	ns	−0.36	0.74	−0.41			
Extract viscosity	0.22	0.25	ns	−0.30	0.51	−0.37	0.76		
Diastatic power	0.17	0.16	ns	0.26	−0.34	0.64	−0.36	−0.18	
α-amylase activity	−0.23	ns	−0.33	0.52	ns	0.40	ns	ns	0.53

† ns = Not significantly different from zero at the 0.05 probability level.

Quantitative Trait Loci

The number of primary QTL (those detected by both SIM and sCIM) ranged from two to four per trait, with a total of 35 primary QTL (Fig. 1 and 2). In addition, there were 36 secondary QTL detected by only one of the two interval mapping methods. For many primary QTL, effects were reasonably constant across environments. For some primary QTL, effects were present in some environments and absent in others. For example, QTL effects on kernel plumpness were strong in environments MB92 and QC93 but generally absent in other environments. This difference is reflected in the phenotypic standard deviations for kernel plumpness: 80 g kg^{-1} in MB92 and 110 g kg^{-1} in QC93, compared to 20 to 40 g kg^{-1} in the other environments. Substantial amounts of QTL×E interaction caused peaks on the QTL×E scans (Fig. 1 and 2). In some cases, when QTL effects in different environments were of opposite direction (i.e. crossover interactions were present), peaks were present only on the QTL×E scans. Approximately 10 primary QTL showed this pattern, as did many secondary QTL.

Both parents contained primary QTL alleles that caused increased levels of fine-grind extract, fine-coarse difference, soluble protein, and extract β-glucan (Fig. 2). Alleles from TR306 caused increased levels of kernel plumpness, kernel weight, and grain protein at all primary QTL (Fig. 1), while alleles from Harrington were the source of increased diastatic power and α-amylase activity at all primary QTL (Fig. 2) with significant main effects. Although TR306 was the only source of decreased extract viscosity at primary QTL, Harrington carried alleles at two secondary QTL that caused reduced extract viscosity (Fig. 2).

Because of the large number of QTL detected, we have chosen to focus on primary QTL, and on regions of the genome that affected multiple traits. These regions are listed by chromosome, as shown in Fig. 1 and 2. All chromosomes, except chromosome 2 (2H, Shepherd and Islam, 1992), contained regions with at least one important QTL.

Chromosome 1 (7H)

Harrington alleles near the centromere (cM position 98) were associated with reduced levels of kernel weight, kernel plumpness, and grain protein. This region also contained QTL that affected soluble protein, extract viscosity, and α-amylase activity, but these effects were not consistent across environments. A second region (cM position 160) affected kernel weight, diastatic power, and fine-coarse difference.

Chromosome 3 (3H)

One region (near cM position 25) affected fine-coarse difference, extract β-glucan, and extract viscosity. In all cases, Harrington alleles were associated with higher levels of these traits.

Chromosome 4 (4H)

On this chromosome, Harrington alleles caused decreased kernel plumpness and grain protein levels. Composite interval mapping indicated that the plumpness effect may be due to two separate QTL, with Harrington alleles giving reduced plumpness at both.

Chromosome 5 (1H)

Two notable QTL regions were present: one near cM position 18, where Harrington alleles caused increased diastatic power, and a second near cM position 111, where Harrington alleles caused reduced fine-grind extract levels.

Chromosome 6 (6H)

A major QTL, at which the Harrington allele caused a large and consistent increase in α-amylase activity, was located near cM position 109. The RFLP marker nearest to this QTL was detected with an α-amylase 1 probe. Harrington alleles in the same region caused increased diastatic power and extract viscosity levels. Harrington alleles in another region of chromosome 6 (near cM position 15) increased the levels of fine-coarse difference, extract β-glucan, and extract viscosity.

Chromosome 7 (5H)

Harrington alleles near cM position 0 were associated with increased fine-grind extract, and decreased levels of kernel plumpness, kernel weight, and grain protein. At the opposite end of this chromosome (cM position 236), QTL were detected for all traits except kernel plumpness, kernel weight, and grain protein. At this position, Harrington alleles were always associated with effects conferring better grain or malt quality.

Averaged across environments, main effects of primary QTL were responsible for proportions of pheno-

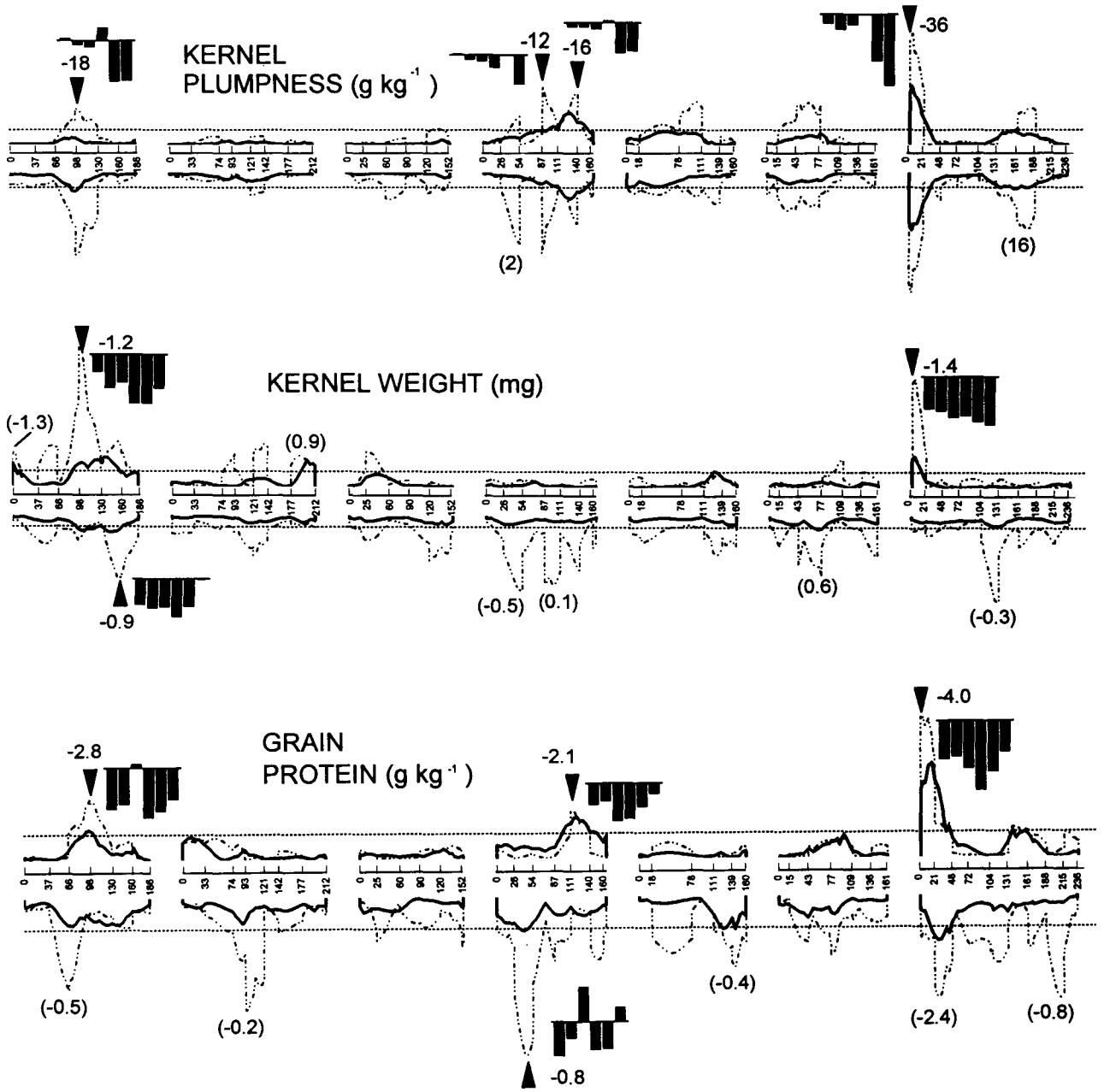

Fig. 1. Scans of a test statistic for simple interval mapping (SIM, solid lines) and simplified composite interval mapping (sCIM, broken lines) for QTL main effects (above axes) and QTL × environment interactions (below axes). Scans are shown for three grain quality traits as indicated. Barley chromosomes 1 to 7 are shown left to right, each oriented with the plus arm on the left. Horizontal scales show approximate cM positions of background markers. Horizontal dashed lines show thresholds for testing SIM, estimated from 5000 permutations of the data to maintain the experiment-wise type-I error rate below 5%. There was no threshold for testing the sCIM scans. Positions of primary QTL (QTL detected by both SIM and sCIM) are shown by darkened triangles located at the peaks of the sCIM scans. Estimated QTL main effects (i.e. the average effect of substituting two Harrington alleles for two TR306 alleles) are shown for primary QTL (numbers beside the darkened triangles) and for secondary QTL (in parentheses). Bar charts beside each primary QTL depict the relative magnitudes of estimated QTL effects for each environment (shown in the order SK92a, SK92b, SK93c, AB92c, MB92, QC93).

typic variance (Table 5, Model 1) ranging from 3% (for extract viscosity) to 61% (for α-amylase activity). Interactions of primary QTL with environments were responsible for an additional proportion of phenotypic variance ranging from 3 to 4% for kernel weight and soluble protein, to 20% for kernel plumpness (Table 5, Model 2 vs. Model 1). Models that also included secondary QTL most effectively explained additional variance for kernel weight (Table 5, Model 3). For all traits, models that included all 51 background markers explained between 47 and 75% of the phenotypic variance (Table 5, Model 4).

DISCUSSION

All grain and malt quality traits showed quantitative variability due to the presence of multiple QTL and

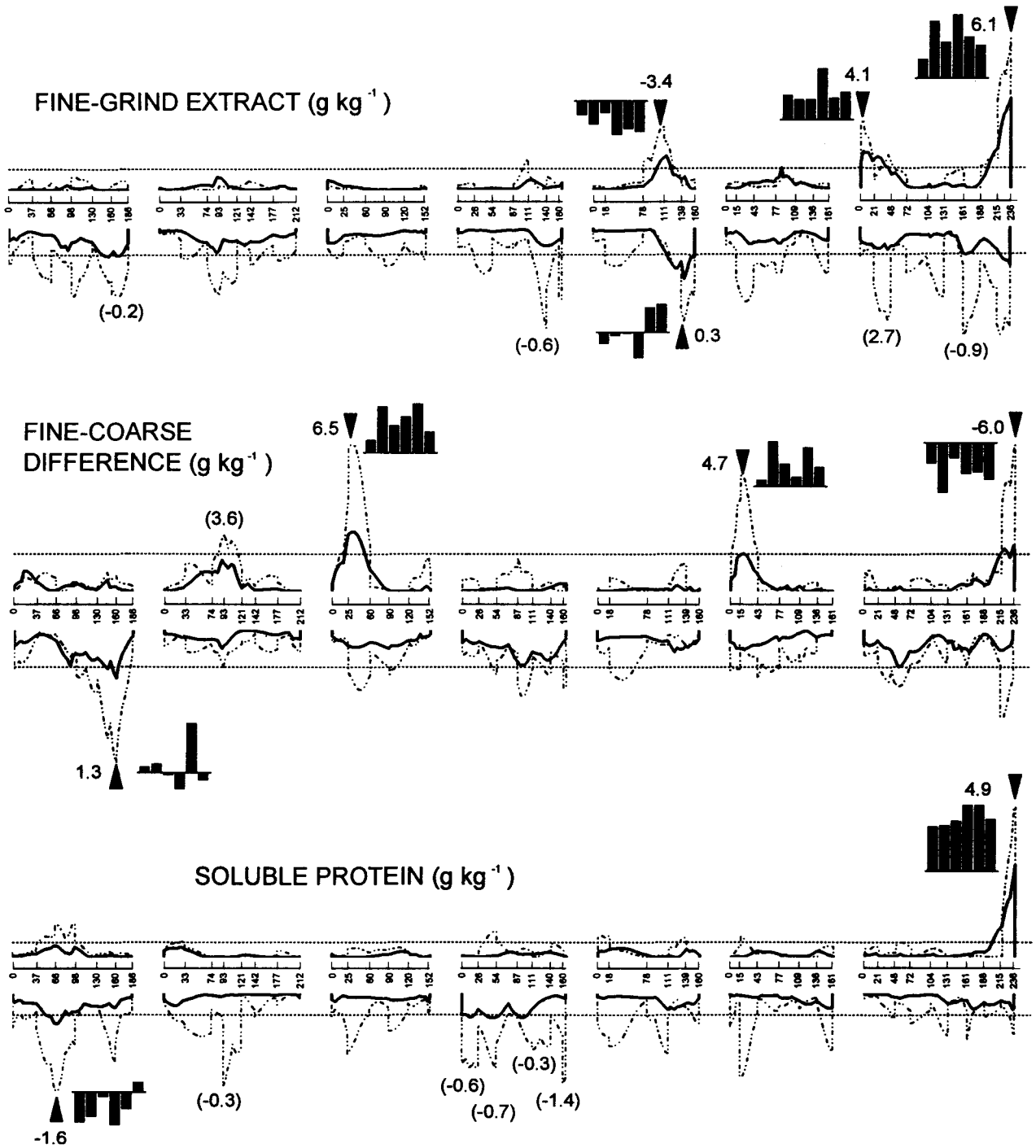

Fig. 2. Scans of a test statistic for simple interval mapping (SIM, solid lines) and simplified composite interval mapping (sCIM, broken lines) for QTL main effects (above axes) and QTL by environment interactions (below axes). Scans are shown for seven malt quality traits as indicated. Barley chromosomes 1 to 7 are shown left to right, each oriented with the plus arm on the left. Horizontal scales show approximate cM positions of background markers. Horizontal dashed lines show thresholds for testing SIM, estimated from 5000 permutations of the data to maintain the experiment-wise Type I error rate below 5%. There was no threshold for testing the sCIM scans. Positions of primary QTL (those detected by both SIM and sCIM) are shown by darkened triangles located at the peaks of the sCIM scans. Estimated QTL main effects (i.e. the average effect of substituting two Harrington alleles for two TR306 alleles) are shown for primary QTL (numbers beside the darkened triangles) and for secondary QTL (in parentheses). Bar charts beside each primary QTL depict the relative magnitudes of estimated QTL effects for each environment (shown in the order SK92a, SK92b, SK93c, AB92c, MB92, QC93).

environmental variance. There was no direct estimate for heritability in these studies because progeny were not replicated within environments. Heritability estimates based on the proportion of variance attributed to background markers ranged from 47 to 75% (Table 5, Model 4); these are in general agreement with classical studies on genetic parameters of grain and malt characters, as reviewed by Sparrow (1971). Many of the traits reported

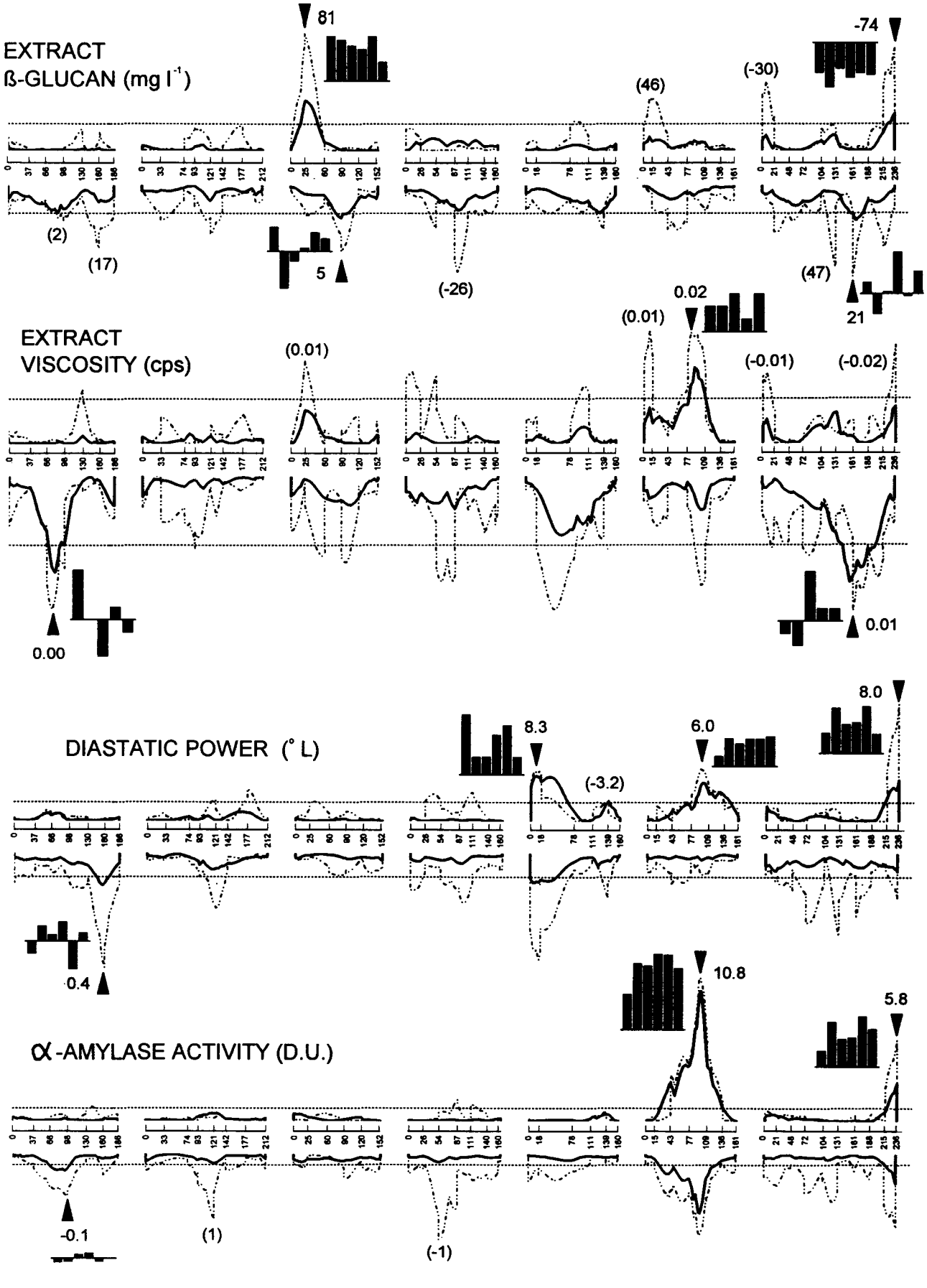
EXTRACT
ß-GLUCAN (mg l-1)
81
-74
(-30)
(46)
(2)
(17)
5
(-26)
(47)
21
EXTRACT
VISCOSITY (cps)
(0.01)
0.02
(0.01)
(-0.01)
(-0.02)
0.00
0.01
DIASTATIC POWER (° L)
8.3
6.0
8.0
(-3.2)
0.4
α-AMYLASE ACTIVITY (D.U.)
10.8
5.8
-0.1
(1)
(-1)

Table 5. Average percentages of within-environment phenotypic variance explained by four models of QTL effect for grain and malt quality traits.

Trait	Model†			
	1	2	3	4
Kernel plumpness	16	36	41	70
Kernel weight	27	30	50	75
Grain protein	18	22	32	59
Fine-grind extract	23	29	37	61
Fine-coarse difference	14	20	23	51
Soluble protein	29	32	40	69
Extract β-glucan	12	17	29	52
Extract viscosity	3	11	21	47
Diastatic power	26	32	34	60
α-amylase activity	61	65	67	73

† Model 1, main effects for primary QTL; Model 2, main effects and QTL by environment interactions for primary QTL; Model 3, main effects and QTL by environment interactions for primary and secondary QTL; Model 4, a separate effect for each background marker within each environment.

here have not been subjected to classical genetic studies.

Simple and Composite Interval Mapping

About half of the QTL detected here were statistically significant with SIM and also obvious with sCIM; these were designated as primary QTL. The others (the secondary QTL) were indicated by only one of the two methods. At some secondary QTL, there was a significant peak on the SIM scan, but no strong peak on the sCIM scan. More often, sCIM generated a distinct peak, usually a QTL×E peak, in a region where the SIM scan was not statistically significant. Thus, sCIM served to suggest possible additional QTL more often than it cast doubt on QTL that had been indicated by SIM. None of the additional QTL suggested by sCIM explained a substantial amount of genetic variance. Thus, from the point of view of QTL detection, SIM might have been sufficient. However, sCIM may have been useful in improving the accuracy of estimation of QTL positions (Tinker and Mather 1995a). This is impossible to evaluate here because the true QTL positions are unknown.

Quantitative Trait Loci vs. Trait Distributions and Correlations

It is useful to examine QTL relative to general characteristics of quantitative trait distributions for parents and progeny. These comparisons illustrate how trait distributions depend on underlying QTL, and provide interesting contrasts between "old" and "new" methods of quantitative trait investigation. Information about QTL regions that affect multiple traits can be used to speculate about biological factors that cause genetic correlations among traits.

The trait levels in the parents, and the predominant directions of QTL effects, were consistent with what was generally known about Harrington and TR306. Harrington was the primary source of QTL alleles for increased diastatic power and α-amylase activity, while TR306 was the primary source of QTL alleles for increased kernel plumpness, kernel weight, and grain protein. Small amounts of transgressive segregation for some of the above traits were due to the effects of secondary and(or) undetected QTL. For fine-coarse difference, soluble protein, and extract β-glucan, both parents contributed QTL alleles with favorable effects, resulting in larger amounts of transgressive segregation (Table 3). Although TR306 contributed primary QTL alleles for increased fine-grind extract and reduced extract viscosity, there were no DH progeny with higher fine-grind extract or lower extract viscosity than Harrington. This may have been due to epistasis, which was not investigated here.

Relative positions of QTL for different traits are reflected in genetic correlations among traits. While the correlations in Table 4 were not entirely genetic, the non-genetic component should have been small because the data were averaged over environments. The strong positive correlation between grain protein and kernel plumpness was due in part to pleiotropic and/or closely linked QTL on chromosomes 1, 4, and 7. Kernel weight, for which there were QTL in two of these regions (chromosomes 1 and 7), was positively correlated with both traits. It is possible that Harrington alleles in these regions caused reduced grain filling, perhaps with proportionally more reduction in storage protein (hordein) accumulation than in starch deposition. Negative correlations between fine-grind extract and kernel plumpness or grain protein were due partially to QTL effects near cM position 0 on chromosome 7. If Harrington alleles in this region caused smaller kernels by reducing the amount of hordein deposition, the resulting malt might produce greater amounts of extract. Negative correlation between grain protein and extract has been found elsewhere (Foster et al., 1967), but genetic associations between kernel plumpness and grain protein or extract have not been consistent (Rasmusson and Glass, 1965; Piper and Rasmusson, 1984).

In the malting industry, kernel weight and plumpness are generally expected to be negatively associated with grain protein and positively associated with malt extract. These expectations may be based primarily on environmental correlations, such as those reported by Rutger et al. (1967). For a given cultivar, samples with plump grain will have less protein and more starch than those with thin grain. The correlations observed in the present study indicate that the genetic relationships among these traits can oppose the environmental ones. Thus, selection for plump or heavy kernels could result in correlated responses toward lower extract and higher grain protein due to chromosome regions such as that near cM position 0 on chromosome 7. Marker-assisted selection could give a breeder the ability to separately manipulate QTL that affect kernel weight or plumpness without affecting extract (e.g., regions on chromosomes 1 and 4).

The strong positive correlation between extract β-glucan and fine-coarse difference was partially due to QTL on chromosomes 3, 6, and 7. These QTL may be pleiotropic, affecting both traits via effects on endosperm modification. With incomplete breakdown of cell walls, greater amounts of β-glucan would be extracted. Starch granules protected by undegraded cell walls, would be less susceptible to hydrolysis, particularly in coarsely ground malt. This would lead to higher fine-coarse difference values.

The positive correlation between extract β-glucan and extract viscosity was expected because β-glucans are one of the causes of increased viscosity. However, comparison of the positions of primary QTL for these two traits does not reveal a basis for genetic correlation. There was only one primary QTL with a main effect on extract viscosity, and there were no QTL that affected β-glucan in this region. There were, however, similar QTL×E interactions for both traits near cM position 161 on chromosome 7, and additional similarities were observed when secondary QTL were considered (e.g., at both ends of chromosome 7). Thus, positive genetic correlation between extract viscosity and extract β-glucan seems to be the result of multiple QTL with minor pleiotropic effects.

Positive correlations among fine-grind extract, α-amylase activity, diastatic power, and soluble protein may have been due to linked QTL or a pleiotropic QTL near cM position 236 on chromosome 7. This region may affect a key step in the germination process (possibly water uptake or gibberellic acid synthesis) resulting in a cascade of effects through the malting process. Elsewhere, diastatic power has been negatively correlated with extract (Foster et al, 1967), possibly due to an association between protein and diastatic power which was not found in the present study.

Interactions between QTL and the Environment

While the genomic positions of QTL are presumably constant, the effects of QTL alleles may vary with environment. Reasons for these QTL×E interactions could range from direct influences of the environment on gene expression, to complex influences of the environment on plant growth and development. This study could not determine the cause of QTL×E interactions, but it demonstrates the importance of studying QTL effects in more than one environment. In a single environment, many QTL would not have been detected, or they would have been detected with effects opposite to those in other environments. Even the effects of important primary QTL (e.g., those that affected α-amylase activity) varied in magnitude across environments, often by a factor of two or more (Fig. 1 and 2).

Environments were considered fixed, with representation from specific years and locations, so we can speculate about sources of observed QTL×E interaction. The two easternmost environments, where QTL effects on kernel plumpness were strongest, may have provided conditions where the presence of Harrington alleles led to incomplete kernel filling. These factors could have involved sink formation, availability of photosynthate, or duration of grain fill.

Several other QTL regions had important effects on grain or malt quality. The QTL at (or near) the *Amy1* locus on chromosome 6 had a large effect on α-amylase activity, and must be a major factor in Harrington's high enzyme levels. Alleles from Harrington at a QTL on chromosome 5 caused increased diastatic power. Marker-assisted selection could be used to transfer these Harrington alleles to cultivars with lower enzyme levels. There were also regions where TR306 alleles favorably affected grain or malt quality: on chromosome 1 (plumpness and kernel weight) and chromosome 3 (β-glucan and fine-coarse difference). Since the DH population already contains progeny with known combinations of Harrington and TR306 alleles, this provides opportunities for recurrent mating and selection of new allele combinations. Introgression of combinations of alleles from both Harrington and TR306 into other genetic backgrounds should also be possible.

Comparison to Quantitative Trait Loci Detected in Other Barley Crosses

In a six-row barley cross, 'Steptoe'/'Morex', some of the most important QTL related to grain and malt quality were found near the amylase loci *Amy2*, *Bmy1*, and *Bmy2* (Hayes et al., 1993). In Harrington/TR306, these amylase loci were not polymorphic, and these regions had no important effects on grain or malt traits; instead, the region near *Amy1*, and regions near the ends of chromosome 7 were most important. Only the hordein region of chromosome 5 showed effects on diastatic power in both Steptoe/Morex and Harrington/TR306. Han et al. (1995) studied several components of β-glucan and β-glucanase in the Steptoe/Morex cross. They found numerous QTL for these traits, two of which were in the same chromosome regions as primary extract β-glucan QTL in Harrington/TR306: one (near locus Dor4A on Chromosome 3 in Steptoe/Morex) affected malt β-glucan, and the other (near locus ABG463 on Chromosome 7 in Steptoe/Morex) affected β-glucanase activity in finished malt. While these two were the most important β-glucan QTL in Harrington/TR306, their importance in Steptoe/Morex was minor relative to other QTL that affected these traits.

In a cross between 'Blenheim' and 'E224/3', Chalmers et al. (1993) detected a QTL for milling energy that may coincide with the QTL we detected for extract on the plus arm of chromosome 7. Milling energy, a trait not measured here, is intended as an indicator of potential extract. Thomas et al. (1996) investigated QTL that affected several other malt quality traits in Blenheim/E224/3. Differences in mapped markers make it difficult to accurately compare QTL positions, but there do not seem to be any cases of QTL positions that are important in both crosses.

While the above comparisons have provided evidence for some orthologous loci in different genetic backgrounds, there have been more differences than similarities. The most important loci that affected grain or malt quality in Harrington/TR306 were relatively minor in the other two crosses, and vice versa. For marker-assisted selection, this implies that the transfer of favorable alleles detected in one cross into different genetic backgrounds may not achieve predicted results. However, the parents of the three crosses compared here were chosen from several distinct pools of malting or non-malting germplasm. Thus, these QTL may cumulatively represent a significant proportion of the genetic variability in malt quality traits available to barley breeders. Opportunities

exist to test combinations of alleles affecting malt quality from different genetic backgrounds (e.g., in a two-row/six-row cross), and to create unique combinations of agronomic and malting traits.

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