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Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm

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Abstract. Quantitative trait locus (QTL) and QTL × environment (E) interaction effects for agronomic and malting quality traits were measured using a 123-point linkage map and multi-environment phenotype data from an F_1-derived doubled haploid population of barley (*Hordeum vulgare*). The QTL × E interactions were due to differences in magnitude of QTL effects. Highly significant QTL effects were found for all traits at multiple sites in the genome. Yield QTL peaks and support intervals often coincided with plant height and lodging QTL peaks and support intervals. QTL were detected in the vicinity of a previously mapped Mendelian maturity locus and known function probes for α- and β-amylase genes. The average map density (9.6 cM) should be adequate for molecular marker-assisted selection, particularly since there were few cases of alternative favorable alleles for different traits mapping to the same or adjacent intervals.

Key words: QTL – RFLP mapping – marker-assisted selection – Barley

Introduction

The current enthusiasm for developing medium-density genome maps in crop species is driven, in large part, by a desire to locate quantitative trait loci (QTL). The QTL may be targets for map-based cloning or molecular marker-assisted selection (MMAS). In the latter case, if linkages can be established between genes controlling quantitative traits and molecular markers, indirect selection may provide greater gains than direct selection.

QTL for a range of agronomic, quality, and stress resistance traits have been reported in a number of crop species (reviewed by Paterson et al. 1991a). These estimates of QTL effects are based on a range of genetic reference populations and analysis procedures. Principal advantages of barley for QTL analysis are that (1) there is intraspecific polymorphism in agronomically meaningful cross combinations (Kleinhofs et al. 1993), and (2) immortal genetic reference populations of doubled haploids can be readily produced, and these populations simplify map construction and QTL estimation (Hayes et al. 1993).

Breeding populations typically exhibit genotype interaction when tested in diverse environments. In the case of such interactions, one would expect that at least some of the genes underlying QTL would also show genotype × environment interaction. QTL × environment (E) interaction would be expressed as (1) significant effects detected only in a subset of the total number of environments, (2) changes in the magnitude of significant effects of QTL across environments, and (3) opposite favorable alleles at a QTL in distinct environments. The nature of the interaction is extremely important. Change in rank (crossover) interactions (i.e., item 3 above) could have significant impacts on

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MMAS. Magnitude changes, on the other hand (i.e., types 1 and 2, above) should be of less consequence.

The potential of QTL analysis for introgression of alleles from exotic germplasm with minimum linkage drag has been demonstrated (Young and Tanksley 1989). The utility of MMAS for "ultimate traits" – such as yield and quality – in agronomically relevant autogamous germplasm has not been as well demonstrated. We have, therefore, based our analyses on a doubled haploid population derived from the cross of two six-row spring barley varieties that occupy significant acreage in the United States. 'Steptoe' is the dominant feed barley in the northwestern U.S.; 'Morex' is the six-row spring U.S. malting quality standard.

This report summarizes QTL analyses based on the evaluation of a population of 150 F_1-derived doubled haploid (DH) lines derived from the cross of 'Steptoe' × 'Morex' in three irrigated and two dryland environments in the western United States. Agronomic data were obtained in all environments, and quality data from four environments. Our intent is to use these data to highlight key issues in QTL mapping: (1) importance and type of QTL × E interaction; (2) magnitude of QTL effects; (3) resolution of QTL, and (4) implications for MMAS.

Materials and methods

The development of the genetic reference population and subsequent map construction have been described by Kleinhofs et al. (1993). Briefly, 150 doubled haploid lines were derived from the F_1 of the cross of 'Steptoe' × 'Morex', using the *Hordeum bulbosum* technique, as described by Chen and Hayes (1989). A 295-point map provided an average density of 4 cM, with considerable overlap in certain regions (Kleinhofs et al. 1993). By selecting relatively evenly spaced markers, we generated a 123-point "skeleton" linkage map providing an average marker density of 9.6 cM. The map was produced by G-MENDEL (Liu and Knapp 1990) with the following constraints: recombination $\leq 30\%$ and $P = 0.001$. Locus designations shown in Tables 2 and 3 are those used by Kleinhofs et al. (1993). The 37.7 cM interval between *ABG473* and *CDO504* on the long arm of chromosome 7 – identified by the separate analysis of chromosome 7 markers – was not included in the QTL analyses. Centromeres were mapped on all chromosomes except chromosome 5 as described by Kleinhofs et al. (1993). When relevant for the purposes of discussion, the recombination intervals shown in Tables 2 and 3 were converted to cM using the Kosambi mapping function (Kosambi 1944).

In 1991, the 150 DH lines and the parents were evaluated in field experiments at Aberdeen, Idaho; Crookston, Minnesota; Bozeman, Montana; Ithaca, New York; Prosper, North Dakota; Klamath Falls, Oregon; and Pullman, Washington. Severe drought in North Dakota and New York, and hail damage in Minnesota, led to these data not being included in the final analysis. At each location, plot size and management were in accordance with local practice: the minimum plot size was 6.6 m^2. An irrigated and a dryland experiment were grown in adjacent fields at Bozeman, Montana. The Washington experiment was grown under dryland conditions. The remaining experiments were irrigated. A randomized complete block design with partial replication was employed at each location. The population of 150 lines, along with the parents, formed one complete block. A subset of 50 DH lines – selected at random from the total population – together with the parents composed the second block. Partial replication was justified based on limited seed supply and the consideration that the primary determinant of the power of tests of hypotheses about QTL genotype means is the number of replcations of QTL genotypes (i.e., the number of individuals in the genetic reference population), not the number of times each individual line is replicated (Knapp et al. 1990). Agronomic traits were measured following standard procedures. Malting quality characters were measured on a composite 400-g sample of each DH line and parent following the standard procedures employed by the USDA/ARS Cereal Crops Research Unit, Madison, Wisconsin. Protocols are available upon request.

QTL analyses were performed using QTL-STAT (B. H. Liu and S. J. Knapp, unpublished). The QTL parameters were estimated using least squares interval mapping methods (Haley and Knott 1992; Knapp et al. 1990). QTL genotype means were estimated, and the hypothesis of "no QTL" was tested against the hypothesis of "one QTL" for every marker bracket. The non-linear relationship between the expected value of marker genotypes and QTL effects was established, based on the assumption of Hardy-Weinberg linkage disequilibrium between flanking markers and putative QTL. Instead of estimating QTL parameters and test statistics at 1-cM intervals within every marker bracket and selecting the location that maximized the likelihood ratio or minimized the error sums of squares, as is often done when interval mapping, we estimated QTL location directly using non-linear least squares (Knapp et al. 1990). Hypotheses about QTL and QTL × E effects were tested using Wald statistics (Knapp 1989; Knapp and Bridges 1990). QTL effects and QTL × E effects were considered significant if they exceeded a Wald statistic of 10.0, which is approximately equal to $P = 0.001$. We specified Wald Support Intervals (WSIs) $\geq 90\%$ at Wald = 10, following the LOD Support Interval (LSI) concept described by van Ooijen (1992).

Results

A range of phenotypic expression for agronomic and quality traits was found in all environments. Mean values for the parents in each environment are presented in Table 1. 'Steptoe' was higher yielding and earlier than 'Morex' in all environments except Oregon. The agronomic trait values are representative of parental performance. 'Morex' consistently had higher grain protein, α-amylase, diastatic power, and malt extract. The grain protein and enzymatic trait values are representative, but the malt extract values for 'Morex' are lower than usual. A more typical average extract for 'Morex' would be 78%. There were positive and negative transgressive segregants for all agronomic traits. For malting quality traits, the parents tended to fall at the extremes of the frequency distributions.

The magnitude and nature of QTL × E interaction indicates the appropriateness of basing selection decisions on average QTL effects or environment-specific

Table 1. Agronomic trait means and malting quality means for 'Steptoe' (S) and 'Morex' (M) evaluated in various environments

Agronomic trait means

	Yield (kg/ha)		Lodging (%)		Height (cm)		Heading date (days after January 1)	
	M	S	M	S	M	S	M	S
Idaho	7951	8629	55	75	116	111	180	179
Montana (dryland)	1738	4099	0	0	97	82	184	181
Montana (irrigated)	4666	7066	50	40	100	98	183	181
Oregon	8329	5344	0	0	110	90	189	191
Washington	5969	6270	0	0	133	112	182	181
$\bar{x}$	5731	6282	21	23	111	99	184	183

Malting quality means

	Grain protein (%)		Alpha-amylase (20 Deg units)		Diastatic power (Deg)		Malt extract (%)	
	M	S	M	S	M	S	M	S
Idaho	14.8	12.1	36.2	26.2	97	74	76.7	74.1
Montana (irrigated)	14.1	12.0	39.0	21.0	140	62	76.3	70.8
Oregon	15.3	11.7	35.8	19.6	147	62	77.5	71.5
Washington	13.2	10.0	37.5	23.6	128	54	77.3	73.8
$\bar{x}$	14.4	11.5	37.1	22.6	128	63	76.9	72.6

effects. In this set of experiments, environments were a highly significant source of variation for all traits in all marker intervals. Overall, there was little QTL × E interaction. When there was significant QTL × E interaction (Wald ≥ 10), it was invariably a difference in magnitude rather than a change in rank phenomenon. Effects were either significant in a subset of environments, or the magnitude of significance varied among environments. What QTL × E interaction did occur was, with the exception of grain protein, limited to the agronomic traits. In the case of grain protein, there was one case of significant QTL × E interaction on chromosome 3. The WSI spanned *ABG396-PSR156*. Wald statistics and support intervals for QTL × E and QTL effects associated with agronomic traits on chromosome 2 are typical of those measured elsewhere in the genome (Table 2). QTL × E and QTL support intervals generally coincided, and the latter usually exceeded the former. For plant height, lodging, and heading date, QTL × E WSI's were always coincident with QTL WSI's. For grain yield, there were eight chromosome regions showing significant QTL × E interaction. Of these, there were three regions for which there were no corresponding significant average QTL effects: one on chromosome 1 (*ABC455-ABG461*), one on chromosome 5 (*Aga6-CDO99*), and one on chromosome 7 (*ABC483-CDO57B*). Given the nature and magnitude of QTL × E interaction in these data, we considered it appropriate to proceed with an analysis and interpretation of average QTL effects. This is also in line with breeding objectives, as a high level of consistent cultivar performance is considered, in our target environments, to be superior to narrow, environment-specific adaptation.

Average QTL effects exceeding the Wald ≥ 10 threshold were found for all characters at multiple sites throughout the genome. Table 3 shows the difference between QTL genotypes, expressed in the units at the head of each column, for the support interval surrounding each Wald peak (indicated in bold). The letter suffix denotes the parent ('Steptoe' or 'Morex') contributing the larger value allele. For grain yield, protein, malt extract, diastatic power, and α-amylase, the larger value allele is positive. For lodging, the larger value allele is negative. Plant height and heading date alleles can be considered neutral, in that the two parents made comparable contributions (Table 4) and only extreme values can be considered to have a negative impact on agronomic fitness.

Additional QTL and QTL × E effects, significant at threshold ≤ Wald = 10, were found for all traits. These may represent the effects of minor genes (Heun 1992) and the interactions of such genes with distinct environments. However, their utility for routine MMAS is questionable. For example, even a Wald peak of 20.6, such as that for yield in the *WG789A-ABG380* interval on chromosome 1, gave a QTL genotype difference of 354 kg ha^{-1}, a value well below the level of mean separation resolution achieved in most yield trials.

Results will be presented for each chromosome using the information found in Table 3. Issues relating to genetic mechanisms, resolution, and utility will be addressed in the Discussion.

Chromosome 1

Significant QTL effects were found for all traits except grain protein. 'Steptoe' contributed positive alleles

Table 2. Wald statistics and Wald support intervals (in bold type) for QTL × environment and QTL effects for agronomic traits on chromosome 2

Marker interval		% Recombination	Yield		Lodging		Height		Heading date	
			QTL × E	QTL	QTL × E	QTL	QTL × E	QTL	QTL × E	QTL
ABG313A	ABG703	7.9	**17.5**	0.2	3.4	0.8	3.8	46.7	4.2	140.9
ABG703	CHS1B	11.0	**11.7**	0.1	2.1	0.9	10.6	62.7	9.8	180.7
CHS1B	ABG8	7.2	**12.5**	**0.9**	3.1	0.3	**19.9**	123.2	**15.5**	377.9
ABG8	RbcS	4.6	**16.1**	**1.7**	**3.1**	0.6	**15.9**	102.1	**24.1**	458.9
RbcS	ABG2	11.5	**15.9**	**10.9**	**8.1**	1.4	**21.4**	**164.4**	**31.6**	**764.3**
ABG2	ABG459	9.0	**16.4**	**7.9**	**8.7**	**4.1**	**11.2**	126.7	**24.5**	348.6
ABG459	Pox	6.8	**10.5**	**5.1**	**8.7**	**6.1**	6.4	76.5	**12.9**	149.5
Pox	Adh8	5.5	**7.6**	**2.6**	**13.9**	**4.9**	3.0	56.6	6.8	87.8
Adh8	ABG19	11.7	6.8	0	**8.3**	**9.7**	3.8	20.3	5.4	9.9
ABG19	ABC162	6.6	4.0	3.8	**3.4**	**12.8**	1.9	7.5	1.4	3.4
ABC162	ABG14	8.3	2.1	0	**4.2**	**4.8**	0.8	15.7	1.2	0.3
ABG14	His3C	10.3	0.6	**7.4**	2.6	2.0	0.3	24.7	1.2	**11.4**
His3C	Ksu15	11.9	2.7	**1.7**	0.9	0.5	0.6	12.5	6.4	**18.4**
Ksu15	Crg3A	22.1	5.2	**10.7**	2.5	1.5	2.6	28.9	1.5	**22.8**
Crg3A	Gln2	16.4	**15.9**	**3.4**	0.8	**8.8**	2.3	38.3	2.8	**8.7**
Gln2	ABC157	7.4	**12.9**	**1.2**	1.3	**10.4**	1.5	**44.5**	2.2	**7.9**
ABC157	ABC165	7.4	**15.0**	**1.6**	1.6	**13.8**	1.2	**53.1**	1.8	**7.7**
ABC165	Pcr1	7.5	**6.9**	**6.4**	1.5	**18.0**	1.0	41.9	1.6	**13.2**
Pcr1	ABA5	8.6	**11.3**	**4.1**	1.2	**14.8**	1.0	32.3	4.6	**11.6**

Table 3. QTL genotype differences for agronomic and malting quality traits where Wald ≥ 10. Value in bold type indicates Wald peak. Adjacent values indicate the support interval. The letter suffix indicates the parent contributing the larger value allele. S, 'Steptoe'; M, 'Morex'. Marker intervals in bold type indicate centromere location

Chromosome 1 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
ABA301	Plc	3.4			3S					
Plc	BCD129	7.5			3S					
BCD129	Glx	8.3			**3S**					
Glx	WG789A	5.5			3S	0.8M				
WG789A	ABG380	4.9	**354S**			**0.9M**				
ABG380	ABC158	7.7	301S			0.9M				
ABC158	ksuA1A	6.1	251S			0.8M				
ksuA1A	ABC154A	3.4								
ABC154A	Brz	7.3								
Brz	ABC156D	5.8								**0.7M**
ABC156D	ABG22A	12.8						**3.4M**	8.4M	0.6M
ABG22A	ABG701	3.9							**10.2M**	
ABG701	ABG11	4.4			**4S**	0.9S				
ABG11	ABC455	5.6				0.9S			10.6M	
ABC455	**Amy2**	6.9		11S		0.8S		**3.3M**	**11.2M**	**0.9M**
Amy2	Ubi1	16.1		12S		**1.1S**				
Ubi1	ABC310B	4.0		**12S**		0.9S				
ABC310B	ABC305	6.7		10S						
ABC305	PSR129	4.9		11S						
PSR129	ABG461	13.0								
ABG461	Cat3	19.7								

Table 3. (*Continued*)

Chromosome 2 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
ABG313A	ABG703	7.9								**0.9M**
ABG703	Chs1B	11.0								
Chs1B	ABG8	7.2								
ABG8	Rbcs	4.6	102S							**0.9M**
Rbcs	ABG2	11.5	**263S**		**8M**	**5.1M**			6.6M	0.7M
ABG2	ABG459	9.0	222S	5S					5.3M	
ABG459	Pox	6.8	129S	6S			**0.7M**		**7.8M**	
Pox	Adh8	5.5		6S					6.1M	
Adh8	ABG19	11.7		9S			**0.7M**		5.8M	
ABG19	**ABC162**	6.6		**11S**					5.1M	
ABC162	ABG14	8.3		6S				**2.8M**	6.9M	
ABG14	His3C	10.3	220S			1.0M				
His3C	ksuF15	11.9	107S			1.0M				
ksuF15	Crg3a	22.1	**266S**			**1.2M**				
Crg3a	Gln2	16.4	149S	9M		0.8M				
Gln2	ABC157	7.4	87S	9M	4M	0.7M				
ABC157	ABC165	7.4	99S	11M	**5M**	0.8M				
ABC165	Pcr1	7.5	198S	**12M**	4M	0.9M				
Pcr1	ABA5	8.6	160S	12M		0.8M	**0.5S**			

Chromosome 3 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
ABA303	ABC171	23.0								
ABC171	ABG57	13.5								
ABG57	ABG471	3.6								
ABG471	**Dor4A**	19.8	443S							
Dor4A	ABG396	6.4	**734S**	**30M**	**8M**					
ABG396	ABG703A	9.4	737S			**1.1S**				
ABG703A	PSR156	9.3	723S							
PSR156	ABG377	7.6								
ABG377	ABG453	10.5				0.9S	0.2M			
ABG453	ABC307B	10.2				**1.1S**	0.3M			
ABC307B	CDO113B	12.3					**0.3M**	**1.6M**		
CDO113B	His4B	16.9	**519S**							
His4B	ABG4	14.1								
ABG4	mPub	7.3								
mPub	ABC174	13.4								
ABC174	ABC166	11.6								
ABC166	ABC172	11.0								

Chromosome 4 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
WG622	ABG313B	10.5			**2M**				10.2M	0.6M
ABG313B	CDO669	4.6				0.8S			**10.6M**	0.7M
CDO669	BCD402B	14.0				1.0S		**2.5M**	9.7M	**0.8M**
BCD402B	TubA1	10.3			1S	**1.1S**		2.4M		0.6M
TubA1	ABG3	4.8			2S					0.4M
ABG3	**ABG484**	5.4			**2S**					0.5M
ABG484	WG464	10.4			2S					0.6M
WG464	ABG472	15.8		11S						0.5M
ABG472	ABG500B	16.1		**14S**			0.3M			0.5M
ABG500B	ABG397	7.0		12S			**0.4M**			
ABG397	Bmy1	25.4		14S		1.5S				
Bmy1	ksuH11	3.3				**1.5S**			11.1S	

Table 3. (*Continued*)

Chromosome 5 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
Aga6	Hor2	2.5			2M				**13.1M**	**0.5M**
Hor2	Hor1	10.5			**2M**				12.8M	0.4M
Hor1	ABA4	6.6			1M					**0.8M**
ABA4	CDO99	8.0			1M					0.7M
CDO99	Ica1	11.2								0.4M
Ica1	ABG500A	7.9								0.6M
ABG500A	ABG494	9.9								**0.6M**
ABG494	Glb1	7.6								0.4M
Glb1	ABC160	8.8								0.4M
ABC160	ABG464	14.7								0.5M
ABG464	His3B	9.6							6.4M	0.5M
His3B	iPgd2	16.6						1.3M	6.4M	**0.5M**
iPgd2	ABG702	12.6						1.4M	**7.6M**	0.4M
ABG702	ABA2	6.4						1.4M		0.5M
ABA2	ABG373	8.3						**1.5M**		**0.6M**
ABG373	ABG387A	5.3						1.0M		

Chromosome 6 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
PSR167	Nar1	6.3				1.0M				0.4S
Nar1	ABG378	5.2				**1.2M**				**0.5S**
ABG378	Cxp3	9.0				1.1M				0.4S
Cxp3	PSR106	16.7								
PSR106	ABG387B	4.5								
ABG387B	ABG458	14.5	299M							
ABG458	Rrn1	6.3	311M							
Rrn1	ABG474	7.1	368M		1S					
ABG474	**ksuD17**	4.1	371M		1S				3.1M	
ksuD17	ksuA3D	7.3	**386M**	**13S**	2S				**5.8M**	
ksuA3D	Nar7	8.7	321M	12S	**2S**			0.4M	5.6M	
Nar7	Nir	5.5			2S			0.4M	2.9M	
Nir	PSR154	12.3						**0.4M**	3.2M	

Chromosome 7 Marker interval		% Recombination	Yield (kg/ha)	Lodging (%)	Height (cm)	Heading date (days)	Grain protein (%)	Alpha-amylase (20 Deg units)	Diastatic power (Deg)	Malt extract (%)
ABC483	ABG705	27.6								
ABG705	ABG395	7.9								
ABG395	Rrn2	3.6							8.8M	
Rrn2	**Ltp1**	4.5					**0.7M**		**9.3M**	
Ltp1	ABC76	5.8					0.7m			
ABC706	Ale	5.4					0.6M			
Ale	ABC302	10.1								
ABC302	CDO57B	13.0								
CDO57B	mSrh	5.4			**4M**					
mSrh	ABG473	6.5						**1.6M**		
CDO504	WG908	7.7						0.7M		
WG908	ABG495A	8.8						1.1M		
ABG495A	ABG496	6.2						0.7M		
ABG496	ABC482	7.4						0.9M		
ABC482	ABG707	7.2						1.4M		
ABG707	ABG463	9.1						**1.4M**		
ABG463	ABA304	8.6								

for yield, 'Morex' contributed all positive alleles for malting quality. There was no overlap of malting quality and yield QTL. The maximum QTL genotype difference for yield was 354 kg ha^{-1}. On the short arm, the Wald peaks coincide and the support intervals overlap, for yield and heading date. The favorable allele for yield came from 'Steptoe', and the larger value heading date allele from 'Morex'. There were overlapping WSI's for lodging and heading date and an adjacent QTL for height on the long arm of the chromosome. These alleles, all contributed by 'Steptoe', are not reflected in an average yield QTL effect for 'Morex'. The environment-specific effect for Oregon in this region, accounting for significant QTL × E interaction, was due to a positive yield effect from 'Steptoe'. Resolution of the agronomic trait QTL ranged from 18.8 cM for yield to 39.2 cM for lodging. Key QTL for α-amylase, diastatic power, and malt extract were well resolved and of high magnitude. There were two α-amylase QTL, one coinciding with the interval bounded by *Amy2*. The *Amy2* locus has been mapped with a clone specific to a member of the α-amylase multi-gene family located on chromosome 1 (Khursheed and Rogers 1988). The second α-amylase QTL is located on the opposite chromosome arm. In both cases, QTL peaks were also the WSI's. The diastatic power WSI's – 17.0 and 12.5 cM – coincided with the malt extract WSI's – 6.9 and 18.9 cM – and both α-amylase peaks. This pattern of coincident QTL for these three characters was observed at several locations in the genome and may be explained by trait correlation and relationship. Diastatic power is a measure of total hydrolytic enzyme activity, including α-amylase. Malt extract percentage is a measure of the soluble carbohydrate in the malted barley.

Chromosome 2

'Steptoe' was the source of favorable alleles for yield and protein. 'Morex' contributed favorable alleles for all malting quality characters. There were coincident Wald peaks, or overlapping support intervals, for the 'Steptoe' yield QTL allele with heading date, height, and lodging QTL from 'Morex'. The yield WSI extending from *ABG8* to *Pox* overlapped with a lodging QTL, also contributed by 'Steptoe', an exception to the general relationship of contrasting yield and lodging QTL alleles. In this case, the yield Wald peak was coincident with large-effect, well-resolved QTL for height and heading date contributed by 'Morex'. This chromosome region is the site of one of the early maturity (*Ea*) loci (Nilan 1964). Beavis et al. (1991) also found that plant height QTL in maize were often associated with mapped qualitative loci. The QTL genotype differences and the Wald values for these QTL (Table 2) were the highest found anywhere in the genome. The yield QTL, in contrast, were poorly resolved, spanning 32.2 and 93.9 cM. The three QTL for grain protein were resolved to single marker intervals. The two grain protein QTL contributed by 'Morex' were within the WSI for diastatic power, as was the α-amylase QTL. The diastatic power support interval overlapped with one of the malt extract QTL. The *Bmy2* locus (Kreis et al. 1987) is within the *ABG19-ABC162* interval (Kleinhofs et al. 1993). The second malt extract QTL was separated from the first malt extract QTL by an 18.4 cM interval and corresponds to no other enzyme QTL. For the purposes of MMAS, the overlapping WSI's for yield and quality QTL agronomic would require prioritization of trait importance and allele value.

Chromosome 3

Chromosome 3 was the site of the largest yield QTL, and both positive alleles were contributed by 'Steptoe'. One QTL, with an average QTL genotype difference of 519 kg ha^{-1} (Wald = 50.0), occupied 17.6-cM interval on the long arm of the chromosome and had no other corresponding agronomic trait QTL. The second, even larger-effect region (Wald = 99.4) accounted for a difference of approximately 734 kg ha^{-1}. The peak occupied a 6.4-cM region, and the support interval spans a 46-cM interval. The yield peak coincides with clearly resolved, single-interval peaks for lodging and height with the larger value alleles contributed by 'Morex'. The yield effect in this region, while apparently related to height and lodging, was also significant in the Montana dryland experiment, where no lodging occurred. There were few malting quality QTL, with 'Morex' contributing coincident protein and α-amylase peaks in the *ABC307B-CDO113B* interval lying between the WSI's for the two yield QTL 'Steptoe' alleles. The α-amylase effect did not have the corresponding diastatic power and malt extract effects seen elsewhere in the genome. The one case of significant QTL × E interaction for a quality trait (grain protein) coincides with the WSI for yield spanning the interval from *PSR156-ABG396*. In this case, a modest but significant QTL for protein was contributed by 'Morex' in Idaho, a location experiencing the most severe lodging. MMAS for both yield loci and the α-amylase QTL would be facilitated by greater QTL resolution. However, there are no additional markers for this region on the 295-point map.

Chromosome 4

There were no yield QTL on chromosome 4, despite lodging and height QTL alleles contributed by 'Steptoe' and height QTL alleles contributed by 'Morex'. As seen on chromosomes 1 and 2, there were overlapping

WSI's for α-amylase, diastatic, power and malt extract, with the favorable alleles coming from 'Morex'. 'Steptoe' contributed a well-resolved, high value QTL allele for diastatic power in a 3.3-cM interval flanked by *Bmy1*, a β-amylase locus (Kreis et al. 1987). As diastatic power is a measure of both β- and α-amylase activity, this provides an additional example of coincident QTL and known function genes. This high β-amylase activity did not, however, correspond to a malt extract QTL.

Chromosome 5

This chromosome was remarkable for the paucity of agronomic trait QTL and the absence of any QTL contribution from 'Steptoe'. The only agronomic trait QTL came from 'Morex', which contributed the maximum difference of 2 cm in height. This interval was also coincident with significant QTL × E interaction for yield. Idaho was again the only environment where a significant effect for this region was detected, with 'Morex' contributing the larger value allele. No grain protein effects were found in the intervals flanked by the hordein loci (*Hor1* and *Hor2*), despite the large contribution of these storage proteins to total grain protein (Shewry et al. 1980). It may be that the molecular polymorphism in this population was not related to gene function polymorphism. There was, however, a coincident peak of a diastatic power QTL with the *Aga6-Hor2* interval. The second diastatic power QTL on the long arm overlapped with the α-amylase QTL and one of the WSI's for malt extract. These malt extract loci, however, are the most poorly resolved QTL in the genome. There were five fairly distinct peaks, indicated in bold type, but the overlapping support intervals spanned the entire length of the chromosome. Presumably, introgression of an intact chromosome 5 from 'Morex' would have no adverse effect on agronomic performance.

Chromosome 6

In contrast to the balance of the genome, on chromosome 6 'Morex' contributed a favorable yield QTL allele and 'Steptoe' a favorable QTL allele for malt extract. The poorly resolved yield QTL overlapped with lodging and height QTL where 'Steptoe' contributed the larger value allele. Overlapping α-amylase and diastatic power QTL alleles both came from 'Morex'. The separation of favorable yield and quality QTL would simplify MMAS for QTL on this chromosome.

Chromosome 7

This was the most sparsely mapped chromosome, with a large gap at the end of the short arm and a 37.7-cM interval between ABG473 and CDO504. As with chromosome 5, the only agronomic trait QTL was for plant height, with 'Morex' contributing an allele accounting for a 4-cm difference. This region was also the site of significant QTL × E interaction for yield, with 'Morex' making a significant positive contribution in Washington. Grain protein and diastatic power QTL overlapped, but had no corresponding α-amylase or malt extract QTL. Likewise, the two α-amylase QTL had no corresponding diastatic power or malt extract QTL.

Discussion

As one objective of this report is to present an overview of QTL and QTL × E effects in a sample of barley germ plasm, we cannot, in this context, do full justice to the issue of map density on QTL detection and resolution. However, with these data, map density had no appreciable effect on QTL detection, magnitude of QTL effects, or resolution of QTL. This is not to say that higher marker density (i.e., 5 cM) might, in some cases, allow for greater QTL resolution. However, in most cases the higher mean density of our 295-point map was due to marker clustering in regions other than QTL peaks. Analyses based on relatively evenly spaced markers can serve to identify regions where increased marker density would be desirable, such as on chromosome 3, where yield and α-amylase QTL occupy adjacent, wide intervals (17.6 and 12.6 cM, respectively). In the absence of alternative favorable alleles mapping to the same or adjacent intervals, an average marker density of 10 cM should be adequate for MMAS. Given a constant population size, increasing maker density will have a modest impact on QTL resolution. The degree of resolution required for map-based cloning may best be achieved by using alternative genetic stocks, such as isogenic lines, rather than attempting to saturate a mapping population with markers (van Ooijen 1992).

QTL were detected that controlled grain yield and malting quality in progeny derived from a cross of agronomically relevant germ plasm. Highly significant effects were found for all traits based on the evaluation of 150 doubled haploids in a representative sample of environments using a genome map providing an average marker density of 9.6 cM. This level of map density should be adequate for MMAS, particularly in the absence of contrasting favorable alleles mapping to the same or adjacent intervals. In this germ plasm, there was only one such case, on chromosome 2, where a 'Steptoe' yield QTL allele coincided with the support intervals for 'Morex' diastatic power and malt extract QTL alleles. The conservative criteria we employed for QTL detection limit both the number of QTL and their resolution. However, QTL that would be declared as

significant at lower Wald values would be well below the limits of detection provided by most field experiments.

The non-linear model analysis allowed for hypothesis tests regarding QTL × E interaction. Such an approach may be of assistance in cases, such as the one described by Paterson et al. (1991b), where the value of average and environment-specific QTL complicates MMAS. All QTL × E interactions in these data were due to changes in the magnitude of significant response. Malting quality traits were remarkably free of interaction effects, while grain yield showed the greatest interaction.

Enumeration of the number of QTL and their total contribution may be useful for comparative purposes. A tabulation of the number of QTL for each trait and the cumulative coefficient of determination (r^2) value for each character is presented in Table 4. For the purposes of this tabulation, the number of QTL was determined by considering two QTL with overlapping support intervals as a single QTL. However, peak intervals were used for estimating multilocus r^2 values. The pattern of favorable QTL allele contribution was supported by the population frequency distributions. There was significant transgressive segregation for agronomic traits, and the two parents contributed approximately the same number of favorable alleles for height and heading date. 'Morex' contributed four reduced lodging QTL and one significant average effect QTL for yield. All three cases of significant QTL × E interactions for yield were due to a favorable allele that was significant in only one of the five environments. An offensive selection program for grain yield and agronomic fitness (i.e., reduced lodging and optimum plant height) should be effective. Selection for malting quality, on the other hand, would require a defensive strategy of maintaining the favorable allele complement already present in 'Morex'.

Characterizing genetic mechanisms underlying QTL, and the interrelationships of QTL, is an enormous challenge. Most, but not all, of the positive yield QTL alleles detected in this population could be ascribed to negative lodging QTL alleles coming from the opposite parent. Nonetheless, these results are not trivial, as straw strenth remains a principal limiting factor in intensive barley production. QTL for malting quality traits were often coincident, which is reasonable, given the reaction products measured by α-amylase and diastatic power protocols and the causative relationship between these enzymatic characters and malt extract. QTL without coincident peaks for related traits, such as the 'Steptoe' high diastatic power QTL allele on chromosome 4, are of particular interest and are promising targets for further understanding the biochemistry of the malting process.

Without a further separation of trait components, we can only speculate on genome organization and the existence of multilocus clusters, the presence of which has been eloquently described by Allard (1988) and hypothesized by Hayes et al. (1993). However, with the exception of chromosome 5, QTL for all characters were relatively dispersed, both within chromosomes and throughout the genome. There was, of course, a clustering of effects, but whether this was due to linkage, pleiotropy, or correlation remains to be determined.

Some of the observed QTL effects can be related to known function genes and previously mapped Mendelian factors. Highly significant α-amylase effects were found on chromosome 1 in the vicinity of the *Amy2* locus. Significant protein and diastatic power QTL were found in the vicinity of *Amy1* on chromosome 6, but no α-amylase effects were detected. Likewise, no grain protein QTL were detected on chromosome 5 in the vicinity of the hordein loci, although large diastatic power effects were detected in these regions. The *Bmy2* locus is within the WSI for diastatic power QTL on chromosome 2, and a large diastatic power effect was found in the *Bmy1-ksuH11* interval on chromosome 4. In terms of the agronomic traits, the large heading date effect on chromosome 2 maps to the same region as the *Ea* maturity locus (Nilan 1964). The traits that we have analyzed from a QTL standpoint have traditionally been addressed via experiments designed to estimate genetic parameters – i.e., genetic variances, heritability, and combining ability (reviewed by Hockett and Nilan 1985). Ultimately, we hope to reconcile these genetic parameter estimates with QTL-based analyses.

In summary, we have found significant QTL effects in a sample of relatively elite germplasm in an auto-

Table 4. Number of QTL alleles contributed by each parent and the corresponding multilocus r^2 values for agronomic and quality traits

Donor of longer value allele	Yield	Lodging	Height	Heading date	Grain protein	Alpha-amylase	Diastatic power	Malt extract
Steptoe	5	4	4	5	1	0	1	1
Morex	1	2	6	4	5	9	8	6
Multilocus r^2	0.58	0.71	0.72	0.67	0.56	0.63	0.67	0.57

gamous species. However, until estimates of QTL effects are validated in selection response experiments, they should be viewed with the same caution as traditionl estimates of genetic parameters. Procedures for distinguishing multiple-linked QTL, for simultaneously estimating multilocus effects, and for estimating epistatic interactions have yet to be implemented. Nonetheless, having detected effects of consistent magnitude and significance, it is now time to empirically validate QTL estimates. The North American Barley Genome Mapping Project is currently supporting QTL validation efforts based on both MMAS-backcrossing and the creation of ideal genotypes based on the designed complementary matings of genotyped individuals.

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