Theor Appl Genet (2002) 105:237–247
DOI 10.1007/s00122-002-0941-1

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QTLs affecting kernel size and shape in a two-rowed by six-rowed barley cross

Received: 14 May 2001 / Accepted: 11 October 2001 / Published online: 14 June 2002

Abstract The suitability of barley (*Hordeum vulgare* L.) grain for malting depends on many criteria, including the size, shape and uniformity of the kernels. Here, image analysis was used to measure kernel size and shape attributes (area, perimeter, length, width, F-circle and F-shape) in grain samples of 140 doubled-haploid lines from a two-rowed (cv Harrington) by six-rowed (cv Morex) barley cross. Interval mapping was used to map quantitative trait loci (QTLs) affecting the means and within-sample standard deviations of these attributes using a 107-marker genome map. Regions affecting one or more kernel size and shape traits were detected on all seven chromosomes. These included one near the *vrs1* locus on chromosome 2 and one near the *int-c* locus on chromosome 4. Some, but not all, of the QTLs exhibited interactions with the environment and some QTLs affected the within-sample variability of kernel size and shape without affecting average kernel size and shape. When QTL analysis was conducted using data from only the two-rowed lines, the region on chromosome 2 was not detected but QTLs were detected elsewhere in the genome, including some that had not been detected in the analysis of the whole population. Analysis of only the six-rowed lines did not detect any QTLs affecting kernel size and shape attributes. QTL alleles that made kernels larger and/or rounder also tended to improve malt quality and QTL alleles that increased the variability of kernel size were associated with poor malt quality.

Keywords *Hordeum vulgare* · Row number · Quantitative trait loci · Kernel size and shape · Digital image analysis

Communicated by P. Langridge

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Introduction

Barley grain used for malting should be uniform and plump to allow for consistent processing and for high yields of malt extract. Kernel size and uniformity are therefore important factors in the development of new cultivars of malting barley and in the selection of lots of barley grain for malting.

One important factor affecting the size, shape and uniformity of barley kernels is the number of rows of kernels on the barley spike. Barley spikes can be two-rowed or six-rowed. In both types, there are three spikelets at each node of the rachis but in two-rowed barley only the central spikelet is fertile, while in six-rowed barley all three spikelets are fertile. Two-rowed barley grain is generally preferred for malting, although six-rowed cultivars are preferred in some markets. Kernels of six-rowed cultivars are expected to be smaller and more variable in size and shape than those of two-rowed cultivars, because kernels from lateral florets tend to be smaller and less symmetrical than those from central florets. Row number is genetically determined, and is influenced mainly by a locus on chromosome 2L (Leonard 1942) that is now designated *vrs1* (BGS6, Lundqvist et al. 1997). The *vrs1.a* allele is present in most six-rowed cultivars while the *Vrs1.b* allele is present in most two-rowed cultivars. Other alleles at the same locus [*Vrs1.t, Vrs1.d and vrs1.c* (BGS6, 66 and 58 respectively, Lundqvist et al. 1997)] can modify the development and fertility of the lateral spikelets.

The importance of *vrs1* (and/or other tightly linked loci) in determining kernel size has been confirmed by comparisons of near-isogenic two-rowed and six-rowed lines (Hockett and Standridge 1975; Takahashi et al. 1975; McGuire and Hockett 1983) and comparisons of two-rowed and six-rowed progeny from crosses segregating at *vrs1* (Start and Riggs 1986; Powell et al. 1990; Jui et al. 1997). Furthermore, QTL mapping experiments have detected QTLs for kernel weight or plumpness that apparently coincide with the *vrs1* locus (Kjaer and Jensen 1996; Marquez-Cedillo et al. 2000)

The *int-c* (BGS178, Lundqvist et al. 1997) locus on chromosome 4S can also affect spike morphology, and its effects interact with those of *vrs1* (Woodward 1947, 1949). Most cultivars of two-rowed barley (including Harrington, the two-rowed parent used in this study) carry the *int-c.b* allele. Their lateral spikelets are normally small, have no anthers and do not set seed. Most cultivars of six-rowed barley (including Morex, the six-rowed parent used in this study) carry the *Int-c.a* allele. Their lateral spikelets are large, fertile and sessile. Plants that carry both the *Vrs1.b* and *Int-c.a* alleles (i.e., two-rowed plants with the *Int-c.a* allele from six-rowed barley) can be distinguished from the *int-c.b* two-rowed homozygotes because their lateral spikelets, although sterile, are noticeably inflated and contain anthers. Plants that are homozygous for both the *vrs1.a* and *int-c.b* alleles (i.e., six-rowed plants with *the int-c.b* allele from two-rowed barley) can be distinguished from the *Int-c.a* six-rowed plants because their lateral spikelets are pedicellate rather than sessile. A third allele, *Int-c.h*, can cause lateral spikelets of two-rowed genotypes occasionally to set seeds (Gymer 1977). This effect has also sometimes been observed in plants carrying *Int-c.a* (J. Franckowiak, personal communication).

Other loci have also been reported to affect spike morphology and kernel development in barley (Woodward 1947, 1949). Mutations at the locus *vrs2* (BGS314, Lundqvist et al. 1997) on chromosome 7(5H)L can reduce the size of lateral spikelets on the upper and lower portions of six-rowed spikes. Mutations at the locus *vrs3* (BGS315, Lundqvist et al. 1997) on chromosome 5(1H)L can promote seed development in lateral spikelets on the upper two-thirds of spikes that would otherwise be two-rowed. Mutations at the locus *vrs4* (BGS124, Lundqvist et al. 1997) on chromosome 3L can affect the formation and development of lateral spikelets, and cause the formation of additional spikelets. The *sls* (BGS227, Lundqvist et al. 1997) locus on chromosome 5(1H)S can affect the development of lateral spikelets in the progeny of crosses between two-rowed and six-rowed parents (Lundqvist et al. 1997).

Several QTLs affecting kernel weight and/or kernel plumpness have been detected and mapped in two-rowed barley (Backes et al. 1995; Thomas et al. 1995; Tinker et al. 1996; Bezant et al. 1997; Mather et al. 1997), in six-rowed barley (Han and Ullrich 1994), and in populations derived from two-rowed by six-rowed crosses (Kjaer and Jensen 1996; Marquez-Cedillo et al. 2000). In these, kernel size was assessed as mean kernel weight (often expressed as thousand kernel weight) and/or as kernel plumpness, the proportion or percentage of grain that does not pass through a screen of a defined mesh size (usually either 1.98 or 2.38 mm). Mean kernel weight provides an estimate of the average kernel size in a variable sample or lot of grain, but does not provide any information on variation in size among kernels nor on kernel shape. Kernel plumpness depends on average kernel size, on kernel shape and on the distribution of kernel sizes and shapes within the sample, but it does not fully describe the range of kernel sizes that may be present. With digital image analysis, it is possible rapidly to measure the dimensions of individual cereal kernels (Berman et al. 1996; Symons et al. 1996) and thus to investigate within-sample variation for both kernel size and shape. Image analysis has been used successfully in wheat classification (Symons and Fulcher 1988b), wheat grading and cultivar identification (Draper and Keefe 1989), and in studies of variation in oat kernel morphology (Symons and Fulcher 1988a). In barley, it has been used to study differences in kernel morphology between lateral and central kernels (Gebhardt et al. 1993), and to identify cultivars (Shrestha 1996). Compared to standard methods for the assessment of the quality of barley grain for malting, image analysis is rapid and inexpensive. It has therefore been investigated as a predictor of malt quality (Freeman et al. 1995; Van Laarhoven et al. 1997; García del Moral et al. 1998). Edney et al. (1999) used multivariate analysis of variance to relate malt extract, friability and diastatic power to variables related to kernel size and shape assessed by image analysis in a set of contrasting samples selected from the Harrington by Morex barley mapping population. They found no clear relationship between kernel shape and malt quality, but they reported that samples with larger kernels tended to have higher diastatic power, but were less friable, and produced less extract than samples with smaller kernels. Frégeau-Reid et al. (1996) used image analysis to assess variation in kernel size and shape among doubled-haploid progeny derived from another cross between two-rowed and six-rowed parents. They reported that the *vrs1* locus was responsible for between 68 and 91% of the total variation for kernel size, and between 7 and 40% of the total variation of kernel shape.

Here, image analysis was used to quantify the sizes and shapes of kernels of doubled-haploid lines in a barley mapping population for which QTLs affecting grain and malt quality traits had already been mapped (Marquez-Cedillo et al. 2000). Our objectives were to map QTLs affecting kernel size and shape, and their variability, and to compare the positions of these QTLs to those of loci affecting spike morphology and/or grain and malt quality traits.

Materials and methods

The mapping population consisted of 140 F_1-derived doubled-haploid progeny from the cross of Harrington with Morex. Harrington is a two-rowed malting cultivar from western Canada (Harvey and Rossnagel 1984) while Morex is a six-rowed malting cultivar from the Midwestern U.S.A (Rasmusson and Wilcoxson 1979). Harrington carries the alleles *Vrs1.b* and *int-c.b*, while Morex carries *vrs1.a* and *Int-c.a*. Harrington, Morex and the 140 doubled-haploid lines were grown in three locations in 1995 and five locations in 1996 (Marquez-Cedillo et al. 2000). Samples of each line and each parent from two locations (Brandon, Manitoba, Canada; and Saskatoon, Saskatchewan, Canada) and from both years were used here. Sub-samples from these site-years had been evaluated for grain and malt quality traits at the Grain Research Laboratory of the Canadian Grain Commission in Winnipeg, Manitoba, Canada (1995 samples) and the USDA-ARS Cereal Crops Research Unit

in Madison, Wisconsin, U.S.A. (1996 samples) using standard procedures of the American Society of Brewing Chemists (ASBC 1992) to measure thousand kernel weight (g), test weight (kg hl^{-1}), kernel plumpness (%), grain protein content (%), the ratio of soluble to total protein, alpha-amylase activity (DU), diastatic power (° Lintner) and fine-grind malt extract (%). Additional sub-samples of 300 randomly selected kernels were drawn for image analysis. These kernels were laid flat, crease-side down, under the objective of a NC-70x black and white camera (DAGE MTI, Michigan City, Ind.) with a 50-mm Canon Macro lens. Images were taken in artificial lighting. The images were digitized via the software KS400 (v 2.0) (Carl Zeiss Imaging, Hallbergmoos, Germany). For each kernel, the following variables were recorded: width (mm), length (mm), perimeter (mm), area (mm^2), aspect ratio (F-shape = width/length) and circular shape factor (F-circle = 4πArea/perimeter^2). For both F-circle and F-shape a perfect circle gives a value of unity. SAS PROC CHART (SAS 1988) was used to screen the data to remove outlier values that apparently represented specks of dust or pairs of kernels touching each other. The mean and the within-sample standard deviation of each variable were computed for each line. SAS PROC CORR (SAS 1988) was used to calculate Pearson's correlation coefficients on the means over site-years of kernel size and shape variables as assessed by image analysis.

Detection and mapping of QTLs were performed by simple interval mapping with the software package MQTL (Tinker and Mather 1995), using the same 108-marker linkage map (Fig. 1) and genotypic data previously used to map QTLs for grain and malt quality traits in the same population (Marquez-Cedillo et al. 2000). These analyses were done on the whole population and also on sub-populations consisting of 72 two-rowed lines that had been scored as carrying the Harrington allele at the *vrs1* locus (i.e., the *Vrs1.b* allele) and 68 six-rowed lines that had been scored as carrying the Morex allele at the *vrs1* locus (i.e., the *vrs1.a* allele).

QTL analysis consisted of four steps: (1) performing interval mapping to find evidence of QTLs, (2) estimating thresholds for inferring QTL presence, (3) inferring the presence of QTLs and estimating their positions, and (4) estimating the additive effects at putative QTLs. Significance thresholds for the test statistics were established by 1,000 permutations to keep the genome-wise Type-I error rate below 5% (Churchill and Doerge 1994). QTLs were declared at the genomic positions of test-statistic peaks for main effects and/or QTL by environment interactions that met or exceeded the threshold. When evidence for the QTL main effect and a QTL by environment interaction were near the same position, it was inferred that a single QTL was present at the interaction position. Effects 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) was estimated for two full models relative to a reduced model. One full model included QTL main effects and environmental main effects. The other full model included QTL main effects, QTL by environment interactions and environmental main effects. The reduced model included only the environmental main effects.

Results

Kernels of Harrington were larger than those of Morex, and kernels of the two-rowed doubled-haploid lines were larger than those of the six-rowed doubled-haploid lines (Tables 1, 2). Kernels of Morex were a little rounder than those of Harrington, yet kernels of two-rowed lines were a little rounder than those of the six-rowed lines (Table 2).

Kernel width and area were more variable within samples of Harrington than within samples of Morex (see the within-sample standard deviations in Table 2). At 2 site-years (Brandon 1995 and Saskatoon 1996) kernel length and perimeter were less variable (Table 2) in samples of Harrington than in samples of Morex. Kernel width was less variable within samples of two-rowed lines than within samples of six-rowed lines. Kernel length, perimeter and area were usually less variable within samples of two-rowed lines than within samples of six-rowed lines, except for those from the 1995 experiment at Brandon (Table 2) for which samples of two-rowed lines were more variable than samples of six-rowed lines.

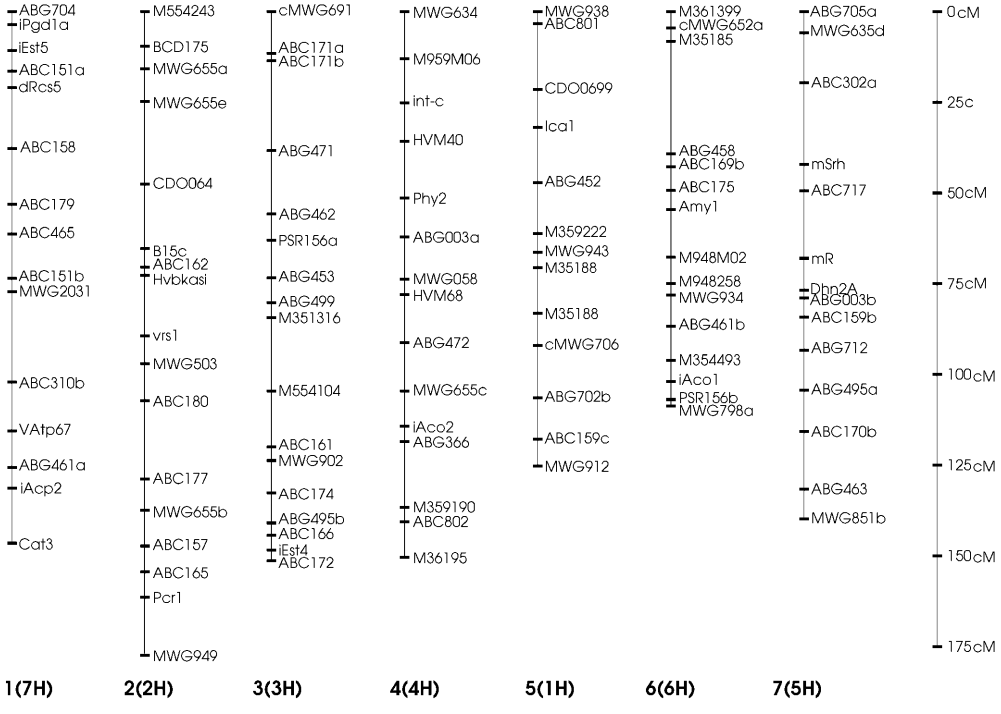

Fig. 1 Linkage map of genetic markers for the barley cross Harrington × Morex

Table 1 Mean thousand kernel weight for Harrington and Morex barley, and means and among-line standard deviations (SD) for thousand kernel weight for two-rowed (n = 72) and six-rowed (n = 68) doubled-haploid lines derived from the F_1 generation of a cross between Harrington and Morex grown at two locations in 1995 and 1996

Year	Location	Thousand kernel weight (mg)					
		Harrington mean	Morex mean	Two-rowed lines		Six-rowed lines	
				Mean	SD	Mean	SD
1995	Brandon	40.4	37.2	45.8	3.3	33.9	3.7
	Saskatoon	47.2	42.2	50.4	3.6	35.8	4.0
1996	Brandon	48.4	42.2	50.5	3.3	36.5	4.2
	Saskatoon	38.0	33.6	43.4	4.3	30.4	3.4

Table 2 Means and mean within-sample standard deviations (SD) for six kernel size and shape traits assessed by image analysis for Harrington, Morex, and two-rowed and six-rowed doubled-haploid lines derived from the F_1 generation of a cross between Harrington and Morex barley

Year	Location	Width (mm)		Length (mm)		Perimeter (mm)		Area (mm^2)		F-circle		F-shape	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Harrington													
1995	Brandon	3.43	0.33	9.02	0.60	21.75	1.41	20.87	2.74	0.55	0.03	0.38	0.03
	Saskatoon	3.30	0.29	8.78	0.78	21.03	1.73	19.13	2.76	0.54	0.04	0.38	0.03
1996	Brandon	3.41	0.29	8.53	1.25	20.98	2.95	19.55	3.66	0.56	0.05	0.40	0.04
	Saskatoon	3.20	0.32	8.12	0.61	19.68	1.41	17.04	2.45	0.55	0.04	0.39	0.04
Morex													
1995	Brandon	3.25	0.23	8.17	0.70	19.96	1.55	18.74	2.44	0.59	0.03	0.40	0.03
	Saskatoon	3.20	0.24	8.43	0.75	20.35	1.67	18.54	2.57	0.56	0.04	0.38	0.03
1996	Brandon	3.22	0.24	7.70	0.76	19.02	1.71	16.99	2.53	0.59	0.04	0.42	0.04
	Saskatoon	2.97	0.30	7.49	0.72	18.30	1.61	15.07	2.47	0.56	0.05	0.40	0.04
Two-rowed doubled-haploid lines (n = 72)													
1995	Brandon	3.39	0.30	9.35	0.87	22.38	1.92	21.15	2.74	0.53	0.05	0.37	0.04
	Saskatoon	3.46	0.30	9.15	1.08	22.06	2.32	21.39	3.02	0.56	0.06	0.38	0.04
1996	Brandon	3.52	0.30	8.67	0.82	21.28	1.86	20.71	2.95	0.58	0.05	0.41	0.04
	Saskatoon	3.37	0.31	8.16	0.73	20.05	1.66	18.67	2.74	0.58	0.04	0.41	0.04
Six-rowed doubled-haploid lines (n = 68)													
1995	Brandon	2.99	0.31	8.75	0.79	20.70	1.77	17.53	2.71	0.51	0.05	0.34	0.04
	Saskatoon	3.00	0.34	8.51	1.08	20.31	2.37	17.16	3.16	0.52	0.06	0.36	0.04
1996	Brandon	3.08	0.36	7.97	0.82	19.40	1.88	16.65	3.12	0.55	0.05	0.39	0.04
	Saskatoon	2.85	0.39	7.78	0.75	18.77	1.76	14.92	2.97	0.53	0.05	0.37	0.04

All kernel size and shape traits as assessed by image analysis were correlated with each other, except for length with F-shape and with F-Circle, and also the perimeter with F-shape and with F-Circle (Table 3). Correlation coefficients ranged from 0.452 (between kernel area and F-circle) to 0.983 (between kernel length and perimeter).

QTL analysis of grain and malt quality traits

For thousand kernel weight, the Harrington allele(s) at or near the *vrs1* locus on chromosome 2 exhibited a large positive effect, and a strong QTL by environment interaction (Fig. 2). In the analysis of the two-rowed sub-population, the only QTL detected for thousand kernel weight was on chromosome 7(5H), near the marker ABC302A (at the 19.6 cM position). At that QTL, the Morex allele increased kernel weight.

For other grain and malt quality traits, the results of QTL analysis on the basis of the data from the four environments considered here were similar to those reported by Marquez-Cedillo et al. (2000) on the basis of data from eight environments. In the analyses of data from the whole population and from the two-rowed sub-population, at least one QTL was detected for each trait. Some of these QTLs exhibited QTL by environment interaction. In the analysis of the six-rowed sub-population, no QTLs were detected for any trait (data not shown).

QTL analysis of kernel size and shape traits assessed by image analysis

Significant QTL and QTL by environment interactions were detected for kernel size and shape variables in analyses of the whole population (Fig. 3) and the two-rowed sub-population (Fig. 4), but not in analyses of the six-rowed sub-population (data not shown). In what follows, significant QTL and QTL by environment interactions are presented for each chromosome.

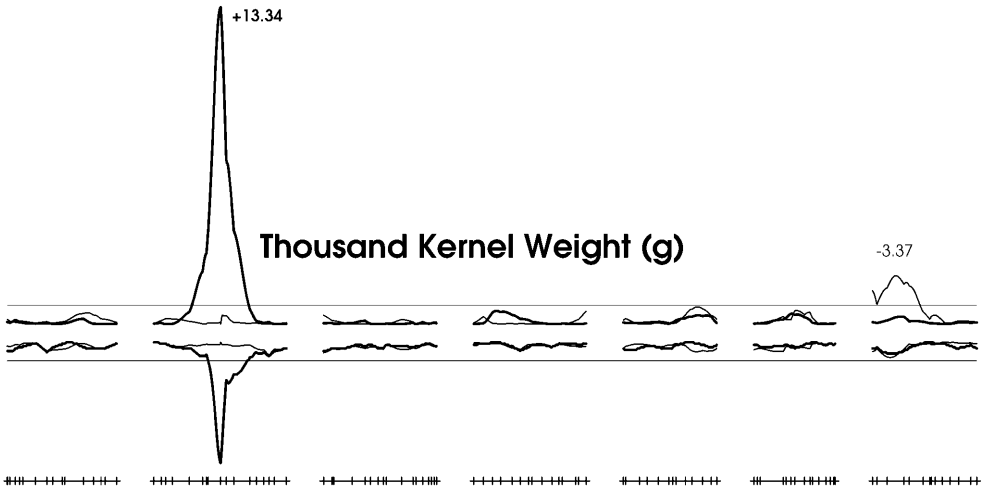

Fig. 2 Test statistic scans for thousand kernel weight for simple interval mapping of QTL main effects (above axes) and QTL by environment interactions (below axes) for the barley cross Harrington × Morex. Barley chromosomes 1 to 7 (7H, 2H, 3H, 4H, 1H, 6H, 5H respectively) are shown left to right, each oriented with the 'plus' arm on the left. A horizontal scale shows approximate marker positions (tick marks). *Horizontal solid lines* show significance thresholds estimated from 1,000 permutations of the data to maintain the experiment-wise type-I error rate below 5%. *Bold lines* show test statistics from QTL analyses of the whole population. *Thin lines* show test statistics from QTL analyses of the two-rowed sub-population. Estimated QTL effects (i.e., the average effects of substituting two Harrington alleles for two Morex alleles) are shown in *bold* for the whole population and in *regular type* for the two-rowed sub-population

Table 3 Pearson's correlation coefficients of the means over site years for kernel size and shape traits as assessed by image analysis

Item	Length	Perimeter	Area	F-circle	F-shape
Width	0.606*	0.728*	0.950*	0.675*	0.750*
Length		0.983*	0.809*	–0.146	–0.058
Perimeter			0.893*	0.013	0.112
Area				0.452*	0.518*
F-circle					0.965*

*Significant at the 0.05 level

Chromosome 1(7H). In a region near marker ABC465 (at the 61.3-cM position), QTL main effects and interactions for F-circle and F-shape were detected. Based on analysis of data from the whole population, this region had a main effect on the within-sample variability of F-circle (with the Morex allele increasing this variability) and both a main effect and a QTL by environment interaction for the within-sample variability of F-shape (with the Morex allele being the source of a high variability in one of four environments) (Fig. 3). When only the two-rowed progeny were considered, this QTL was found to have main effects on the mean values of F-shape and F-circle (with the Morex allele causing rounder kernels) (Fig. 4).

Chromosome 2. Two QTL regions were mapped on chromosome 2 in the analysis of the whole population (Fig. 3). Neither of these was detected in the analysis of the two-rowed sub-population (Fig. 4). Near marker CDO064 (47.6 cM), there is a minor QTL at which the Harrington allele increased the within-sample variability of kernel area in three of the four environments (Fig. 3). Between the markers HVBKASI (72.7 cM) and *vrs1* (89.4 cM), QTL effects were detected for the mean values of all of the kernel size and shape characteristics assessed by image analysis (Fig. 3). In all cases, the Harrington allele substantially increased these values, causing larger, rounder kernels. In the same region, the Morex allele increased the within-sample variability of kernel width (Fig. 3) (particularly in 1996) and kernel area (particularly in samples from Saskatoon in 1996), and increased the within-sample variability of F-shape and F-circle, also with differential effects across environments (Fig. 3).

Chromosome 3. In the analyses of both the whole population and the two-rowed sub-population, QTLs were mapped between the markers ABG462 (55.8 cM) and M351316 (84.3 cM) for which the Harrington allele made the kernels longer (greater mean kernel length and perimeter) and less round (lower mean F-shape and F-circle) (Figs. 2 and 3). Near the marker ABG495B (140.9 cM), a QTL was found for which the Harrington allele increased mean kernel width, area and perimeter in two of the four environments. This QTL was detected in the analysis of the whole population (Fig. 3) but not in the analysis of the two-rowed sub-population (Fig. 4).

Chromosome 4. In the analysis of the whole population, a QTL was mapped between *int-c* (25.1 cM) and the marker HVM40 (35.7 cM), at which the Harrington allele increased the within-sample variability of kernel width and area (Fig. 3). In the two-rowed sub-population, there was a similar effect, but only for the variability of kernel width and at a somewhat more-distal position on the chromosome (near marker M959M06 at 13 cM).

Chromosome 5(1H). In the analysis of the whole population (Fig. 3), a QTL was mapped in the region near

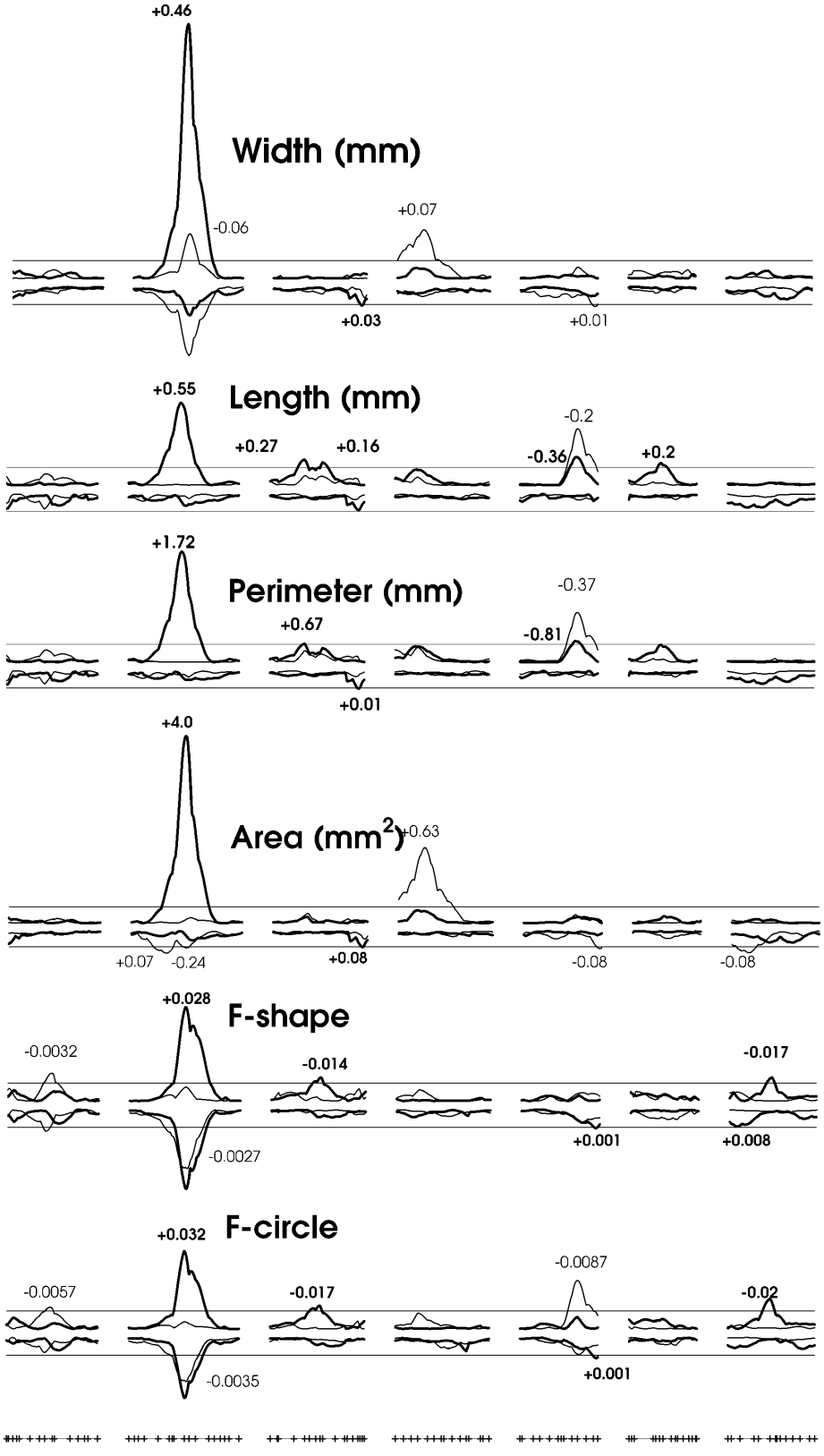

Fig. 3 Test statistic scans for kernel size and shape characteristics as assessed by image analysis for simple interval mapping of QTL main effects (above axes) and QTL by environment interactions (below axes) for the barley cross Harrington × Morex in the Harrington by Morex barley mapping population. Barley chromosomes 1 to 7 (7H, 2H, 3H, 4H, 1H, 6H, 5H respectively) are shown left to right, each oriented with the 'plus' arm on the left. A horizontal scale shows approximate marker positions (tick marks). *Horizontal solid lines* show significance thresholds estimated from 1,000 permutations of the data to maintain the experiment-wise type-I error rate below 5%. *Bold lines* show test statistics from QTL analyses of trait means. *Thin lines* show test statistics from QTL analysis of within-sample standard deviations. Estimated QTL effects (i.e., the average effects of substituting two Harrington alleles for two Morex alleles) are shown in *bold* for the mean and in *regular type* for within-sample standard deviations

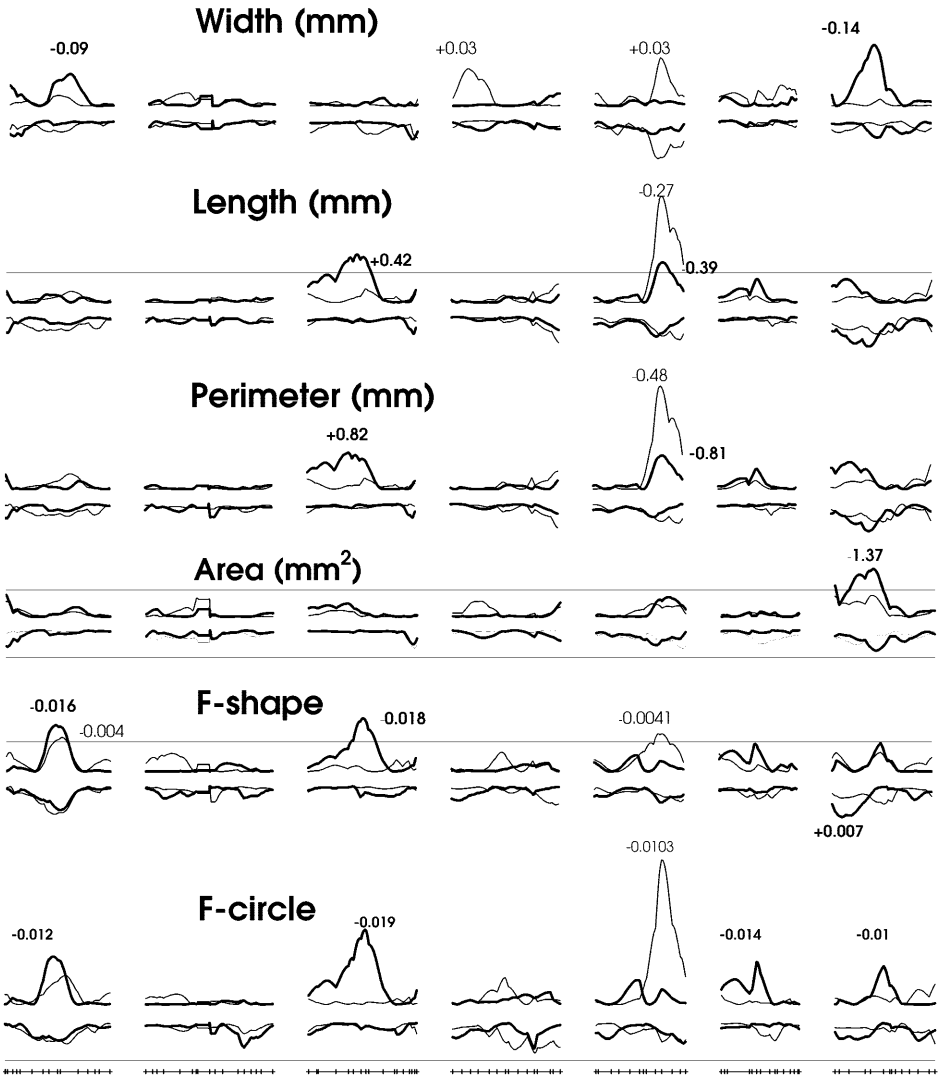

Fig. 4 Test statistic scans for kernel size and shape characteristics as assessed by image analysis for simple interval mapping of QTL main effects (above axes) and QTL by environment interactions (below axes) for the barley cross Harrintong × Morex in the two-row lines of the Harrington by Morex barley mapping population. Barley chromosomes 1 to 7 (7H, 2H, 3H, 4H, 1H, 6H, 5H respectively) are shown left to right, each oriented with the 'plus' arm on the left. A horizontal scale shows approximate marker positions (ticks mark). *Horizontal solid lines* show significance thresholds estimated from 1,000 permutations of the data to maintain the experiment-wise type-I error rate below 5%. *Bold lines* show test statistics from QTL analysis of means. *Thin lines* show test statistics from QTL analysis of within-sample standard deviations. Estimated QTL effects (i.e. the average effects of substituting two Harrington alleles for two Morex alleles) are shown in *bold* for the mean and in *regular type* for within-sample standard deviations

markers M35188 (83.1 cM) and cMWG706 (92 cM) at which the Morex allele increased the mean and the within-sample variability of kernel length and perimeter, and the within-sample variability of F-circle in three of the four site years. In the two-rowed sub-population, the same region affected the mean and the within-sample variability of kernel length and perimeter and the within-sample variability of kernel width, and both F-circle and F-shape (Fig. 4). In this case, the Morex allele increased the values of these variables except for within-sample variability of kernel width, which it decreased in three of the four site-years.

In the analysis of the whole population, a QTL was mapped near marker ABC159c (1,17.9 cM), at which the Harrington allele increased the within-sample variability of kernel width and the mean of both F-shape and F-circle, but decreased the within-sample variability of kernel area. This QTL showed significant interaction with environments, with effects in only two or three of the four environments.

Chromosome 6. Near the marker ABC175 (49.3 cM) there is a QTL at which the Harrington allele was found to increase mean kernel length in the analysis of the whole population (Fig. 3) and to decrease the mean F-circle in the analysis of the two-rowed sub-population (Fig. 4).

Chromosome 7(5H). In the analysis of the whole population, a QTL was detected between markers MWG635D (5.9 cM) and ABC302A (19.6 cM), at which the Harrington allele increased the mean F-shape but decreased the within-sample variability of kernel area, but not in all environments (Fig. 3). Within the two-rowed sub-population, the Harrington allele increased the mean F-shape in three environments (Fig. 4).

Another QTL was found between markers ABC717 (49.4 cM) and the locus responsible for smooth awns *mR* (68.0 cM) (re-designated as *raw1*, BGS312, Lundqvist et al. 1997). There, the Harrington allele decreased the mean kernel size (kernel width and kernel area, but only within the two-rowed sub-population) and roundness (F-shape and F-circle in the whole population, F-circle in the two-rowed sub-population) (Figs. 2 and 3).

Discussion

In this study, QTLs affecting kernel size and shape variables were detected on each chromosome of the barley genome and these included loci affecting only the means of kernel size and shape characteristics, loci affecting only the within-sample variability for these characteristics and loci affecting both the means and the variability.

A major QTL on chromosome 2, at or near the locus *vrs1*, affected kernel plumpness, thousand kernel weight and the means of all kernel size and shape characteristics assessed by digital image analysis. This was expected, because *vrs1* is the major locus determining the row-number phenotype in barley. This QTL explained between 19 and 76% of the phenotypic variance for the kernel size and shape traits. This region also affected the within-sample variability of kernel area, kernel width, F-shape and F-circle in some environments, but explained only 1 to 15% of the total among-sample variance for these traits, with most of the rest of the variance being due to the environment. Direct effects of this QTL on kernel size and shape are presumably due to differences in the size and shape between kernels from central florets and those from lateral florets, with kernels from central florets generally expected to be larger and more symmetrical than those from lateral florets. The QTL effects estimated here were consistent with this expectation and with previous observations (Frégeau-Reid et al. 1996; Kjaer and Jensen 1996); the Harrington (two-rowed) allele apparently increased kernel size and roundness and decreased within-sample variation. These effects were associated with some favourable effects of the Harrington allele on grain quality traits (higher test weight, kernel plumpness and kernel weight) and diastatic power, but also with some unfavourable effects on protein traits (higher grain protein and lower soluble-to-total protein ratio). This chromosome region had no significant effects on alpha-amylase activity or malt extract.

Other genes affecting plant morphology are known to be closely linked with *vrs1* and these may have indirect effects on kernel size and shape. The principal effects of these loci are on culm length (Neatby 1929; Swenson and Wells 1944), rachis internode length (Swenson and Wells 1944) and peduncle length (J. Franckowiak, personal communication), all traits that could influence kernel size and shape via effects on spike density and/or stress tolerance. Thus, QTL effects in the *vrs1* region may not be due exclusively to the effects of *vrs1* itself.

QTL effects were also detected at or near *int-c* on chromosome 4. Consistent with the fact that alleles of *int-c* affect the development of lateral spikelets, the QTL in this region influenced the within-sample variability of kernel size. These effects were detected for both kernel width and kernel area in the analysis of the whole population, but only for kernel width in the analysis of the two-rowed sub-population. While it seems reasonable for *int-c* to have differential effects in two-rowed and six-rowed genotypes, it is not obvious why an effect on the variation of kernel area would be detected in the whole population but not in either sub-population. For both kernel width and kernel area, it was the Harrington allele that caused greater variability. This allele (and/or other Harrington alleles in the same region) lowered the malt extract and the soluble-to-total protein ratio, perhaps indicating that the lack of uniformity interfered with thorough and uniform modification. This association is consistent with the preference of maltsters for uniform lots of grain. The same allele(s) increased test weight and grain protein. The Harrington allele(s) in this region may also increase alpha-amylase activity (Marquez-Cedillo et al. 2000).

Several other QTL regions detected here are on the same chromosomes as loci known to affect the development of central and lateral spikelets [*vrs4* on chromosome 3 and *vrs2* on chromosome 7(5H)]. Neither *vrs2* nor *vrs4* have been mapped precisely, so it is not possible to say how closely they coincide with the estimated QTL positions. Several of the QTLs on these chromosomes were detected in the two-rowed sub-population but not in the six-rowed sub-population, indicating probable epistasis between these QTLs and *vrs1*. The QTL on chromosome 3 affected the means but not the standard deviations for kernel size and shape attributes, and had no apparent effects on other grain or malt quality characteristics. Within the two-rowed sub-population, the Morex allele at a QTL on chromosome 7(5H) (near the morphological marker *mR*) that caused kernels to be rounder had unfavourable effects on test weight and kernel plumpness. In the analysis of the whole population, the same allele apparently caused kernels to be rounder but had no effect on grain or malt quality. At another QTL on chromosome 7(5H), the Morex allele caused kernels to be more elongated, and increased kernel plumpness, test weight and grain protein.

Another locus that might be expected to affect kernel size variability is *sls* (small lateral spikelet), which is on the ‘plus’ arm of chromosome 5(1H). Harrington and Morex apparently carry contrasting alleles at this locus (J. Franckowiak, personal communication). Two QTLs with effects on kernel size and shape variability were detected on chromosome 5(1H), but both were on the ‘minus’ arm, apparently quite distant from *sls*. The locus *vrs3* is also on chromosome 5(1H) but there is no indication that Harrington and Morex carry contrasting alleles at this locus. Furthermore, *vrs3* is on the ‘plus’ arm but distal to *sls*. It should not be considered as a candidate gene for either of the QTLs detected here. At one of the QTLs on chromosome 5 (1H) (between 83.1 and 92.0 cM), the Harrington allele decreased kernel size and increased kernel size and shape uniformity. These effects were associated with a lower test weight, a lower grain protein content and a higher malt extract. At the other QTL on this chromosome (near 117.9 cM), the Harrington allele caused kernels to be rounder and had inconsistent effects on kernel size and shape variability, but had no effect on grain and malt quality traits.

QTL effects on kernel size and shape characteristics were also detected in regions of the genome where no lo-

ci for spike morphology are known, i.e., on chromosomes 1(7H) and 6. The QTL on chromosome 1(7H) affected kernel roundness and its variability, the Morex allele giving less-uniform kernels. This effect was associated with unfavourable effects on diastatic power. At the QTL on chromosome 6, the Morex allele causes kernels to be shorter in the whole population and rounder in the two-rowed sub-population. This allele also caused higher diastatic power, but it reduced alpha-amylase activity and test weight.

Given the extreme morphological differences between the two-rowed and six-rowed spikes, it is not surprising that QTL mapping gave different results in the two sub-populations. Loci that affect kernel size and shape in two-rowed genotypes may or may not affect these traits in six-rowed genotypes, and vice versa. This differential expression is a form of epistasis (i.e., interaction of QTLs with *vrs1*), and is consistent with a previous report of possible epistasis for kernel size and shape characteristics (Frégeau-Reid et al. 1996). However, the complete absence of significant QTLs for kernel size and shape characteristics in the analysis of the six-rowed sub-population is surprising. It can not be attributed to sample size; the six-rowed sub-population had almost as many lines as the two-rowed sub-population in which numerous QTLs were detected. It may be that greater within-sample variation in kernel size and shape in the six-rowed sub-population reduced the power of QTL detection. However, this also seems unlikely, given that, for most traits, there were no large differences in within-sample variation between the two-rowed and six-rowed sub-populations (Table 2). Thus, it may be that the population used here is segregating for QTLs affecting the size and shape of kernels on two-rowed spikes but not for any QTLs affecting the size and shape of kernels on six-rowed spikes. Perhaps the presence of both central and lateral kernels on six-rowed spikes has a stabilizing effect on average kernel size, and even on the within-sample variability. Such an effect might be related to compensation during grain filling. Alternatively, genetic effects may be exerted separately on central and lateral kernels and these effects may be confounded in analyses based on heterogeneous mixtures of the two kernel types. Results reported by Gebhardt et al. (1993) provide some support for this idea. In a study of a historical set of six-rowed barley genotypes, Gebhardt et al. (1993) manually separated central and lateral kernels from spikes, and assessed samples of each kernel type. They reported no significant changes in the weight of central kernels over time, but found that lateral kernel weight was higher in two recent genotypes than in older cultivars, indicating that selection may have had differential effects on central and lateral kernels. Furthermore, the most-recent line had smaller differences between the central and lateral kernel area than did the older cultivars. In QTL mapping, detection of loci that differentially affect central and lateral kernels might require separate analyses of the two kernel types. In the present study, this would have entailed manual separation of central and lateral kernels from a representative number of spikes of each of the 68 six-rowed lines. In a six-rowed mapping population, this would be even more laborious (because all progeny lines would require manual separation) but perhaps more fruitful; there would be greater potential to detect and map QTLs that could be directly exploited within the six-rowed germplasm pool.

Most of the kernel size and shape variables assessed here exhibited QTL by environment interaction. When only the magnitude of QTL effects vary among environments, there may be a significant main effect peak (a peak pointing upwards in Figs. 1, 2 or 3) at the same genomic location as the QTL by environment peak (a peak pointing downwards). One example of this is the QTL on chromosome 1(7H) affecting the within-sample variability of F-shape, where effects of the Harrington allele were negative (i.e., the Harrington allele caused a reduction in the variability) in all four environments, but varied in magnitude from –0.0067 to –0.0018. At some other positions where QTL by environment peaks were significant, there were no significant main-effect peaks. At these locations, there may be a reversal of QTL effects between site years. For instance, at a QTL on chromosome 2 in the region of *vrs1*, the Harrington allele increased the within-sample variability of F-circle and F-shape at Brandon in 1995 (Fig. 3) but decreased this variability at Brandon in 1996 and at Saskatoon in both years. A significant QTL by environment interaction can also occur without a significant main effect in cases where there is little or no effect in one or more site years. The QTL by environment interaction peak for within-sample variability of kernel area near marker CDO064 on chromosome 2 was due to the effect of the Harrington allele(s) in all environments, except in the year 1996 in the experiment grown in Brandon.

In this study, digital imaging provided more-complete descriptions of kernel size and shape than is possible with conventional measures such as thousand kernel weight and the percentage of plump kernels. Application of QTL mapping to data from digital image analysis permitted the detection of regions of the genome that affect kernel size and shape, and allowed us to determine which QTLs affect specific kernel size and shape characteristics. Furthermore, because digital image analysis provided data on individual kernels, we were able to compute estimates of within-sample variability and map QTLs affecting trait variability. In the two-rowed by six-rowed mapping population studied here, the most important QTLs coincided with loci known to affect spike morphology and kernel development, but these were not the only QTLs detected. Therefore, it seems that the same approach could be used within two-rowed or six-rowed populations to detect loci that underlie the more subtle variation in kernel size and shape that is expected in such populations.

As might be expected, in this study we found QTL regions common to both kernel size and shape characteristics as assessed by image analysis, and to kernel size as measured by conventional methods (for instance on chro-

mosomes 2 and 4). In general, bigger kernels were rounder and heavier. We also found QTL regions in common between grain protein content and some kernel size and shape characteristics, mainly on chromosome 5, where the alleles causing more variability of kernel width were responsible for lowering grain protein content. These alleles may affect kernel width by enhancing starch deposition, with a dilution effect on protein content.

Based on a subset (ten samples from each of two environments) of the image analysis and malt quality data used here, Edney et al. (1999) have already reported that malt quality variables such as malt extract and friability are associated with kernel area variables assessed by image analysis. The mapping analyses reported here permit consideration of the genetic basis for these associations. In the present study only a few QTLs were common to kernel size and shape characteristics and malt quality characteristics. QTL alleles that increased size and shape attributes, and that decreased the variability of such traits (for instance on chromosome 2), had favourable effects on malting quality (diastatic power). On chromosome 4, there were QTL alleles that increased the variability of kernel width and area, and decreased the ratio of soluble to total protein and diastatic power. On chromosome 5(1H) there were QTLs that decreased the variability of kernel size and that improved malt quality characteristics like the ratio of soluble to total protein and malt extract. This is consistent with the idea that uniform grain will germinate and modify at a uniform rate, resulting in superior malt. Malt quality traits were also affected by QTLs that had no effect on any of the kernel size and shape characteristics assessed by image analysis, notably those on the 'plus' arm of chromosome 5(1H) and the 'minus' arm of chromosome 7(5H) (Fig. 2 and Marquez-Cedillo 2000). These QTLs may be related more with physiological characteristics, such as plant hormone levels, enzyme synthesis and enzyme activation, that are essential for the production of quality malt.

Acknowledgements This research was supported by the Natural Sciences and Engineering Research Council of Canada, and by scholarships awarded to the first author by Agriculture and Agri-Food Canada and the 'Fonds pour la formation de chercheurs et l'aide à la recherche'.

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