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Association Between rDNA Alleles and Quantitative Traits in Doubled Haploid Populations of Barley

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ABSTRACT

Doubled haploids (DH) were generated from reciprocal F_1 hybrids which were heterozygous for alleles at the *Nor-H3* locus on chromosome 5H of barley. The r-DNA alleles did not deviate significantly from the expected 1:1 ratio and the DH progenies were classified into two groups based on the allelic constitution of the *Nor-H3* locus. The DHs were grown in a randomized, replicated field experiment and a range of agronomic and quality traits were recorded. The *Nor-H3* locus was associated with a significant portion of the genetic variation for: yield, thousand corn weight, water sensitivity and milling energy requirement of the grain. However, the magnitude of the differences between groups was dependent on the direction of the cross. The milling energy requirement of the grain was consistently associated with alleles at the *Nor-H3* locus. These results are presented in relation to the dynamics of rDNA evolution and variability. The potential of molecular markers in conjunction with doubled haploids to map quantitative traits in barley is also discussed.

NUCLEAR ribosomal DNA (rDNA) is typically found as tandemly repeated multigene families, with up to several thousand gene copies per genome (Appels and Honeycutt 1986; Rogers and Bendich 1987). The rDNA is located at the nucleolar organizers (NOR) which can be physically mapped by observing the nucleolar constriction in metaphase chromosomes and by *in situ* hybridization (Appels *et al.* 1980; Miller *et al.* 1980). Each repeat unit of rDNA contains a single rDNA transcription unit as well as an intergenic spacer (IGS) region that separates the transcription units of adjacent repeat units. The intergenic region has been found to be highly variable in a range of plants: *Triticum aestivum* (Appels and Dvořák 1982); *Triticum dicoccoides* (Flavell *et al.* 1986); *Hordeum vulgare* (Saghai-Maroof *et al.* 1984); *Vicia faba* (Rogers and Bendich 1987) and maize (Zimmer, Jupe and Walbot 1988). This variation primarily involves the number of base-pair repeats in the IGS region which can be detected by restriction enzyme and Southern blot analysis (Flavell 1983). The availability of rDNA-specific probes has allowed the IGS to be mapped intrachromosomally in wheat (Snape *et al.* 1985) and two Mendelian loci which determine IGS variability in barley have been located to chromosomes 6H and 5H (Saghai-Maroof *et al.* 1984).

The population dynamics of rDNA IGS variability has been examined by Allard and colleagues (Allard 1988; Saghai-Maroof *et al.* 1984) who observed that a class of ribosomal spacer DNA that was originally infrequent became predominant in a composite cross population of barley. The population had undergone 54 generations of selfing but there was no conscious or direct selection for rDNA. Flavell *et al.* (1986) examined variation in the IGS of rDNA of tetraploid wild emmer wheat (*T. dicoccoides*) originating from 12 sites in Israel. Considerable rDNA spacer length variation was detected but more importantly the rDNA variability was significantly correlated with a range of environmental variables. Variation in the rDNA IGS region has been observed in a maize population ("Hays Golden") subjected to mass selection for high grain yield (Rocheford, Osterman and Gardner 1990). Directional selection for yield, plant height and maturity was practised for 29 generations and significant changes in rDNA IGS hybridization pattern were detected. These three experiments clearly demonstrate that rDNA allele frequency changes are associated with selective forces operating on both cultivated and natural plant populations. However, these studies were unable to establish whether selection (natural or artificial) was operating on the rDNA loci or alleles tightly linked to them.

Bailey (1971) and Burr *et al.* (1988) have outlined the advantages of recombinant inbred lines in gene mapping. These include the fact that recombinant inbred lines represent a permanent population that can be used indefinitely for mapping. In some cases recombinant inbred lines are generated by several rounds of selfing so that homozygosity is approached. Alternatively doubled haploids (DH) can be generated from F_1 hybrids. DH extracted from F_1 hybrids are likely to exhibit a higher linkage disequilibrium rela-

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tive to other generations due to the reduced opportunities for recombination. Furthermore, the absence of within-family segregation resulting in greater additive genetic variation associated with DHs indicates that this approach is well suited to relating variation detected at the nucleic acid level to whole plant phenotypic variation. This approach has been used previously to analyse the effects of morphological (POWELL, CALIGARI and JINKS 1985; POWELL *et al.* 1985a, 1990; THOMAS *et al.* 1991) and isozyme loci (POWELL, ELLIS and THOMAS 1990b) on quantitative traits in random inbred lines of barley. In this paper we report on the associations between rDNA alleles and a range of quantitatively controlled traits of economic importance in DH populations of barley.

MATERIALS AND METHODS

Plant material: Microspore-derived plants were regenerated from reciprocal crosses between the spring barley cultivar Blenheim (*B*) and the SCRI breeding line E224/3 (*E*). Methods for plantlet regeneration from microspores have been described by FINNIE, POWELL and DYER (1989). Fifty-nine microspore derived lines from the $B \times E$ (B86–13) cross and 56 lines from the $E \times B$ (B86–19) cross were field multiplied in 1988. For each cross, the DH were grown, together with their parents (each entered twice) and other control cultivars, in a field trial in 1989. The trial design was a generalized lattice grown in two replicates and the trials were sprayed with fungicides to control foliar pathogens. During the growing season heading date (HD) was recorded as the number of days after 1 June by which the majority of the plot had reached growth stage (GS) 53. Height (cm) from the ground to the collar was recorded at GS 85 (Ht). At harvest the plots were harvested and the yield recorded as tonnes per hectare after drying to a constant moisture. The proportion of a cleaned sample of grain from each plot that passed over a 2.2-mm sieve (GT22), a 2.5-mm sieve (GT25) and a 2.8-mm sieve (GT28) was recorded. The GT25 proportion was retained for all subsequent measurements. Thousand corn weight (TCW) was calculated from the weight of 100 grains. The specific weight (SPW) of each sample of grain after cleaning was measured using a chondrometer and expressed as kg/hl. Germinative energy (GE3D), germinative capacity (GC5D) and water sensitivity (1WS3D, 1WS5D, 2WS3D, 2WS5D) were measured according to the Institute of Brewing's (1971) recommended methods. GE3D, GC5D, 1WS3D and 1WS5D were measured approximately 4 weeks after harvest. Both GE3D and GC5D were sufficiently high to suggest that dormancy had broken but 1WS3D and 1WS5D were quite low, indicating that there was considerable water sensitivity. The water sensitivity test was therefore repeated 4 weeks later (2WS3D and 2WS5D) and the figures suggested that much of the water sensitivity had disappeared. Percentage grain nitrogen was assessed by near infra-red reflectance (ALLISON, COWE and MCHALE 1978) predicted diastatic power (DPP) following papain extraction of grain (ALLISON and SWANSTON 1974) and milling energy (joules) (ME) by the modified comparamill (COWE, CUTHBERTSON and SWANSTON 1989). Hot water extract (1°/kg) (HWE) was measured following modified decro-malting (SWANSTON and TAYLOR 1990), which was carried out over 6 months after harvest by which time the effects of any residual dormancy or water sensitivity should be negligible. The trial entries were scored for all the quantitative traits to maintain the structure of the experiments and the standard error used to test the significance of differences in Table 3 was derived from the error variance of the whole trial analyzed as a generalised lattice design. When comparing groups by analysis of variance, the structure of the trial was lost and the data were analysed as if they had been grown in a randomised complete block design.

DNA procedures: To differentiate between alleles at the *Nor-H3* locus, rDNA restriction fragment length polymorphisms were scored. Leaf material was harvested from the 1989 field grown plots of the microspore-derived lines. Total plant DNA was extracted from powdered freeze-dried leaves by a modification of the CTAB extraction procedure (MURRAY and THOMPSON 1980). Following restriction endonuclease digestion (10 μg DNA) with *Eco*RV, the resulting DNA fragments were fractionated on 1% agarose/TAE gels and transferred to HYBOND N^+ charged nylon membrane (Amersham) by alkaline blotting (REED and MANN 1985). The filters were hybridised to ^{32}P-labeled pBG35, a recombinant plasmid containing an 8.6-kb insert of one flax rDNA repeat unit and includes structural genes for 25S, 18S and 5.8S rRNA as well as intergenic spacers (GOLDSBROUGH and CULLIS 1981). Twenty to one hundred nanograms of the intact plasmid served as the template for radioactive probe preparation by oligolabelling (FEINBERG and VOGELSTEIN 1984). Four DH lines from each cross gave inconclusive results and were excluded from the analysis.

RESULTS

The parental and DH means together with the range of inbred lines generated are given for each character in Table 1. Using the error variance of the trial to test for differences between the parental means, Blenheim (*B*) had a significantly earlier heading date, shorter height, higher yield, greater thousand corn weight and greater water sensitivity than E224/3 (*E*) in both trials. In the $B \times E$ trial, Blenheim had a significantly greater proportion of grain passing over a 2.5- and a 2.2-mm sieve but in $E \times B$ Blenheim had a significantly smaller portion passing over a 2.8 mm sieve. Since the objective of this study is to investigate the effects of major genes on quantitative traits it is of interest to estimate the number of genes or more correctly effective factors (*K*) segregating in the population. An estimate of *K* (although an underestimate) can be obtained by a comparison of the difference between the extreme DH progenies to the genetic variance (CROFT and SMICHEN 1965; POWELL *et al.* 1985b) and estimates of *K* range from 3 to 32. The RFLP profiles of the parents and a sample of the DH progeny are given in Figure 1. Polymorphism was detected at one of the two *Nor-H3* loci and this polymorphic locus was mapped to chromosome 5H using the wheat, barley addition lines. The segregation ratios for alleles at the *Nor-H3* locus on chromosome 5H of the DH progenies from the reciprocal crosses are given in Table 2. Segregation of alleles does not deviate significantly from the expected 1:1 ratio. By classifying the DH progeny for the alternative allele at the *Nor-H3* locus the background genetic effects

TABLE 1

Mean scores for the parents and DH progenies together with the minimum (min) and maximum (max) scores: estimates of *K* (the number of effective factors) is also given

	Hd	Ht	Yield	TCW	GT28	GT25	GT22	GE3D	GC5D	1WS3D	1WS5D	2WS3D	2WS5D	*N*	DP	ME	HWE	SPW
Blenheim × E224/3																		
Blenheim	20.5	54.7	5.40	51.2	54.1	82.6	93.8	96.3	97.8	12.4	41.0	65.1	74.5	1.96	50.21	685.6	291.1	72.78
E224/3	21.5	65.7	5.01	46.9	52.1	77.0	90.0	94.1	98.0	35.8	66.5	89.3	96.3	1.95	47.71	695.1	285.7	71.34
S.E.D. (parents)	0.47	1.41	0.130	0.81	3.00	1.81	0.89	1.17	0.68	6.29	9.06	3.77	3.18	0.031	1.74	9.84	3.03	0.444
DH-Min	18.0	44.9	3.49	44.7	24.9	68.2	87.4	89.2	94.5	3.4	17.5	45.6	62.0	1.72	32.51	575.2	272.3	67.11
DH-Mean	21.9	59.8	4.92	48.3	50.3	79.0	92.7	95.9	98.0	29.6	66.3	76.8	88.6	1.95	48.01	675.8	292.1	71.23
DH-Max	27.0	77.5	6.05	53.3	73.9	87.5	97.2	99.4	100.0	71.6	96.0	92.4	99.0	2.24	71.51	786.2	305.4	74.59
K	6	3	6	3	5	3	5		19	8	11	9	10	11	9	6	9	13
E224/3 × Blenheim																		
Blenheim	20.8	53.8	5.40	50.4	38.5	78.3	92.0	92.5	97.8	38.9	52.0	55.7	68.2	1.85	45.86	665.4	291.0	70.38
E224/3	22.4	63.3	4.80	45.7	50.6	78.4	91.1	96.0	98.8	84.7	96.3	95.0	98.1	1.83	43.49	682.8	295.0	70.45
S.E.D. (parents)	0.61	1.63	0.130	0.65	2.42	1.51	0.94	2.58	0.68	5.01	3.99	4.66	3.45	0.026	2.12	10.09	2.93	0.435
DH-Min	18.0	41.0	3.83	40.8	20.5	61.9	85.3	75.5	94.0	22.1	33.5	49.2	58.0	1.70	38.14	605.5	281.9	67.81
DH-Mean	22.7	54.5	4.86	47.4	41.2	76.0	91.4	94.5	98.6	62.2	78.3	79.5	89.3	1.86	50.83	667.1	292.2	70.69
DH-Max	28.8	76.5	5.62	52.8	63.4	85.5	96.5	100.0	100.0	87.1	97.3	94.7	99.0	2.19	67.75	754.8	302.0	73.99
K	9	4	12	6	5	5	6	32	16	5	7	5	7	9	5	6	8	9

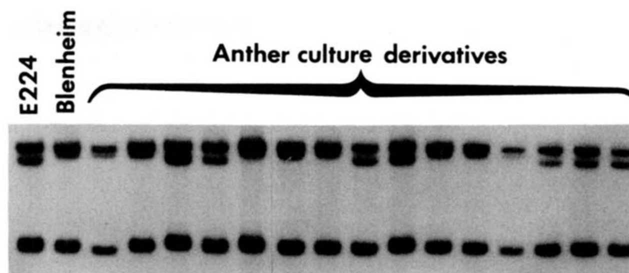

FIGURE 1.—Segregation of alleles at the *Nor-H3* locus. DNA extracted from the parents and anther culture derivatives and digested with *Eco*RV and hybridized with pBG35 (a flax rDNA repeat unit).

TABLE 2

Segregation ratios for alleles at the *Nor-H3* locus in a doubled haploid population derived from Blenheim (*B*) × E224 (*E*) and E224/3 × Blenheim

Cross	Number of DH lines with Blenheim (*B*) and E224/3 (*E*) alleles		$\chi^2_{(1)}$
	B	*E*	
B × *E*	26	29	0.16
E × *B*	30	22	1.23

will be nullified. In order to test for differences between the mean values of the DH grouped for the alternative alleles at the *Nor-H3* locus an analysis of variance was performed in which the sums of squares associated with *Nor-H3* (the between groups item) is tested against the between genotypes within groups mean squares.

The analyses of variance for the two crosses studied are given in Tables 3 and 4. If one considers the *B* × *E* cross (Table 3) there are highly significant differences ($P < 0.001$) between DH families for 16 of the traits measured. This indicates that there are significant levels of genetic variation available for manipulation. However, the between groups item was only significant for: GT28 ($P < 0.05$) and milling energy ($P < 0.001$). In the case of the reciprocal cross there were significant differences between groups for: yield, TCW, 1WS3D and milling energy. Thus, the classification of DH progeny based only on the allelic constitution of the *Nor-H3* locus results in significant differences between group means for both agronomic and quality attributes in barley.

The genetical variation associated with DH families will contain only fixable components of genetic variation since dominance and dominance-related epistatic effects are absent. The expected mean squares may be used to obtain estimates of the total genetic variation (G) and for both crosses significant levels of genetic variation is available for manipulation. The variances between DH lines will contain both additive genetic variation (D) and that ascribable to homozy-

TABLE 3

Analysis of variance for the Blenheim × E224 cross

	Mean squares (d.f.)			
	DH families (54)	Differences between groups (1)	Families within groups (53)	Error (54)
Hd	7.2986***	2.2500	7.3908	0.9455
Ht	152.534***	81.568	153.873	9.959
Yield	0.58960***	2.12694	0.56060	0.07423
TCW	10.109***	0.001	10.300	2.634
GT28	226.73***	1215.10*	208.08	33.10
GT25	36.38***	49.61	36.13	13.11
GT22	7.929***	0.726	8.064	3.058
GE3D	8.661*	2.240	8.782	5.094
GC5D	3.008			2.208
1WS3D	578.6***	160.7	586.5	156.3
1WS5D	969.5***	5.1	957.7	253.9
2WS3D	250.13***	11.51	254.63	58.92
2WS5D	162.97***	29.38	165.49	45.14
N	0.033022***	0.002771	0.033592	0.004604
DPP	119.19***	311.51	115.56	1.959
ME	3948.3***	58605.5***	2916.9	272.6
HWE	101.78	172.31	100.45	1.949
SPW	3.689***	0.000	3.758	2.549

*** $P < 0.001$; * $P < 0.05$.

TABLE 4

Analysis of variance for the E224 × Blenheim cross

	Mean squares (d.f.)			
	DH families (51)	Differences between groups (1)	Families within groups (53)	Error (54)
Hd	8.655***	8.021	8.6678	1.946
Ht	196.18***	0.27	200.10	15.35
Yield	0.24012***	1.62832**	0.21236	0.09611
TCW	14.394***	95.626**	12.770	2.381
GT28SV	283.85***	11.00	289.31	37.06
GT25	66.555***	24.631	67.393	9.645
GT22	13.525***	3.334	13.729	3.413
GE3D	39.38			30.24
GC5D	1.785*	0.302	2.835	1.679
1WS3D	364.84***	1510.39*	341.93	89.23
1WS5D	326.84***	1209.63	309.19	48.82
2WS3D	184.78***	645.43	175.57	60.59
2WS5D	118.43**	415.24	112.43	47.28
N	0.0178***	0.0155	0.0178	0.0052
DPP	101.53***	239.91	98.76	19.67
ME	2218.6***	8670.1*	2089.5	381.7
HWE	62.91*	0.15	64.16	36.26
SPW	3.763	12.209	3.59	2.336

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

TABLE 5

Estimates of the total genetic variation (*G*) and the amount associated with the rDNA locus *Nor-H3*

	Blenheim × E224			E224 × Blenheim		
	G total	*G* rDNA	% *G* rDNA	*G* total	*G* rDNA	% *G* rDNA
Hd	3.18	0.00	0.00	3.35	0.00	0.00
Ht	71.29	0.00	0.00	90.42	0.00	0.00
Yield	0.26	0.017	6.46	0.07	0.011	16.00
TCW	3.74	0.00	0.00	6.01	0.82	13.6
GT28	96.82	9.33	9.64	123.40	0.00	0.00
GT25	11.64	0.13	1.10	28.5	0.00	0.00
GT22	2.44	0.00	0.00	5.1	0.00	0.00
GE3D	1.78	0.00	0.00	4.57	0.00	0.00
GC5D	0.40	0.00	0.00	0.55	0.00	0.00
1WS3D	211.15	0.00	0.00	137.8	11.46	8.3
1WS5D	357.8	0.00	0.00	139.0		
2WS3D	95.61	0.00	0.00	62.1		
2WS5D	58.9	0.00	0.00	35.6		
N	0.0142	0.00	0.00	0.0063	0.00	0.00
DPP	52.29			40.93		
ME	1837.85	515.7	28.11	918.45	64.55	7.0
HWE	29.09			13.83	0.00	0.00
SPW	0.57			0.71	0.085	12

gote × homozygote interaction (*I*). These components of the genetic variation represent that portion which is available for selection in a breeding programme. An estimate of the genetic variation associated with *Nor-H3* can be calculated from the reduction in *G* when it is estimated from the between genotypes within groups component compared to the estimates from the between genotypes component of variation (Al-Banna, Jinks and Pooni 1984; Powell, Caligari and Jinks 1985; Powell *et al.* 1985a). The percentage of *G* associated with *Nor-H3* is given in Table 5. Alleles at *Nor-H3* are associated with 16%, 14% and 12% of the genetic variation for yield, thousand grain weight and specific weight, respectively, in *E* × *B* (B86–19). In addition 8% of the genetic variation for 1WS3D is associated with *Nor-H3*. In the reciprocal cross 9.6% of the variation for the >2.8

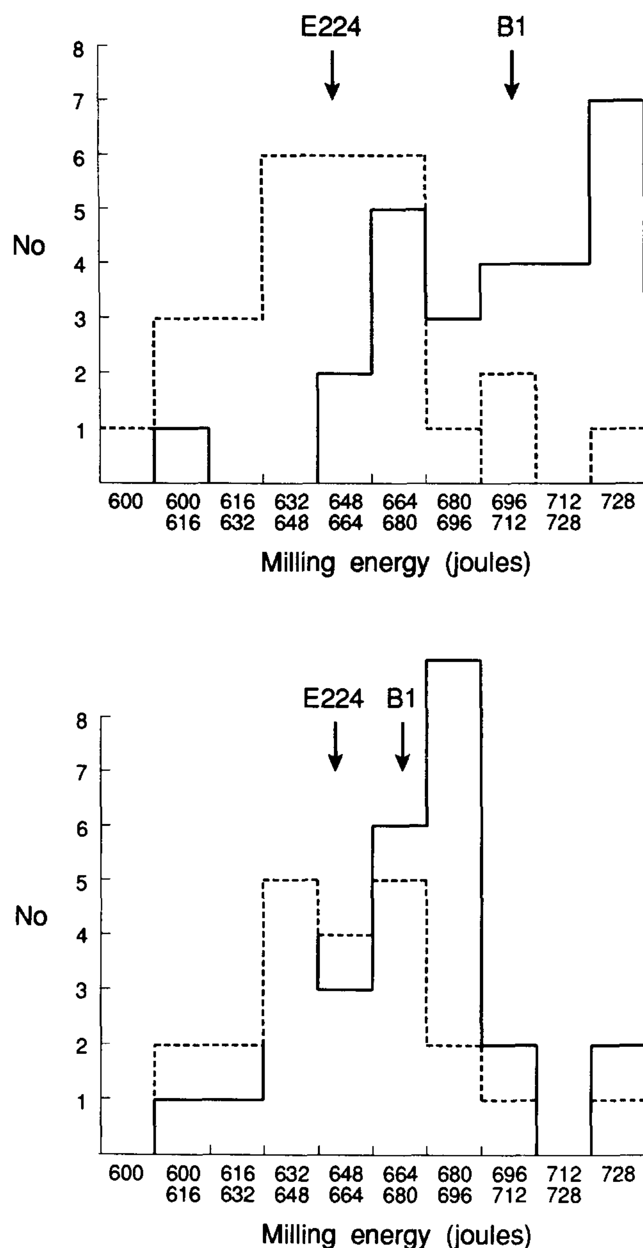

FIGURE 2.—Frequency distributions for milling energy in two doubled haploid populations. The two distributions obtained within each cross are due to selection for alleles at the *Nor-H3* locus. The E224/3 alleles is represented by (- - - -) and the Blenheim allele by (——).

mm sieve fraction was associated with *Nor-H3*. For both crosses the *Nor-H3* locus was associated with a relatively large proportion of the genetic variation for milling energy. However, the magnitude of the association was greatest in $B \times E$. This is illustrated graphically in Fig. 2. For $B \times E$ there are two distinct frequency distributions. Selection of DH progeny possessing the *Nor-H3* allele from E224/3 would result in a population with a significantly lower milling energy score and a higher hot water extract (Table 3). Over 28% of the genetic variation for milling energy is associated with allelic variation at the *Nor-H3* locus which is located on the short arm of chromosome 5H.

DISCUSSION AND CONCLUSIONS

This study has used DH to demonstrate associations between alleles at the *Nor-H3* locus on chromosome 5H and a number of agronomic and quality characters in barley. In order to predict the level of expression of a given quantitative trait a high level of linkage disequilibrium is required. DH extracted from F_1 hybrids are likely to exhibit a higher linkage disequilibrium relative to other generations and are therefore well suited to identify such associations. The *Nor-H3* locus was found to be associated with genes conditioning yield, TCW and milling energy. There has been much speculation on the potential of molecular markers to dissect quantitatively determined traits. The present study demonstrates that a significant proportion of the genetic variation for some important quantitative traits is determined by relatively small regions of the barley genome. However, the effects of the *Nor-H3* locus were not consistent over reciprocal crosses, even though both crosses shared the same nuclear DNA. These results may suggest that cytoplasmic and or maternal effects may be involved in the manifestation of the effects of the rDNA locus on quantitative traits. However, since the reciprocal crosses were grown in separate trials no direct comparison can be made between the two populations. TANKSLEY and HEWITT (1988) have stressed the dangers inherent in evaluating the effects of molecular markers in a single cross. Chromosome segments from *Lycopersicon chmielewskii* introduced into cultivated tomato were identified as having an effect on soluble solids content. The effect of the introduced segments was dependent on genetic background and emphasises the need to analyze a range of genotypes. Environmental and genotype by environment ($G \times E$) interaction may also complicate the identification and interpretation of such experiments. The availability of homozygous, fixed DH families will allow extensive replication and studies in a number of sites and seasons. The importance of $G \times E$ interactions in influencing the effects of marker loci on quantitative traits can therefore be critically assessed.

A comparison of the parental scores should allow us to predict whether the Blenheim or E224 alleles will have a positive or negative effect on the expression of a given QTL. However, a number of examples were identified (*e.g.*, DP and ME; Table 3) in which the expression of a QTL is in the opposite direction to that predicted by the parental values. These examples probably represent gene dispersion in the parents and similar results have been reported for tomato (TANKSLEY, MEDINA-FILHO and RICK 1982) and barley (POWELL *et al.* 1990). This conclusion is supported by the observation that there were no significant differences between the parents for some of these characters (Table 1). It must be stressed that the

microspore-derived DHs used in this study were regenerated via an embryogenic route rather than via a callus phase and are therefore less likely to be associated with reduced vigour (FINNIE, POWELL and DYER 1989; POWELL 1990). A cytological, biochemical and molecular analysis of microspore derived DHs of barley produced on a maltose based medium exhibited low levels of gametoclonal variability (FINNIE *et al.* 1991). However, Blenheim possess the denso dwarfing gene and a significant excess of genotypes possessing this gene was observed in the DH populations (THOMPSON *et al.* 1991). Alleles at the denso dwarfing gene locus have been shown to be associated with a range of agronomic traits (POWELL *et al.* 1985a; THOMAS, POWELL and SWANSTON 1991) and differences between the mid-parental value and the mean of the DH populations may therefore reflect the differential transmission of this region of the barley genome in the DH populations. Alternatively differences between the mean performance of DH families and the mid-parental values may reflect the presence of additive × additive type epistasis. Alleles at the *Nor-H3* locus on chromosome 5H did not deviate significantly from the expected 1:1 ratio (Table 2).

It is also desirable to establish whether associations between a marker locus and a QTL are due to linkage disequilibrium or pleiotropy. In the present study no explicit conclusions can be drawn on the causes of the detected associations between *Nor-H3* and QTLs since only DH extracted from F_1 hybrids were analyzed. However, inbred lines generated by several rounds of selfing from the same cross could be included in the analysis as they would provide an opportunity to reexamine the extent of associations between *Nor-H3* and QTLs following several rounds of gametogenesis. The G*Pert* locus has been mapped to chromosome 5H of barley (THOMAS, POWELL and WOOD 1984) and a series of DH and single seed descent (SSD) families have been used to examine the effect of G*Pert* on QTLs. The significant associations between the G*Pert* locus and additive genetic variation for TCW, plot, main stem and single plant yield in three spring barley crosses was found to diminish when comparisons were made between DH and SSD families (POWELL, CALIGARI and JINKS 1985a). These observations indicate that the associations between G*Pert* and QTLs are due to linkage disequilibrium rather than pleiotropy. Recently JENSEN (1989) working with two marker genes on chromosome 5H established linkage between *s* (short rachilla hair) and a quantitative trait controlling thousand grain weight. JENSEN concluded that the association was unlikely to be due to pleiotropic effects because the recombination frequency between *s* and TCW (0.26 ± 0.09) differed significantly from zero. These independent studies provide further support in our findings that *Nor-H3* may be linked to genes controlling TCW and these in turn may be influencing the expression of traits such as yield.

The milling energy requirement of barley cultivars has been proposed as a method for predicting genotypes with the potential for good malting quality (ALLISON, COWE and MCHALE 1976). Cultivars with high milling energy are associated with poor malting performance and SWANSTON (1987) found that high milling energy was one of the deleterious characters associated with genotypes with quantitative brown rust resistance derived from *Hordeum laevigatum*. In this study, both parents have *H. laevigatum* in their pedigree and there appeared to be little difference in their reaction to brown rust, hence we can assume that we have located genes other than those associated with the quantitative brown rust resistance. For both crosses studied in this experiment *Nor-H3* was associated with a significant portion of the genetic variation for milling energy thus providing strong evidence for the conclusion that genes controlling milling energy requirement in barley are located on chromosome 5H. Two other studies lend further support to this conclusion. THOMAS, POWELL and SWANSTON (1991) established that the G*Pert* locus on chromosome 5H was associated with high milling energy requirement, although this appeared to be due to the association of the gene with low TCW. In a study of the milling energy requirements of the Triticeae, FORSTER and ELLIS (1990) identified homoeologous group 5 chromosomes as possessing genes conditioning milling energy requirement. From this study, it would seem that the *Nor-H3* locus is potentially an excellent genetic marker for the indirect selection of endosperm texture in barley. It would also seem that this locus is not associated with hot water extract but there were no significant differences between the parents (Table 1) and little evidence of significant genetic variation in either cross (Tables 3 and 4), suggesting that there were insufficient differences between the parents to detect any differences.

Two previous studies have focused on rDNA IGS variability and its association with agronomic traits. ROCHEFORD, OSTERMAN and GARDNER (1990) working with a mass-selected maize population proposed that "spacer hybridisation fragment patterns may serve as markers of favourable alleles." However, changes in the frequency of IGS hybridization pattern was more pronounced in one out of the two populations studied. The barley composite cross population studied by SAGHAI-MAROOF *et al.* (1984) was generated from a wide genetic base with diverse parents from contrasting geographical regions. Changes in the IGS hybridization pattern may therefore have been due to loss of unadapted genotypes. ALLARD (1988) working with the same barley population found consistent associations between superior reproductive ca-

pacity and marker loci. In the present study adapted barley genotypes were studied and the significant associations observed between *Nor-H3* and traits of importance are likely to be even more pronounced if diverse parents were included in the analysis. Nevertheless, allelic variation at the *Nor-H3* locus is responsible for a significant proportion of the genetic variation for yield, TCW, water sensitivity and milling energy. Thus although adapted germplasm was used there is evidence that the *Nor-H3* locus is associated with traits that have a strong reproductive advantage.

An important feature of the methods described in this report is the creation and use of homozygous recombinant inbred lines. Such families may be monitored for the segregation of alleles at marker loci of known chromosomal location. The effects of such loci can therefore be evaluated in an almost constant genetic background. Studies of this nature provide further impetus for the identification of regions of the barley genome which have large effects on the expression of quantitative traits in plants.

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