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Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.)

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Abstract Winterhardiness in cereals is the consequence of a number of complex and interacting component characters: cold tolerance, vernalization requirement, and photoperiod sensitivity. An understanding of the genetic basis of these component traits should allow for more-effective selection. Genome map-based analyses hold considerable promise for dissecting complex phenotypes. A 74-point linkage map was developed from 100 doubled haploid lines derived from a winter × spring barley cross and used as the basis for quantitative trait locus (QTL) analyses to determine the chromosome location of genes controlling components of winterhardiness. Despite the greater genome coverage provided by the current map, a previously-reported interval on chromosome 7 remains the only region where significant QTL effects for winter survival were detected in this population. QTLs for growth habit and heading date, under 16 h and 24 h light, map to the same region. A QTL for heading date under these photoperiod regimes also maps to chromosome 2. Contrasting alleles at these loci interact in an epistatic fashion. A distinct set of QTLs mapping to chromosomes 1, 2, 3, and 5 determined heading date under 8 h of light. Under field conditions, all QTLs identified under controlled environment conditions were determinants of heading date. Patterns of differential QTL expression, coupled with additive and additive×additive QTL effects, underscore the complexity of winterhardiness. The presence of unique phenotype combinations in the mapping population suggests that coincident QTLs for heading date and winter survival represent the effects of linkage rather than pleiotropy.

Key words Barley · Vernalization · Photoperiod
Winterhardiness · QTLs

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Introduction

Winterhardiness in cereals is the final expression of a number of interacting characters that can include vernalization requirement, photoperiod response, and low-temperature tolerance. Progress in developing superior winter-habit barley varieties may best be achieved based on an understanding of the chromosome locations of genes controlling these characters. Thomashow (1990), reviewed the wealth of physiology data regarding mechanisms of cold injury, and the paucity of information regarding the genetic basis of resistance to such stress. Hayes et al. (1993 a) recently reported the presence of a putative multilocus cluster on barley chromosome 7 defined by quantitative trait locus (QTL) effects for a number of winterhardiness component traits. Takahashi and Yasuda (1970) proposed that three major genes-*sh*, *Sh2*, and *Sh3*-located on chromosomes 4, 7, and 5, respectively, were responsible for winter vs spring growth habit in barley (*Hordeum vulgare*). A multiple allelic series at the *Sh2* locus and complex epistatic interactions among loci were postulated to explain the range of growth habit seen in barley germplasm (Nilan 1964; Takahashi and Yasuda 1970). In hexaploid wheat (*Triticum aestivum*), five major genes-*Vrn1*, *Vrn2*, *Vrn3*, *Vrn4*, and *Vrn5*-are reported to control vernalization response (Law 1966; Pugsley 1971, 1972). *Vrn1*, *Vrn4*, and *Vrn3* are located on chromosomes 5A, 5B, and 5D, respectively (Xin et al. 1988). *Vrn2* is located on chromosome 2B, and *Vrn5* is located on chromosome 7B (Law 1966). The homoeol-

ogous relationship of wheat and barley chromosomes is as follows: wheat chromosome 2:barley chromosome 2; wheat chromosome 5: barley chromosome 7; and wheat chromosome 7: barley chromosome 1 (Islam et al. 1981). The homoeologous relationships of the *Sh* and the *Vrn* loci have not been rigorously established.

The most frequently-employed measure of winter vs spring growth habit in cereals is heading date. Takahashi and Yasuda (1970) concluded that at least three internal physiological factors-vernalization response, photoperiod response and earliness-were responsible for heading date in barley. Vernalization responses are strongly influenced by photoperiod (Roberts et al. 1989). Likewise, photoperiod responses can be masked or cancelled by vernalization (Takahashi and Yasuda 1970). Earliness per se has been the subject of much research, particularly in spring-habit barley. Four loci-*Easp*, *Eac*, *Eak*, and *Ea*7, located on chromosomes 3, 4, 5, and 6, respectively-were reported to control both maturity and photoperiod sensitivity in spring barley (Ramage and Suneson 1958; Dormling and Gustafsson 1969; Yasuda and Hayashi 1980; Gallagher et al. 1991). The *Ea* locus on chromosome 2 was described by Nilan (1964) and Hayes et al. (1993b) reported the presence of a large-effect QTL for heading date in the same chromosome region. A lack of common markers precludes drawing a definitive assignment of this QTL effect to the *Ea* locus. The effects of these *Ea* loci in photoperiod- and vernalization-sensitive genetic backgrounds are unknown.

Low-temperature tolerance is an induced response (Thomashow 1990) and thus hardening and maximum expression of cold tolerance are often confounded because the two characteristics often occur in association. This has led to considerable discussion of underlying genetic mechanisms (Cahalan and Law 1979; Brule-Babel and Fowler 1988). Recent evidence points to linkage rather than pleiotropy. Roberts (1989) reported that at least two loci on chromosome 5A were involved with cold hardening in wheat. One locus is, or is tightly linked to, the vernalization gene *Vrn1*, but the other locus appears to be unrelated to this gene. In barley, Doll et al. (1989) reported that doubled haploid lines with good winterhardiness but without a vernalization requirement were recovered from winter×spring crosses. Hayes et al. (1993 a) reported segregation for low-temperature tolerance in the progeny of a winter×spring cross, where the winter parent was photoperiod-sensitive but had no vernalization requirement. Progress in elucidating the genetic mechanisms of winterhardiness, vernalization, and photoperiod responses is hindered by the often quantitative nature of trait expression and complex interactions among traits. The development of molecular-marker technology and quantitative trait locus (QTL) mapping offers a new approach to resolving this complexity (Paterson et al. 1991); Lander and Botstein 1989. By locating individual QTLs with molecular markers, individual QTL genotypes can be determined with relative certainty. This allows for a dissection of complex traits, the measurement of individual gene effects, and the measurement of gene interactions. Ultimately, higher-resolution mapping will allow for the separation of pleiotropic and linkage effects (Paterson et al. 1990) and map-based cloning (Martin et al. 1993).

Several molecular marker maps of barley have been developed (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993). and these maps have been used to locate QTLs affecting yield, quality, and disease-resistance traits (Heun 1992; Hayes et al. 1993 b; Chen et al. 1994 b). Based on analyses of a winter×spring cross, Hayes et al. (1993 a) reported the presence of a QTL on the long arm of chromosome 7 that was associated with low-temperature tolerance in both field and controlled-environment tests. QTL effects for heading date under 24 h light and other winterhardiness-related traits mapped to the same region. However, the presence of individual recombinant lines with unique character attributes led these authors to hypothesize that the association of trait expression was due to linkage rather than pleiotropy. Additional markers have since been mapped in this same population, providing a greater degree of map resolution and genome coverage. Therefore, the objectives of this work were to (1) reassess cold tolerance QTL expression in view of greater map density and coverage, (2) locate QTLs for vernalization and photoperiod response, and (3) determine the importance of epistatic interactions among QTLs.

Materials and methods

Two barley cultivars, "Morex" and "Dicktoo", and 100 F_1-derived doubled haploid (DH) progeny were used in these experiments. Morex, released in 1978 by the Minnesota Agricultural Experimental Station, is the US. six-row spring-barley malting standard. Dicktoo is a six-row winter-feed barley released by the Nebraska Agricultural Experimental Station in 1952. The pedigree of Dicktoo is unknown, and the variety description is mixed. Under field conditions, the particular accession of Dicktoo used in these studies displays prostrate winter growth habit and average maturity compared to other winter-barley genotypes. The DH lines were developed by the *Hordeum bulbosum* method, as described by Chen and Hayes (1989).

The 100 DH lines and the parents were phenotyped for winterhardiness-component traits in field and controlled-environment tests. The cold-tolerance testing procedures were described by Hayes et al. (1993 a) and involved field plots at Bozeman, Montana and Corvallis, Oregon, USA. The heading dates of the 100 DH lines and the two parents were also measured in a series of controlled-environment tests involving six combinations of vernalization and photoperiod. In all cases, the vernalization treatment consisted of 6 weeks of hardening seedlings at 6°C with an 8 h-light/16-h dark photoperiod regime. In all experiments, seedlings for the unvernalized treatment were established 1 week prior to the end of vernalization so that plant material from both treatments was transplanted at the same time and at approximately the same growth stage. Heading date, with and without vernalization with an 8-h light/16-h dark photoperiod regime, was assessed in the phytotron facilities of the Martonvasar Research Institute (Martonvasar, Hungary) with the light intensity and temperature program described by Tischner (1993). Heading date, with and without vernalization under 16-h light/8-h dark and under a continuous light regime, was determined under greenhouse conditions at Corvallis, Oregon. Ambient temperatures were 20±2°C, and supplemental illumination was provided by high-pressure sodium lights suspended 1.5 m above the bench surface. Each genotype was replicated twice. These six data sets will subsequently be referred to by the hours of light duration (8 h, 16 h, or 24 h) and a suffix indicating the vernalization treatment, where v=vernalized and uv=unvernalized.

A total of 78 markers were scored on the 100 DH progeny in a cooperative effort involving Linkage Genetics, Inc. (Salt Lake City, Utah), Montana State University (Bozeman, Montana), and Oregon State University (Corvallis, Oregon). The marker nomenclature of Kleinhofs et al. (1993) is employed in this report. Briefly, the prefixes "m", "i", and "ap" designate morphological, isozyme, and sequence-tagged site (STS) markers, respectively. Additional detail on the STS markers can be found in Tragoonrung et al. (1992). Restriction fragment length polymorphisms (RFLPs) were detected with an array of anonymous cDNA and genomic clones and several known-function clones. The prefixes "WG", and "BCD" designate anonymous wheat genomic and barley cDNA clones, respectively (Heun et al. 1991). The prefixes "ABC", and "ABG" designate anonymous barley cDNA and genomic clones developed by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993). *Crh*1 and *Crh*2 are plant calreticulin loci (Chen et al. 1994 b). *Cr*3 is a low-temperature-induced cDNA clone (Sutton and Kenefick 1994). *Dhn*1, 2, 4, and 5 are Dehydrin cDNA clones (Close et al. 1993). The morphological markers rachilla hair (*mS*) and awn texture (*mR*) were scored under a stereomicroscope. Isozyme and storage protein markers were analyzed as described by Nielsen and Johanson (1986). STS markers were assayed as described by Tragoonrung et al. (1992), and RFLP markers were assayed essentially as described by Kleinhofs et al. (1993).

A linkage map was constructed using Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992 a). Seventy-four markers were resolved into 11 linkage groups. Assignments of these linkage groups to the seven chromosomes of barley were made based on previously-mapped markers. QTL analyses were conducted using the interval mapping procedures of Mapmaker/QTL 1.1 (Lincoln et al. 1992 b). The minimum LOD threshold was usually specified at 3.0. However, in certain cases, QTL effects significant at lower LOD scores are reported. Hypotheses regarding epistatic interactions between individually-significant QTLs were tested by classifying individual genotypes for key marker intervals. Only non-recombinant genotypes were used. Because the number of replications of QTL marker genotypes was unbalanced, ANOVAs were conducted using the PROC GLM option in SAS (SAS 1988) to generate Type-III sums of squares. A significant QTL×phenotype interaction mean square ($P< 0.05$) was taken as evidence for epistasis. As there is no dominance variance in a population of completely-homozygous doubled haploids, significant interactions indicate the presence of additive×additive epistasis.

Results

Phenotypic trait expression

The winter-survival data of Hayes et al. (1993 a) are presented for the sake of reference and because of the greater genome coverage that has been achieved since the publication of that report. Morex, and approximately half of the DH lines, experienced complete mortality in Montana. The mean survival of Dicktoo was 43% and the percent survival of the remaining DH lines ranged from 1 to 85 (Fig. 1 h). The standard error was ±11.1%. Less-severe winter conditions at Corvallis led to complete survival of all DH lines, with a range of 1–100% (Fig. 1 i). As these estimates were based on an unreplicated test, no standard error can be calculated. However, as demonstrated by Knapp and Bridges (1990), the primary determinant of tests of hypotheses about QTL genotype-means is the number of replications of QTL genotypes, not the number of times each line is replicated. The survival of Dicktoo was 89% and that of Morex 18%.

Heading-date frequency distributions for the DH population in both field and controlled environments were continuous (Fig. 1 a–g), indicating quantitative inheritance. Population means and ranges, and the numbers of phenotypic transgressive segregants, were quite different in the various vernalization and photoperiod regimes, indicating environmental mediation of gene expression. Under field conditions, the time from planting to heading in the DH population averaged 252 days, with a standard error of 1.2 days, substantially longer than in any of the controlled-environment tests (Fig. 1 g). This may be attributable to a low-temperature-mediated reduction in growth rate during the winter months. Under field conditions, there was limited phenotypic transgressive segregation.

In the controlled-environment tests, population means and ranges for heading date were strongly affected by photoperiod and to a lesser extent by vernalization. In terms of mean performance, vernalization had little effect in the 8-h experiments (Fig. 1 a and b): the average heading date was 108±1.4 days with vernalization and 111±1.6 days without vernalization. Genotypes varied in their response to vernalization. For example, vernalization reduced the time to heading for Morex by 19 days and by 8 days for Dicktoo. Under 16-h light, the time to heading was dramatically reduced, as compared to the 8-h experiments (Fig. 1 c and d). Vernalization at 16-h light had a modest effect on shortening the time to heading. The population mean was 41±0.9 days with vernalization and 51±1.1 days without vernalization. Vernalized Morex headed 9 days earlier and vernalized Dicktoo 10 days earlier. In contrast to the 8-h experiments, there was substantial transgressive segregation for heading date in the 16-h experiments. Approximately 50% of the DH lines were earlier than Morex in both vernalized and unvernalized treatments and 25% of the DH lines were later than Dicktoo in the unvernalized treatment. Under 24-h light, the population means were dramatically reduced, as compared to the 8-h experiments, and there were significant numbers of transgressive segregants (Fig. 1 e and f). Standard errors for the unvernalized and vernalized experiments were ±1.5 and 1.1 days, respectively. Compared to the 16-h experiments, the heading dates of the two parents were more similar. Differences among the progeny were accentuated, particularly without vernalization. In general, genotypes that were early under 16-h light were even earlier under 24-h light, and genotypes that were late under 16-h light were even later under 24-h light. Vernalization had little effect and in some cases even delayed heading. For example, vernalized Morex was 5 days later whereas vernalized Dicktoo was 3 days earlier.

The matrix of phenotypic correlations among traits is shown in Table 1. As reported by Hayes et al. (1993 a) the correlation between winter survival in the two field tests was 0.57. Correlations between the two measures of winter survival and all other traits were consistently positive and of similar magnitude. Correlations between the vernalized and unvernalized treatments within a photoperiod regime were consistently high and positive. Heading-date responses under 16-h and 24-h light were positively cor-

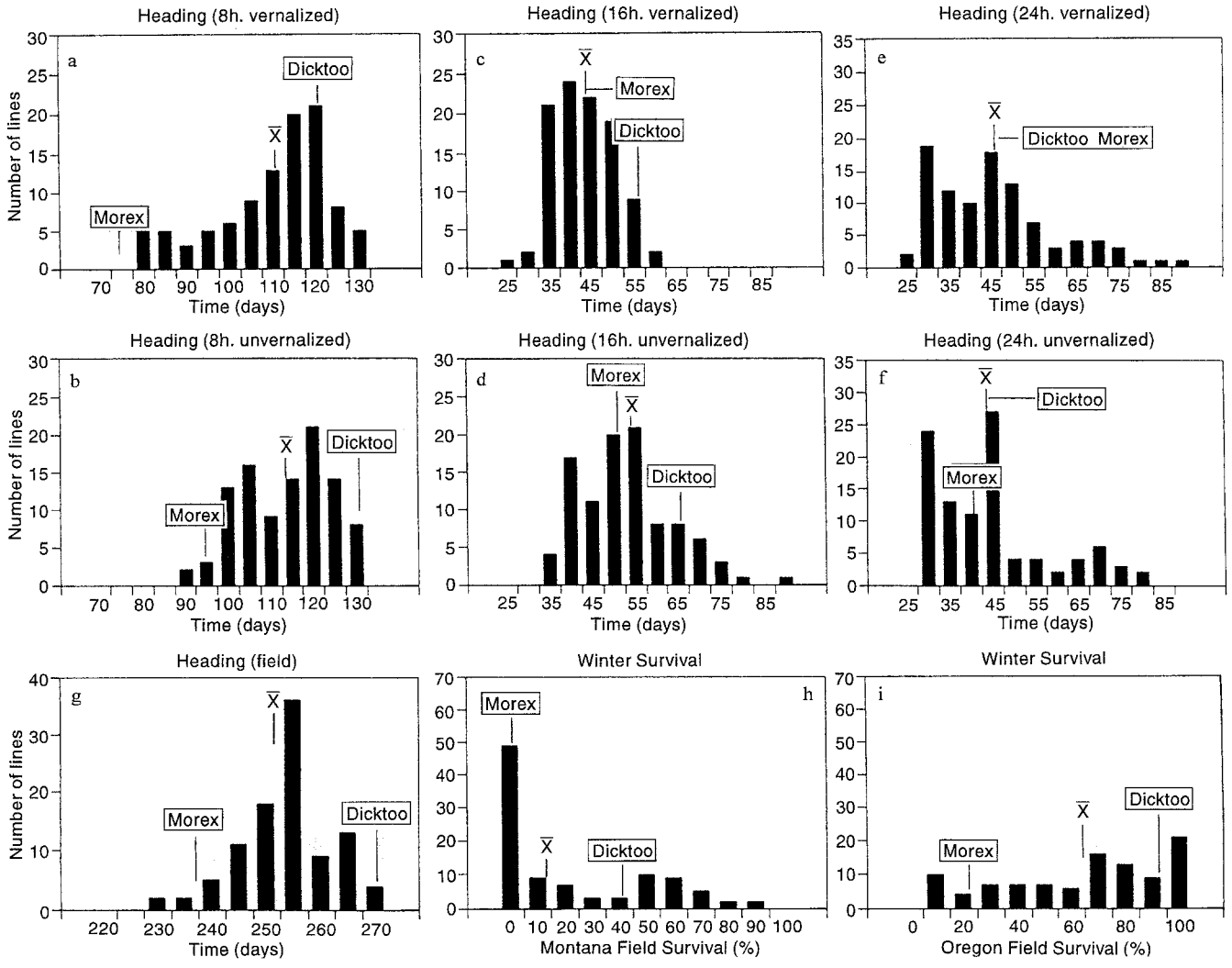

Fig. 1 Frequency distributions for heading date and field survival in a population of 100 doubled haploid barley lines derived from the cross of Dicktoo × Morex

related, but there was little relationship with heading date under 8-h light. Of the controlled-environments measures of heading date,that under 8-h light was the most highly correlated with field heading date, confirming the important role of daylength in determining the heading date of winter barley under field conditions at 45°N latitude.

Map construction

The 78 markers were used to construct the 74-point linkage map presented in Fig. 2. The assignment of linkage groups to chromosomes was based on the large number of markers in common with previously-published maps (Graner et al. 1991; Heun et al.1991; Kleinhofs et al. 1993). Marker orders and distance are generally comparable with the published maps. The map provides only partial genome coverage. Distances between linkage groups on chromosomes 1, 2, 3, and 5 were greater than the 30 cM threshold. These segments are shown in their assumed orientation. The highest resolution was achieved on chromosomes 2, 4, 5, and 7. The map of the long arm of chromosome 7 has been extended and greater resolution has been achieved in the key interval between *mR* and *BCD265b* since the report of Hayes et al. (1993 a). Two Dehydrin loci-*Dhn1* and *Dhn2*-co-segregated and were mapped within the critical interval reported by Hayes et al. (1993 a). Two additional markers -*Dhn4a* and *ABG391* -were mapped distal to this region. The low-temperature-induced clone-*Cr3*-mapped to the short arm of chromosome 2.

Quantitative trait loci

The large QTL effect for cold tolerance on chromosome 7 reported by Hayes et al. (1993 a) was substantiated with the greater density and resolution of the current map. Despite the presence of phenotypic transgressive segregants in both test environments (Fig. 1 h and i), this remains the only region of the genome in which a QTL effect for winter survival was detected. As shown in Table 2, in Montana the winter-survival LOD score for the QTL peak defined by *Dhn1-BCD265b* was 27.1 and this interval ac-

Table 1 Phenotypic correlations among heading date and winter survival for 100 doubled haploid (DH) lines derived from the cross of Dicktoox × Morex

Item[a]	HD 8 hv	HD 8 huv	HD 16 hv	HD 16 huv	HD 24 hv	HD 24 huv	HD Field	SURM
HD8 huv	0.81**							
HD16 hv	0.29**	0.31**						
HD16 huv	0.19	0.27**	0.72**					
HD24 hv	0.19	0.34**	0.66**	0.85**				
HD24 huv	0.12	0.22*	0.60**	0.88**	0.85**			
HD Field	0.66**	0.76**	0.27**	0.21*	0.21*	0.06		
SURM[b]	0.10	0.23*	0.43**	0.61**	0.46**	0.55**	0.37**	
SURO[c]	0.51**	0.59**	0.41**	0.50**	0.44**	0.45**	0.58**	0.57**

[a] HD=heading date
[b] SURM=winter survival in Montana
[c] SURO=winter survival in Oregon
v =vernalized
uv=unvernalized
* Significant at $P \leq 0.05$
** Significant at $P \leq 0.01$

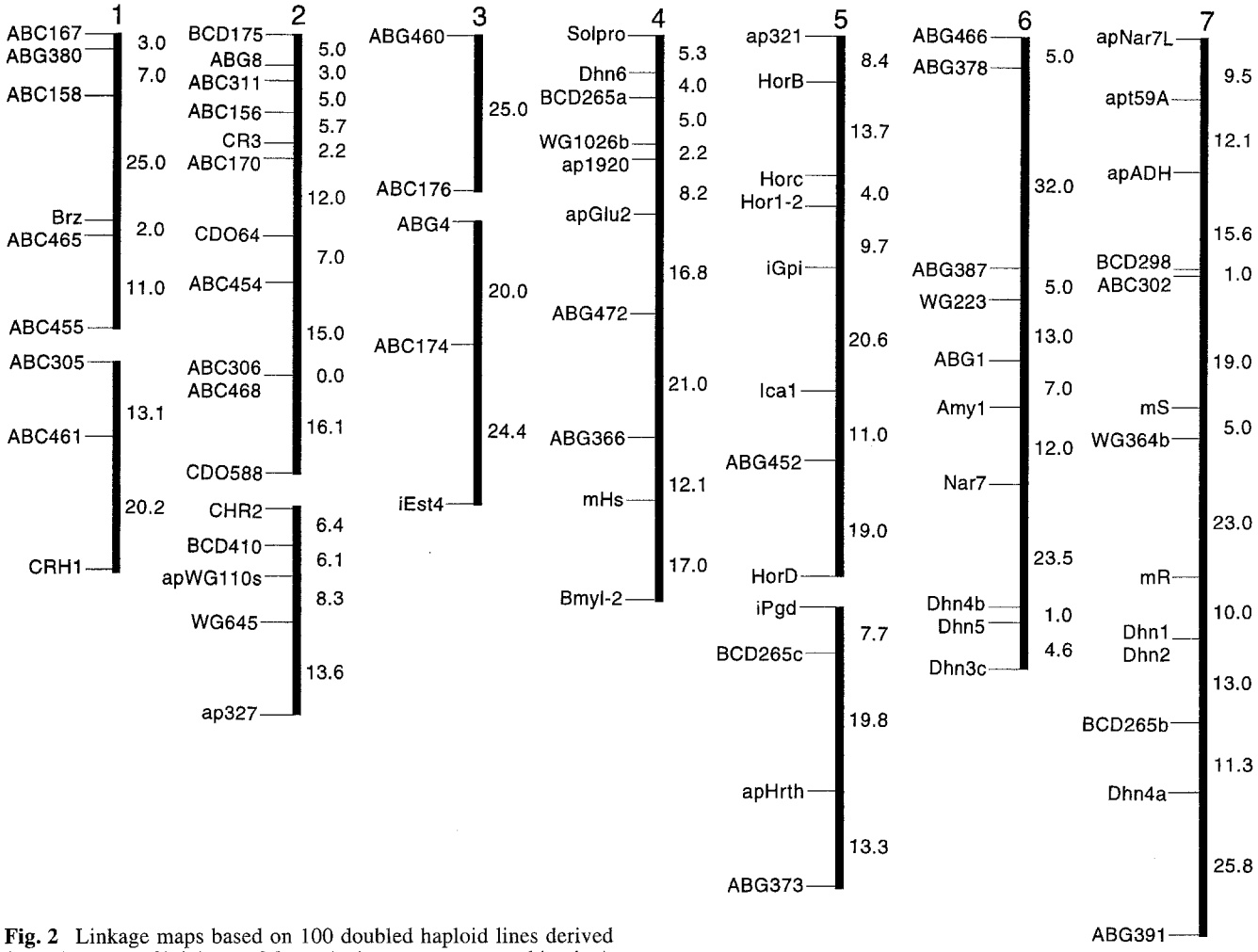

Fig. 2 Linkage maps based on 100 doubled haploid lines derived from the cross of Dicktoo × Morex (units are percent combination)

counted for more than 79% of the variation for winter survival in this population. In Oregon, the LOD score was reduced to 8.1, the QTL peak position shifted to the upstream interval (*mR-WG364b*), and this QTL effect had an r^2 of 0.31. In both data sets, QTL LOD scores declined at the upstream marker *WG364b* and the downstream marker *BCD265b*, while three peaks of approximately-equal magnitude were seen in the intervening intervals. There were only modest differences between peaks in terms of maximum LOD and r^2, so selection of any particular peak as the location of the underlying gene is arbitrary. Multiple adjacent peaks may represent the effects of linked QTLs

Table 2 QTL peak effects for heading date and winter survival of 100 doubled haploid (DH) lines derived from the cross of Dicktoo × Morex)

Trait[a]	Chromosome	Marker interval	% Recombination	LOD	r^2	Weight
SURM	7	*Dhn1-BCD265b*	13.0	27.1	0.79	47.3D[b]
SURO	7	*WG364b-mR*	23.0	8.1	0.31	34.2D
HD8 hv	1	*ABC158-Brz*	25.0	3.0	0.16	10.9D
	3	*ABG4-ABC174*	20.0	2.4	0.13	9.7D
	5	*iPgd-BCD265c*	7.7	10.1	0.40	17.2D
	5	*Hord-ABG452*	19.0	3.8	0.19	11.8D
Multilocus				16.6	0.59	
HD8 huv	1	*ABC158-Brz*	25.0	2.8	0.17	8.8D
	3	*ABG4-ABC174*	20.4	3.6	0.22	9.7D
	5	*iPgd-BCD265c*	7.7	6.8	0.30	11.5D
	5	*Hord-ABG452*	19.0	3.8	0.18	8.8D
	7	*ms-WG364b*	23.0	2.2	0.10	6.6D
Multilocus				17.8	0.64	
HD16 hv	2	*ABC170-CD064*	12.0	6.4	0.29	7.8M
	7	*Dhn1-BCD265b*	13.0	5.1	0.22	7.0D
Multilocus				13.0	0.48	
HD16 huv	2	*ABC170-CD064*	12.0	6.6	0.30	12.0M
	7	*Dhn1-BCD265b*	13.0	12.7	0.49	15.6D
Multilocus				28.3	0.79	
HD24 hv	2	*ABC170-CDO64*	12.0	7.4	0.34	16.1M
	7	*Dhn1-BCD265b*	13.0	7.4	0.34	16.3D
Multilocus				18.8	0.65	
HD24 huv	2	*ABC170-CDO64*	12.0	8.5	0.36	16.4
	7	*Dhn1-BCD265b*	13.0	10.9	0.44	18.5D
Multilocus				32.2	0.83	
HD Field	1	*ABC158-Brz*	25.0	3.3	0.18	7.3D
	2	*ABC170-CDO64*	12.0	3.0	0.15	6.7D
	3	*ABG4-ABC174*	20.4	2.1	0.11	5.9D
	5	*iPgd-BCD265c*	7.7	4.7	0.20	7.8D
	5	*Hord-ABG452*	19.0	2.1	0.11	5.7D
	7	*mS-WG364b*	23.0	4.1	0.17	7.2D
Multilocus				18.2	0.66	

[a] HD=heading date
[b] Letter suffix indicates parent contributing higher value allele, where D=Dicktoo and M=Morex
SURM=winter survival in Montana
SURO =winter survival in Oregon
v =vernalized
uv=unvernalized

(Martinez and Curnow 1992), a "shadow" effect due to the linkage of adjacent intervals with the true location of the QTL, or may simply be a consequence of random errors in phenotyping. Based on the available data, we can conclude that QTL effects underlying the same phenotype measured in very different environments map to the long arm of chromosome 7. Determination of the exact position of the gene or genes detected by these QTL effects will require analysis in alternative genetic stocks, such as near-isogenic lines, that will allow for higher-resolution mapping.

QTLs for heading date under 16- and 24-h light, both with and without vernalization, were detected on chromosomes 2 and 7 (Table 2). Morex contributed the late-maturity QTL on chromosome 2, while Dicktoo contributed the later-maturity allele on chromosome 7. The LOD peaks for the chromosome-2 and chromosome-7 intervals were defined in all treatments by the *ABC170-CDO64* and *Dhn1-BCD265b* intervals, respectively. The interval on chromosome 7 is the same as that defining winter survival in Montana. There was variation in the magnitude of the QTL effects at these two intervals in the four data sets. With vernalization, the effects of the QTLs at the two intervals were similar in the two photoperiod regimes, although the effects were more pronounced under continuous light. For example, the chromosome-2 and -7 interval LOD scores for the 16 hv were 6.4 and 5.1 while the LOD scores for the 24 hv were 7.4 and 7.4. Without vernalization, effects were again larger under continuous light, but under both photoperiod regimes the chromosome-7 effect was larger than the chromosome-2 effect. Under 16-h light, the chromosome-2 and -7 interval allele weights were 12.0 and 15.6 days, respectively. The multi-locus r^2 values for these effects supported the differential achieved in trait expression without vernalization and with continuous light: the

respective r^2 values for the 16 hv, 24 hv, 16 huv, and 24 huv experiments were 0.48, 0.65, 0.79, and 0.83. Takahashi and Yasuda (1970) based their genetic analysis of growth habit on heading date under 24-h light without vernalization in order to maximize differences in trait expression. In terms of vernalization response, however, differential expression was maximized under 16-h light. As measured by the magnitude of QTL effects, the chromosome-7 QTL was more vernalization responsive than the chromosome-2 QTL. For example, the differences in allele effects for the chromosome-2 and -7 QTLs without and with vernalization under 16-h light were 4.2 and 8.0 days, respectively. Under continuous light, these same differences were only 0.3 and 2.2 days, respectively.

Under 8-h light, heading date was determined by a different set of QTLs than those controlling responses at 16-h and 24-h light. As shown in Table 2, QTL effects were found on chromosomes 1, 3, 5, and 7. In all cases, Dicktoo contributed the later allele. Since Dicktoo was among the latest-heading genotypes under 8-h light, these loci may be those governing sensitivity to short daylength. A QTL on chromosome 7, defined by the *mR-WG364b* interval, the same interval defining the Oregon field-survival QTL, was detected at a low LOD (2.2) only in the unvernalized experiment. The QTL was poorly resolved and had a confidence interval spanning the *Dhn1-BCD265b* interval. It may represent a unique QTL or it may be an example of peak shift and thus represent an effect of the same gene or genes determining heading date under 16- and 24-h light. Otherwise, QTL expression was essentially the same with and without vernalization. The QTL with the single largest effect was defined by the *iPgd-BCD265c* interval on chromosome 5.

Heading date under field conditions was controlled by the same QTLs determining heading date under all three controlled-photoperiod regimes. The magnitudes of the chromosome-1, -3, -5, and -7 QTLs were similar under 8-h light and under field conditions. The *ABC170-CDO64* QTL on chromosome 2 had a lesser effect under field conditions than it did under 16-h or 24-h light, and there was a change in allele phase. In the controlled environment tests, Morex contributed the larger value allele at this locus, while under field conditions Dicktoo contributed the larger-value allele. The QTL peak on chromosome 7 under field conditions was the same as that detected in the 8-h experiments, but not that detected in the 16-h and 24-h experiments. As was discussed relative to other QTL effects on the long arm of chromosome 7, additional analyses will be required to determine if this peak shift is of biological significance.

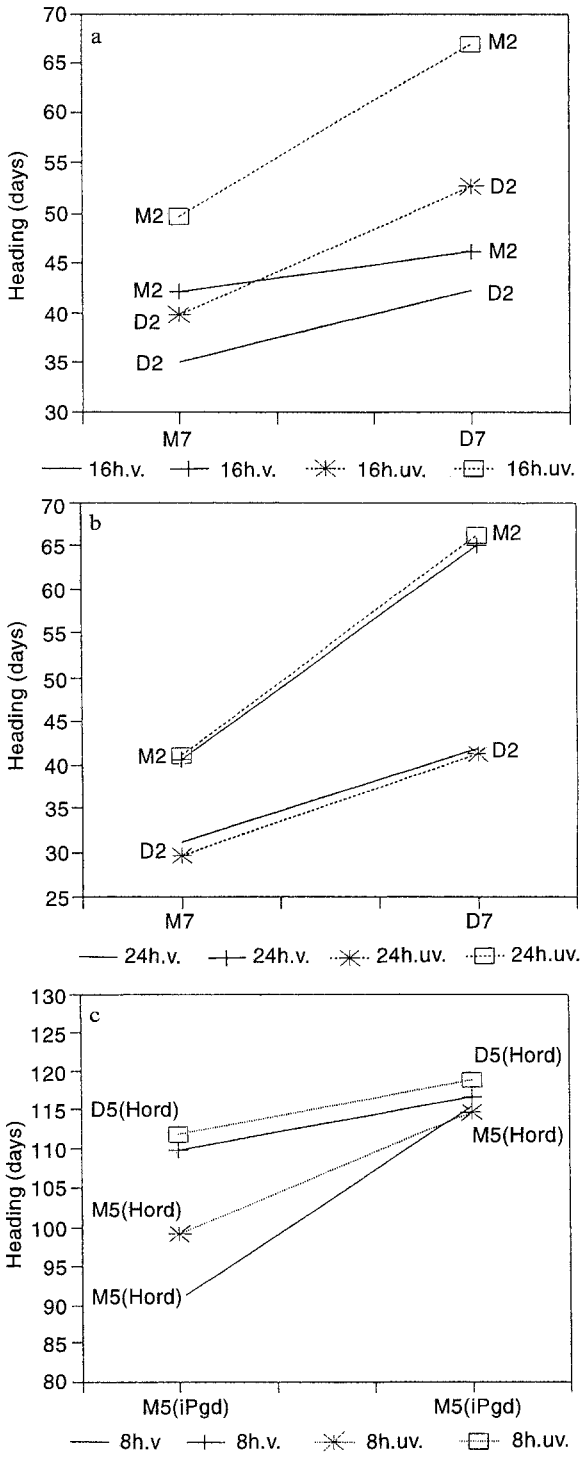

Fig. 3 Two-locus QTL genotype means for heading date under 16-h (**a**), 8-h (**b**) and 24-h (**c**) light

QTL interaction

Takahashi and Yasuda (1970) proposed that alleles at the *Sh* loci on chromosomes 4, 5, and 7 interact in an epistatic fashion to determine spring vs winter growth habit. As interval mapping detects only individual QTL effects or their combined additive effects, we analyzed our data in terms of two-way interactions among individually-significant QTL effects. As described in the Materials and methods, a subset of genotypes that were non-recombinant between flanking markers were used to assess additive×additive epistasis. For any two-locus combination, four QTL combinations are possible. In the subsequent discussion, these QTL genotypes are identified by the chromosome

and the parent subscript, where M=Morex and D=Dicktoo. For example, the four QTL genotypes for 16-h heading date on chromosomes 2 and 7 are identified as 2M7 M, 2M7D, 2D7 M, and 2D7D.

As described above, QTLs for heading date under 16-h light were mapped to chromosomes 2 and 7 (Table 2). The order of mean heading date for the four QTL genotypes was D7M2 (46 days)>M7M2 (43 days)>D7D2 (42 days)>M7D2 (35 days) with vernalization, and D7M2 (67 days)>D7D2 (53 days)>M7M2 (50 days)>M7D2 (40 days) without vernalization. The results are consistent with the finding that Dicktoo contributed the late allele on chromosome 7 while Morex contributed the late allele on chromosome 2. As indicated in Fig. 3 a, no interaction between these two QTLs was detected; thus their effects are essentially additive. Vernalization shortened the mean heading date of D7M2 and D7D2 genotypes by 21 days (P>0.01) and 11 days (P> 0.05), respectively, but had no significant effect on the M7M2 and M7D2 genotypes. This supports the conclusion based on the single-locus data that the heading-date QTL on chromosome 7 was responsive to vernalization.

Under 24-h light, the order of mean heading date was D7M2 (65 days)>D7D2 (42 days)>M7M2 (41 days)> M7D2 (31 days) with vernalization, and D7M2 (66 days)> D7D2 (41 days)=M7M2 (41 days)>M7D2 (30 days) without vernalization (Fig. 3 b). No significant differences were found between the vernalized and the unvernalized treatments. Contrary to the additive QTL effects under a 16-h photoperiod, a significant interaction (P> 0.01) was detected between the two QTLs. The difference between the D7M2 and D7D2 QTL genotypes was significantly greater (P> 0.01) than that of the difference between the M7M2 and M7D2 genotypes (23 versus 10 days, respectively). The mean difference between the D7M2 and the M7M2 genotypes was significantly greater (P> 0.01) than the difference between the D7D2 and the M7D2 genotypes (24 vs 11 days, respectively). Thus, the particular allelic combination M2D7 gave a non-additive effect and accounts for the presence of phenotypic transgressive segregants.

Four QTL peaks were detected for heading date under 8-h light with vernalization and five QTL peaks for heading date under 8-h light without vernalization (Table 2). These individual loci were likewise tested for two-locus interactions. The only significant interaction detected was that for the two chromosome-5 intervals defined by *iPgd-BCD265c* and *HorD-ABG452*, and the interaction was significant (P> 0.01) only with vernalization. As can be seen in Fig. 3 c, the heading dates of the D5(*iPgd*)M5(*HorD*) and M5(*iPgd*)D5(*HorD*) lines were 115 and 110 days, respectively, while those of the M5M5 and D5D5 homozygotes were 118 and 91 days, respectively.

Discussion

Using a 39-point linkage map, Hayes et al. (1993 a) found a QTL for winter survival in the *BCD265b-mR* interval on chromosome 7 in this population. With the current 74-point map, this chromosome region is still the only site of significant QTL effects for this trait. The multiple peaks detected in the three intervals defined by *WG364b, mR, Dhn1/Dhn2*, and *BCD265b,* and the shift in peaks across environments, hampers resolution and only places these effects within a 48.3-cM interval. The presence of large numbers of positive phenotypic transgressive segregants in the two field tests may simply be a consequence of the variability associated with measurement of low-temperature tolerance (Fowler and Gusta 1979). If, however, these represent genotypic transgressive segregants, then there must be other QTLs in regions of the genome that have not been completely mapped, or there may be epistatic interactions between the chromosome-7 QTLs and other genome regions that did not have a significant single-locus effect. More definitive mapping of this low-temperature-tolerance effect will require alternative genetic stocks, such as lines that are isogenic for critical chromosome intervals. The association of the *Dhn1* and *Dhn2* loci with this low-temperature-survival QTL is intriguing, given the commonality of dehydration and cold-stress responses (Steponkus 1980). However, confirmation of cause and effect relationships will require further analyses. Dehydrin loci have been mapped elsewhere in the barley genome where QTL effects for winterhardiness-related traits have not been detected. *Dhn3, Dhn4*, and *Dhn5* mapped to chromosome 6, *Dhn4* to chromosomes 6 and 7, and *Dhn6* to chromosome 4. Of the low-temperature-induced clones isolated by Sutton and Kenefick (1994), only *Cr3* revealed an RFLP in this population. No QTL effects for low-temperature-stress tolerance were detected in the region of this locus, which mapped to chromosome 2. A heading-date effect was detected in the interval defined by the adjacent downstream marker, *ABC170*. Ottaviano et al. (1991) reported no association between high-temperature-induced genes and drought-tolerance QTL effects in maize. Further work is necessary before we can reach the same conclusion relative to low-temperature tolerance in barley.

These data confirm the earlier findings of Hayes et al. (1993 a) regarding the coincident map location of QTL effects for low-temperature tolerance and heading date under 24-h light and provide additional information on the location of genes governing growth habit and response to photoperiod and vernalization. QTLs affecting the time from planting to heading under field condition were detected on chromosomes 1, 2, 3, 5, and 7. The heading-date QTL which mapped to the *BCD265c-Dhn1* interval on chromosome 7 was moderately responsive to vernalization under 16 h light. This QTL effect may correspond to the *Sh2* gene described by Takahashi and Yasuda (1970). The accession of Dicktoo used in these studies does not have a vernalization requirement, yet it carries an allele on chromosome 7 that is vernalization responsive. An epistatic response was seen in genotypes with this chromosome-7 allele and the Morex allele on chromosome 2. Takahashi and Yasuda (1970) reported that there was a multiple allelic series at the *Sh2* locus that accounted for gradations in winter-growth habit. Thus, this accession of Dicktoo may carry

an allele conferring an intermediate level of vernalization response because, even in the epistatic allelic configuration without vernalization and under 16-h light, the latest DH lines headed within 30 days of Dicktoo. Two heading-date QTLs were mapped to the long arm of chromosome 5 with the 8-h light treatments. However, the relationship of these QTLs with *Sh3* and the ea_k cannot be established. Takahashi and Yasuda (1970) reported only a single-point linkage of *Sh3* with *B* (black caryopsis) with a recombination percentage of 35.8. The ea_k locus is downstream from the B locus, showing 11.4% recombination with *trd* (third outer glume). Both *B* and ea_k are on the long arm of chromosome 5. No QTL effects were detected on chromosome 4, site of the *sh* locus. Takahashi and Yasuda (1970) reported 6.3% recombination between *sh* and *mHs*, a common marker in the test populations. These authors reported that certain accessions of US spring barley carried the winter-habit allele (*Sh*) at this locus. Thus, it may be that Dicktoo and Morex have the same allele at the *Sh* locus. Hackett et al. (1992) mapped a QTL for heading date, which they termed a *Vrn* locus effect, near *β-amylase*. The distance between *mHs* and *β-amylase* in our map is 13.3 cM.

The heading-date QTL on chromosome 2 was detected under 16- or 24-h photoperiods. The heading-date QTLs on chromosomes 1, 3, and 5 were detected under 8-h light. Genes affecting heading date in the field or under short daylength were also previously reported on chromosomes 1, 2, 3, and 5 in other barley germplasms (Frey 1954; Woodward 1957; Doney 1961; Gallagher et al. 1991). Again, definitive conclusions regarding the allelism of QTL effects and these previously-reported Mendelian loci are not possible due to the lack of common markers. However, in this population, and in the Steptoe x Morex population (Hayes et al. 1993 b), a heading-date QTL where Morex contributes the larger value allele was mapped to chromosome 2 in the approximate region of the *Ea* locus (Nilan 1964).

These data confirm the overriding importance of photoperiod and vernalization in determining phenotypic expression of heading date. Under long-day conditions (16- and 24-h light) variation in heading date was determined by QTLs on chromosomes 2 and 7. Dicktoo contributed the late allele on chromosome 7 and Morex the late allele on chromosome 2. Independent assortment at these unlinked loci gave rise to the transgressive segregation observed under these photoperiod conditions. Under short-day conditions (8-h light), variation for heading date was determined by QTLs on chromosomes 1, 3, 5, and 7. Dicktoo contributed the late alleles at all loci. As a consequence, the parents were at the extremes of the frequency distribution. Under field conditions, where the population was exposed to a dynamic set of photoperiod and temperature conditions, all QTLs identified under controlled-environment conditions were operating. The change in allele phase at the chromosome-2 locus shows that a particular locus can be a determinant of trait expression but that the test environment can dictate allele value. The low probability of recovering genotypes with late alleles at all QTL loci may explain the position of the parents at the extremes of the frequency distribution for heading date under field conditions.

As demonstrated in this report, epistatic interaction can occur between QTLs and these interactions can be specific to a defined set of environmental conditions, such as photoperiod length. Since additive×additive epistasis may be a determinant of trait expression in doubled haploid breeding populations, realized marker-assisted selection based on single-locus effects may not equate to predicted responses. The potential complexities of three-way and higher-order interactions among significant QTLs, interactions of significant QTLs with genome regions where no QTL effects are detected, and the additional effects of changing photoperiod and temperature under field conditions, underscore the complex genetic basis of these characters.

The QTL for vernalization response under the 16-h light and the significant heading-date effects under 16-h and 24-h light mapped to the same region on chromosome 7 as the QTL for winter survival. These data support linkage rather than pleiotropy as the basis for this relationship. Although most of the DH lines that were vernalization responsive had better winter survival, there were exceptions. For example, DH line 10 which was 24 days earlier to head with vernalization under 24-h light, had 12% and 32% winter survival in Montana and Oregon, respectively. Several DH lines had very-weak vernalization responses but good winter survival. For example, DH lines 51, 77, and 80 were only 2, 3, and 4 days earlier with vernalization, respectively. However, their respective winter survival percentages were 45%, 53%, and 82% in Montana, and 95%, 97%, and 100% in Oregon.

We have shown that genome map-based analyses are useful in dissecting a complex phenotype. Significant QTL effects for components of winterhardiness-low temperature tolerance, vernalization response, and photoperiod response-were detected throughout the genome, and these effects often corresponded to the approximate map locations of previously-mapped Mendelian loci. Vernalization-responsive progeny were recovered from the cross of two parents showing only modest response to vernalization. The most responsive QTL effect mapped to chromosome 7, and this may represent one of the possible alleles in the allelic series at the *Sh2* locus (Takahashi and Yasuda 1970). As no QTL effects were detected on chromosome 4 in the vicinity of the *sh* locus, we surmise that the parental lines probably carry the same allele. QTL effects for heading date were detected in two regions on chromosome 5, but a lack of common markers precludes definitive relationship of either of these effects to the *sh3* locus. Under continuous illumination, an epistatic interaction was detected between QTLs on chromosomes 2 and 7, rather than between loci on chromosomes 4, 5, and 7, as reported by Takahashi and Yasuda (1970). A vernalization response was detected only under 16-h light, supporting the contention that photoperiod and vernalization interact to determine maturity.

These results of this study indicate two future courses of investigation: (1) higher-resolution mapping of critical QTLs in this germplasm base, and (2) assessment of orthologous QTL expression in other germplasms. We are,

therefore, developing near-isogenic genetic stocks in the Dicktoo × Morex background and conducting map-based analyses in other winter barley populations.

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