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Statistical analysis of a linkage experiment in barley involving quantitative trait loci for height and ear-emergence time and two genetic markers on chromosome 4

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Summary. The quantitative traits height and ear-emergence date were analyzed in the F_2 progeny of a cross between a tall winter barley cultivar (Gerbel) and a short spring barley cultivar (Heriot). The trait distributions were found to be related to the genotypes at two biochemical loci, β-amylase (*Bmy1*) and water-soluble protein (*Wsp3*), which are known to lie on the long arm of chromosome 4. Linkages between each trait and the markers were investigated using normal mixture models. The two parental phenotypes and the heterozygote phenotype of *Bmy1* were distinguishable so the model could be used directly to estimate linkage between *Bmy1* and a quantitative trait locus (QTL) for height (*Height*). The Gerbel homozygote and heterozygote phenotype of *Wsp3* could not be distinguished and the model was adapted accordingly. The proportion of plants requiring vernalization was consistent with control by two independent genes acting epistatically, and a normal mixture model based on a two-gene hypothesis was fitted to the distribution of ear-emergence date to estimate linkage between the marker loci and a QTL for ear-emergence date (*Vrn1*). The parameters of each model were the recombination fraction between the marker locus and the QTL and the means and standard deviations associated with each QTL genotype; these were estimated by maximum likelihood. The fitted distributions correspond well to those observed and the order of the loci along the chromosome is inferred to be *Height* – *Vrn1* – *Bmy1* – *Wsp3*, with *Wsp3* being the most distal.

Key words: Barley – Ear emergence – Height – Linkage – Quantitative inheritance – Vernalization requirement

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Introduction

Quantitative traits of agricultural significance, such as yield, often cannot be measured early in plant development. However, if a quantitative trait locus (QTL) lies near a genetic marker then unwanted genotypes can be identified and eliminated from breeding populations, so improving the efficiency of selection. Much work has been carried out on testing for, and estimating, linkage between a marker gene and a QTL. Weller (1986) modelled the distribution of a trait for the three marker genotypes of an F_2 population as a mixture of three normal distributions, one for each QTL genotype. Luo and Kearsey (1989, 1991) also fitted mixture models to simulated F_2, backcross, and doubled haploid populations. Mixture models for linkage to more than one genetic marker have been proposed by Weller (1987), Jensen (1989) and Knapp et al. (1990).

One important trait in barley is the time from sowing to ear emergence which, as in all the *Triticeae*, depends on the rates of plant differentiation and growth (Yasuda 1981; Ellis and Russell 1984). These are determined by the interaction of genotypes, especially their vernalization and photoperiod requirement (Doll et al. 1989), with temperature and day-length (Kirby and Ellis 1980). A vernalization requirement delays flowering so that frost damage at vulnerable developmental stages can be avoided. Takahashi and Yasuda (1970) concluded that the spring/winter habit in barley was controlled by three genes, *sh*, Sh_2 and Sh_3, located respectively on barley chromosomes 4, 1 and 7 (homoeologous to 4H, 7H and 5H) and that the only genetic constitution leading to winter habit was $ShShsh_2sh_2sh_3sh_3$.

The experiment reported here was designed to determine the genetic map distance from the biochemical marker gene loci *Bmy1* (syn. *β-Amy-1*) and *Wsp3* (syn.

Wsp-3, *Ibf-1*), which are known to be on the long arm of chromosome 4 (Thompson et al. 1990; Forster et al. 1991), to the gene on this chromosome controlling vernalization requirement. The existence of a gene controlling height on this chromosome arm was also investigated. Following the flexible approach of Weller (1986) we have modelled the quantitative traits as mixtures of genotype-specific distributions and have used the method of maximum likelihood, which has well understood properties, to estimate all parameters.

Materials and methods

Materials

A cross was made between the barley cultivars Gerbel, a tall, winter type with a six-row ear, and Heriot, a short, spring type with a two-row ear. Twenty-five individual F_2 seeds were taken from each of seven F_1 plants and cut in half. The halves without embryos were each screened for β-amylase (EC 3.2.1.2) and for water-soluble protein following the isoelectric focusing (IEF) protocols described by Thompson et al. (1990) and Forster et al. (1991). Embryo halves were germinated and grown in a glasshouse under non-vernalizing conditions and long days. The date of ear emergence and plant height at maturity (from the base of the culm to the collar) were recorded for the plants which flowered under non-vernalizing conditions. Such plants were scored as V^-. Plants which remained vegetative after 90 days were scored as V^+. The V^+ plants were subsequently vernalized but their flowering times and heights were excluded from the analysis because of the change in environment.

Statistical methods

Linkage between the biochemical marker loci Bmy1 and Wsp3

The alleles at the *Bmy1* and *Wsp3* loci were denoted as *β-Amy-1a*, *β-Amy-1b*, *Wsp-3a*, and *Wsp-3b*, by Forster et al. (1991); here they will be denoted by A_a, A_b, W_a, and W_b, for brevity. If n_{ij} is defined as the number of plants of genotype i at the *Bmy1* locus and genotype j at the *Wsp3* locus [where i=1 (A_bA_b), 2 (A_bA_a) or 3 (A_aA_a) and j=1 (W_bW_b) or 2 ($W_aW_b+W_aW_a$, which were indistinguishable)] the genotype frequencies can be modelled as a multinomial distribution. The likelihood for the recombination fraction θ is then:

$$L(\theta)=\frac{\left[\frac{(1-\theta)^2}{4}\right]^{n_{11}}\times\left[\frac{\theta(2-\theta)}{4}\right]^{n_{12}}\times\left[\frac{\theta(1-\theta)}{2}\right]^{n_{21}}\times\left[\frac{1-\theta+\theta^2}{2}\right]^{n_{22}}\times\left[\frac{\theta^2}{4}\right]^{n_{31}}\times\left[\frac{1-\theta^2}{4}\right]^{n_{32}}\times n..!}{n_{11}!\,n_{12}!\,n_{21}!\,n_{22}!\,n_{31}!\,n_{32}!} \quad (1)$$

where $n..$ is the total number of plants. This expression was maximised numerically to estimate θ.

Linkage between genetic markers and a gene for height

Figure 1 shows the heights of the F_2 plants for each of the *Bmy1* genotypes. The distributions vary according to the marker genotype, indicating the presence of a gene influencing height on chromosome 4. This will be referred to as *Height*.

The model of Weller (1986) expresses the probability density functions f_1, f_2 and f_3 of the heights, X, for the three *Bmy1* genotypes as different mixtures of the probability density functions associated with the three *Height* genotypes H_aH_a, H_aH_b and H_bH_b. For example:

$$f_1(X)=P(H_bH_b|A_bA_b)\,f(X|H_bH_b)+P(H_bH_a|A_bA_b)\,f(X|H_bH_a)+P(H_aH_a|A_bA_b)\,f(X|H_aH_a). \quad (2)$$

The conditional probabilities are functions of the recombination fraction θ so the densities can be expressed as:

$$f_1(X)=(1-\theta)^2 f(X|H_bH_b)+2\theta(1-\theta)f(X|H_bH_a)+\theta^2 f(X|H_aH_a) \quad (3)$$

$$f_2(X)=\theta(1-\theta)f(X|H_bH_b)+[1-2\theta(1-\theta)]f(X|H_bH_a)+\theta(1-\theta)f(X|H_aH_a) \quad (4)$$

$$f_3(X)=\theta^2 f(X|H_bH_b)+2\theta(1-\theta)f(X|H_bH_a)+(1-\theta)^2 f(X|H_aH_a). \quad (5)$$

The trait distributions $f(X|H_bH_b)$, $f(X|H_bH_a)$ and $f(X|H_aH_a)$ are assumed to be normal with means μ_S, μ_H and μ_W and standard deviations of σ_S, σ_H and σ_W. The likelihood is:

$$L(\theta,\mu_S,\mu_H,\mu_W,\sigma_S,\sigma_H,\sigma_W)=\left[\prod_{i=1}^{n_{1.}}f_1(X_i)\right]\left[\prod_{j=1}^{n_{2.}}f_2(X_j)\right]\left[\prod_{k=1}^{n_{3.}}f_3(X_k)\right], \quad (6)$$

where $n_{1.}$, $n_{2.}$ and $n_{3.}$ are the numbers of plants with the *Bmy1* genotypes A_bA_b, A_aA_b and A_aA_a respectively. Weller (1986, 1987) and Luo and Kearsey (1989, 1991) used the method of moments to estimate some of the parameters, thus reducing the dimension of the maximisation problem. Here maximum likelihood estimates for all seven parameters were calculated simultaneously using the NAG implementation of the Nelder-Mead algorithm (Nelder and Mead 1965). Asymptotic standard errors of the estimates were derived by numerical approximation to the second derivatives of the log likelihood at the maximum. Maximum likelihood estimation was also used by Jensen (1989).

There are only two distinct distributions associated with the *Wsp3* locus so modifications to Weller's (1986) method are required. The probability density function for the homozygote W_bW_b is unchanged from (3) above. The second density is associated with the genotypes W_aW_b and W_aW_a and can be expressed as:

$$f_{23}(X)=\tfrac{1}{3}\theta(2-\theta)f(X|H_bH_b)+\tfrac{2}{3}(1-\theta+\theta^2)f(X|H_bH_a)+\tfrac{1}{3}(1-\theta^2)f(X|H_aH_a). \quad (7)$$

Apart from this modification, the *Wsp3* parameters were estimated in the same manner as the *Bmy1* parameters.

Linkage between genetic markers and a gene for vernalization requirement

The segregation ratio of $V^-:V^+$ plants in the F_2 generation was found to be 140:29. This is inconsistent with a theoretical 3:1 ratio but agrees with a 13:3 ratio, i.e., in this cross the vernalization requirement can be considered to be controlled by the epistatic interaction of two unlinked genes. These genes will initially be denoted *V1* and *V2* to avoid associations with established gene loci.

Figure 2 shows that the distributions of ear-emergence date and the numbers of plants with a vernalization requirement varied according to the genotype at the *Bmy1* locus. Hence one

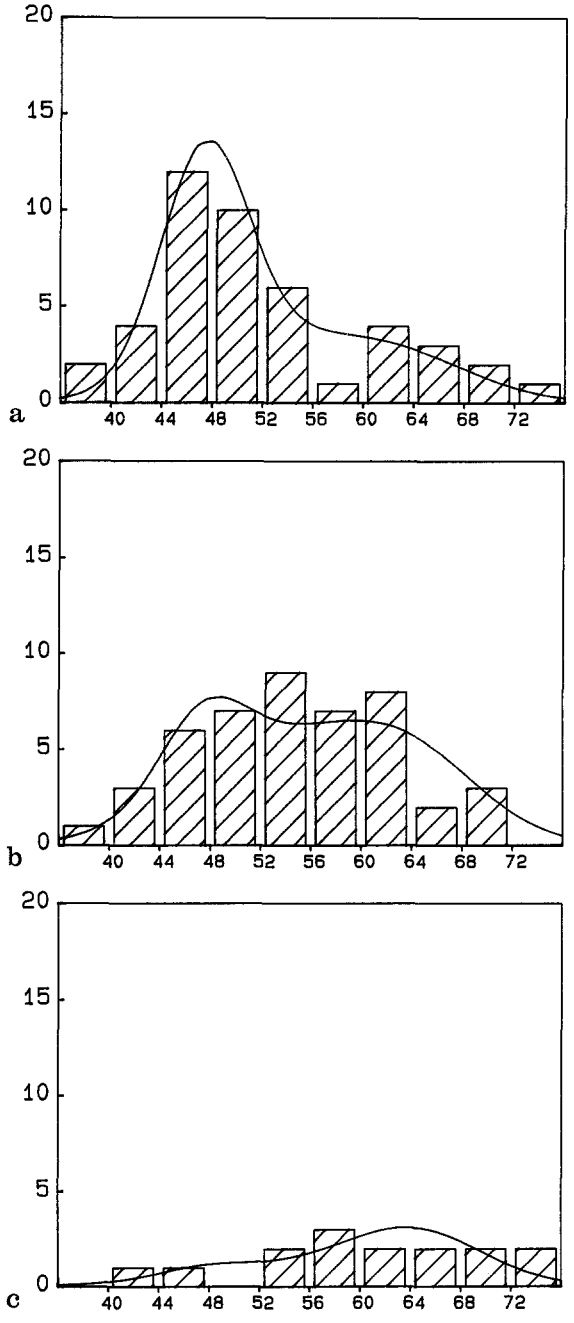

Fig. 1a–c. Observed and fitted height distributions for each genotype of *Bmy1*. **a** Heights for homozygote A_bA_b. **b** Heights for heterozygote A_aA_b. **c** Heights for homozygote A_aA_a. Frequency on y-axis: Height (cm) on x-axis

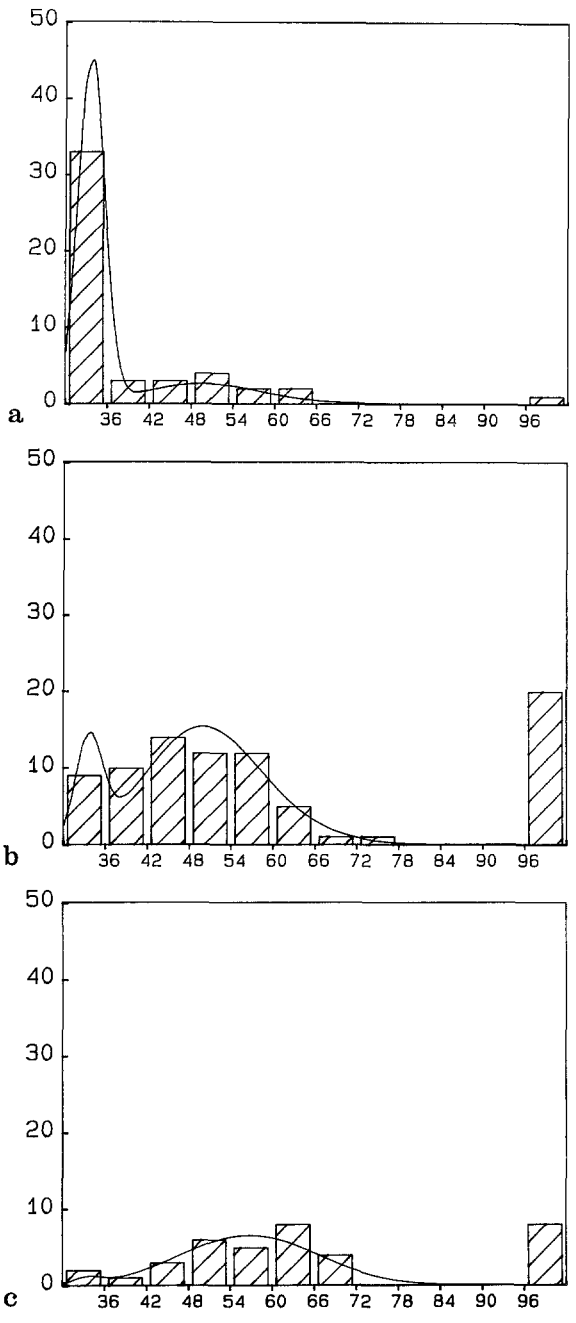

Fig. 2a–c. Observed and fitted distributions of ear-emergence date for each genotype of *Bmy1*. Plants requiring vernalization were coded as having a ear-emergence date of 99 days. **a** Ear-emergence dates for homozygote A_bA_b. **b** Ear-emergence dates for heterozygote A_aA_b. **c** Ear-emergence dates for homozygote A_aA_a. Frequency on y-axis: Ear emergence date (days) on x-axis

vernalization gene, say *V1*, must lie on the long arm of chromosome 4, linked to *Bmy1* and *Wsp3*. The observed data is consistent with the genetic model of Fig. 3. No other two gene model fits the constraints of this data set. The probabilities for the different F_2 genotypes at the *Bmy1*, *V1* and *V2* loci are given in Table 1.

The recombination fraction between *Bmy1* and *V1* can be estimated by classifying each plant as V^- or V^+, and as A_bA_b, A_aA_b or A_aA_a at the *Bmy1* locus. These data were modelled by a multinomial distribution and the maximum likelihood estimate of θ was calculated numerically. The same method was used to estimate the recombination fraction between *Wsp3* and

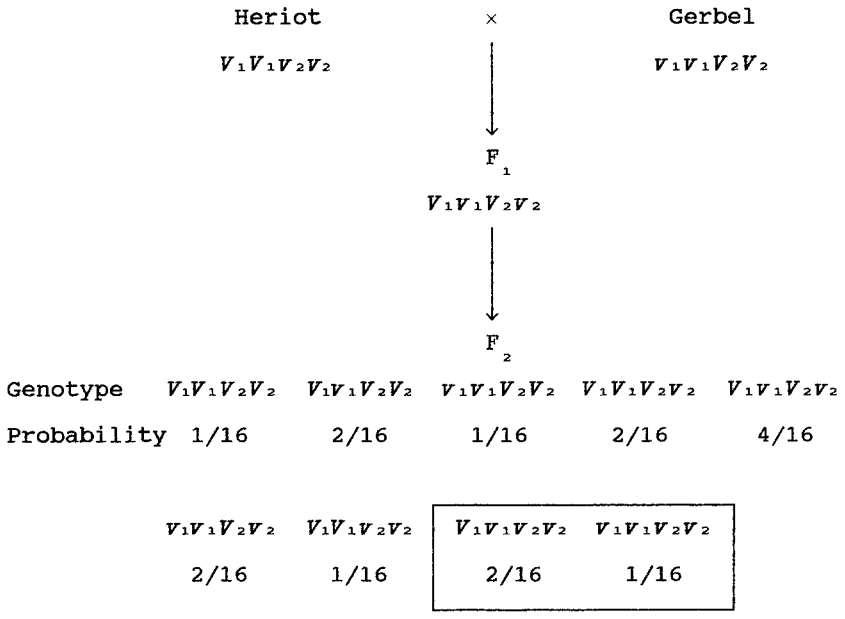

Fig. 3. Inheritance of the unlinked vernalization genes, *V1* and *V2*. The late-flowering genotypes are *boxed*

V1, using the probabilities from Table 1 with the lines for classes A_bA_a and A_aA_a combined.

However, classification of lines as V^- or V^+ ignores the actual ear-emergence dates of those plants that did not require vernalization, although the distribution of these dates varies with the genotypes at the marker loci *Bmy1* and *Wsp3*. If *V1* is regarded as a QTL for ear-emergence date the probability density function of the ear-emergence dates can be modelled for each marker genotype as a mixture of three continuous distributions, together with the probability of a plant requiring vernalization. The mixture probabilities are given in Table 1. The densities of ear-emergence date, Y, for the three *Bmy1* genotypes, conditional on the plant not requiring vernalization, can be written as:

A_bA_b homozygote:

$$f_1(Y) = \frac{(1-\theta)^2 f(Y|V_1V_1) + \frac{3}{2}\theta(1-\theta) f(Y|V_1v_1) + \frac{3}{4}\theta^2 f(Y|v_1v_1)}{1-\frac{1}{4}\theta(2-\theta)} \quad (8)$$

A_aA_b heterozgyote:

$$f_2(Y) = \frac{\theta(1-\theta) f(Y|V_1V_1) + \frac{3}{4}(1-2\theta(1-\theta)) f(Y|V_1v_1) + \frac{3}{4}\theta(1-\theta) f(Y|v_1v_1)}{1-\frac{1}{4}(1-\theta+\theta^2)} \quad (9)$$

A_aA_a homozygote:

$$f_3(Y) = \frac{\theta^2 f(Y|V_1V_1) + \frac{3}{2}\theta(1-\theta) f(Y|V_1v_1) + \frac{3}{4}(1-\theta)^2 f(Y|v_1v_1)}{1-\frac{1}{4}(1-\theta^2)} \quad (10)$$

The ear emergence dates for each genotype at the *V1* locus were again assumed to follow a normal distribution and the parameters were estimated by maximum likelihood.

In the case of *Wsp3* the model has to be adapted as previously described. The probability density function for the homozygote W_bW_b is the same as (8) while the probability density function for genotypes W_aW_b and W_aW_a, conditional on the plant not requiring vernalization, is:

$$f_{23}(Y) = \frac{\frac{1}{3}\theta(2-\theta) f(Y|V_1V_1) + \frac{1}{2}(1-\theta+\theta^2) f(Y|V_1v_1) + \frac{1}{4}(1-\theta^2) f(Y|v_1v_1)}{1-\frac{1}{12}(3-2\theta+\theta^2)} \quad (11)$$

Table 1. Probabilities of the F_2 genotypes for plants segregating at the *Bmy1*, *V1* and *V2* loci

	V^+ (require vernalization)	V^- (no vernalization requirement)			
	V1v1v2v2 *v1v1v2v2*	*V1V1V2V2* *V1V1V2v2* *V1V1v2v2*	*V1v1V2V2* *V1v1V2v2*	*v1v1V2V2* *v1v1V2v2*	
A_bA_b	$\theta(2-\theta)/16$	$(1-\theta)^2/4$	$3/8\,\theta(1-\theta)$	$3/16\,\theta^2$	1/4
A_bA_a	$(1-\theta+\theta^2)/8$	$\theta(1-\theta)/2$	$3/8\,(1-2\theta(1+\theta))$	$3/8\,\theta(1-\theta)$	1/2
A_aA_a	$(1-\theta^2)/16$	$\theta^2/4$	$3/8\,\theta(1-\theta)$	$3/16\,(1-\theta)^2$	1/4
	3/16	1/4	3/8	3/16	

Results

The β-amylase and water-soluble protein phenotypes of Gerbel and Heriot have already been described (Forster et al. 1991). In the case of β-amylase the heterozygote phenotype could also be distinguished. The individual segregation ratios for β-amylase and water-soluble protein were consistent with the expected 1:2:1 and 3:1 ratios respectively and the pairwise segregation confirmed linkage between the two markers. The recombination fraction θ was estimated as 0.085 ± 0.032.

The maximum likelihood estimates and standard errors of the mixture distribution parameters describing the linkage of *Height* with *Bmy1* and *Wsp3* are given in Table 2. The model requires that the means and standard deviations associated with the *Height* genotypes are independent of the position of the genetic marker; this was confirmed by the similarity of the two sets of estimates.

Table 2. Estimates of the recombination fractions and the means and standard deviations associated with the three genotypes at the *Height* QTL

Parameter estimates	Marker			
	Bmy1		*Wsp3*	
	Estimate	SE	Estimate	SE
Recombination fraction	0.255	0.056	0.267	0.066
Mean of short homozygote	47.4	0.98	47.4	1.06
Mean of heterozygote	55.4	1.65	55.6	2.18
Mean of tall homozygote	64.6	2.17	63.4	2.91
SD of short homozygote	3.45	0.70	3.29	0.80
SD of heterozygote	8.27	1.09	9.10	1.33
SD of tall homozygote	5.30	1.29	5.49	1.39
Recombination fraction between *Bmy1* and *Wsp3*	0.085	0.032		

Table 3. Observed and estimated means and standard deviations of the distributions of height for each marker genotype

Locus and genotype	Mean		Standard deviation	
	Observed	Estimated	Observed	Estimated
Bmy1				
Homozygote A_bA_b	51.82	51.56	8.91	7.79
Heterozygote A_aA_b	55.37	55.63	7.74	8.84
Homozygote A_aA_a	61.40	59.99	8.94	8.51
Wsp3				
Homozygote W_bW_b	51.70	51.77	8.91	8.12
Heterozygote W_aW_b and homozygote W_aW_a	57.16	56.76	8.72	9.12

Mean plant heights are estimated to be approximately 48, 55 and 64 cm depending on the genotype at the *Height* locus. The observed and fitted distributions for each genotype of *Bmy1* are shown in Fig. 1. Chi-squared goodness of fit tests failed to detect any significant departure from the models [$\chi^2_{(8)}=8.82$, $P=0.36$ for *Bmy1*; $\chi^2_{(5)}=2.45$, $P=0.78$ for *Wsp3*]. The observed and estimated means and standard deviations of the distribution for each marker genotype also agree well, as shown in Table 3. A mixture of two distributions was fitted to each data set to test the hypothesis of a dominant allele obscuring any difference between the heterozygote and tall genotypes at the *Height* locus. However, a likelihood ratio test rejected this hypothesis in favour of three distributions with $P<0.005$ for linkage to *Bmy1* and $P<0.1$ for linkage to *Wsp3*.

When the F_2 plants were classified simply as V^- or V^+ the ratio was significantly different from a 3:1 ratio, but was consistent with a 13:3 ratio. This indicates control by two unlinked genes, i.e., *V1* and *V2*. The pairwise segregations of the markers indicated linkage between *Bmy1*, *Wsp3* and *V1*. The estimates for recombination fraction and Kosambi map distances (Table 4) indicated that the closest linkage was between *Bmy1* and *V1* [4.3 centiMorgans (cM)]. The map distance between *Wsp3* and *V1* (11.5 cM) was greater than that between *Wsp3* and *Bmy1* which indicates that the order of the loci is *V1* – *Bmy1* – *Wsp3*.

Table 4. Estimates of the recombination fractions and the means and standard deviations associated with the three genotypes at the *Head* QTL

Parameter estimates	Marker			
	Bmy1		*Wsp3*	
	Estimate	SE	Estimate	SE
Recombination fraction	0.138	0.031	0.155	0.033
Mean of spring homozygote	33.7	0.32	33.8	0.32
Mean of heterozygote	49.4	1.35	48.6	1.10
Mean of winter homozygote	59.0	2.76	61.5	1.79
SD of spring homozygote	1.89	0.23	1.89	0.23
SD of heterozygote	8.00	1.08	6.44	0.94
SD of winter homozygote	9.00	2.50	6.07	1.18
Recombination fraction with *V1*	0.043	0.060	0.113	0.110

However, *V1* can be regarded as a QTL, *Head*, affecting ear emergence date. In this case the parameters describing its linkage with *Bmy1* and *Wsp3* are given in Table 4 and, as for *Height*, the estimates of the means and standard deviations are consistent for the two markers. Mean ear-emergence times of about 34, 49 and 60 days are predicted depending on the genotype at the *V1* locus. Chi-squared goodness of fit tests failed to detect any significant departures from the model developed using the two-gene hypothesis [$\chi^2_{(9)}=7.32$, $P=0.60$ for *Bmy1*; $\chi^2_{(2)}=3.99$, $P=0.14$ for *Wsp3*]. The observed and estimated means and standard deviations of the mixture distributions for each marker genotype and the observed and estimated numbers of plants requiring vernalization agree well (Table 5).

When *V1* is regarded as a QTL the estimates of the recombination fraction are larger than when *V1* is regarded as a Mendelian gene, but not significantly so. The precision of the estimates is increased by regarding *V1* as a QTL. In either case the recombination fractions between *Height* and *Wsp3* or *Bmy1* were significantly greater than those between *V1* and *Wsp3* or *Bmy1*. The orientation of *Bmy1* and *Wsp3* has been established by mapping to *ml-o* (Thomas et al. 1992) and hence the order of genes implied by the QTL analyses is *Height* – *V1* – *Bmy1* – *Wsp3* with *Wsp3* being the most distal.

Table 5. Observed and estimated means and standard deviations of the distributions of ear emergence date for each marker genotype

Locus and genotype	Mean		Standard deviation		Late plants	
	Observed	Estimated	Observed	Estimated	Observed	Estimated
Bmy1						
Homozygote A_bA_b	39.69	41.02	12.40	16.95	1	3.07
Heterozygote A_aA_b	60.74	59.31	23.08	22.91	20	18.48
Homozygote A_aA_a	65.43	66.62	19.92	20.47	8	9.07
Wsp3						
Homozygote W_bW_b	40.80	41.80	15.14	17.51	2	2.91
Heterozygote W_aW_b and homozygote W_aW_a	61.30	61.71	21.88	22.17	24	25.31

Discussion

Genes controlling height and ear-emergence date have been mapped to the long arm of chromosome 4 of barley. The mean plant heights corresponding to the three *Height* genotypes were 48, 55 and 64 cm. The spring parent, Heriot, is known to possess the *denso* dwarfing gene (Thomas et al. 1990) derived from Triumph, which has not yet been located to a chromosome. We suggest that the gene for height located here may be the *denso* gene.

Previous studies have also identified a genetic determinant of ear-emergence date on chromosome 4. Takahashi et al. (1957) and Takahashi and Hayashi (1966) mapped a gene (*sh*) controlling spring habit to chromosome 4, 2.1 cM from the locus for yellow spike (*yh*). Nielsen et al. (1983) estimated the distance between *Bmy1* and *yh* to be 6 cM. Chojecki et al. (1989) deduced the genetic distance from *Bmy1* to be about 4 cM. We therefore conclude that *V1* is synonymous with *sh*. We will now use the symbol *Vrn1* (Forster and Ellis 1991) as this gene probably forms part of a homoeoallelic series with *Vrn1* of wheat (Pugsley 1972; Law et al. 1976) which is also linked to the *β-Amy-1* (syn. *β-Amy-2* and homoeoallelic to *Bmy1* in barley) and the *Wsp-3* (syn. *Ibf-1* and homoeoallelic to *Wsp3* in barley) loci (Ainsworth et al. 1983; Liu and Gale 1989, respectively).

Our data suggest a two gene hypothesis for the segregation of vernalization requirement, i.e., *Vrn1* on chromosome 4, linked to *Bmy1* and *Wsp3*, which interacts with an unlinked gene, *Vrn2*. This is supported by the data of Doll et al. (1989) and that of Kjaer et al. (1991). Takahashi and Yasuda (1970) found that three genes were segregating for winter/spring habit in their cross but Gerbel and Heriot could be homozygous for the same allele at the third locus of Takahashi and Yasuda. Linkage between *Vrn1* and the genetic markers could be estimated simply by scoring plants as V^- or V^+: in this case the estimates of the recombination fraction have very large standard errors. If *Vrn1* is regarded as a QTL affecting ear emergence date its position may be estimated with more precision and the hypothesised distributions agree well with the observed data. More complicated genetic mechanisms could be proposed to explain the distribution of ear-emergence dates but the simple two-gene hypothesis is adequate. In particular, it is possible that more than one gene for ear-emergence date lies on chromosome 4, which would bias the parameter estimates. This might be the situation if the gene for height is actually the *denso* dwarfing gene, which is known to be associated with delayed ear-emergence date (Thomas et al. 1990). These hypotheses need to be tested using further genetic markers. Useful markers would include the *yh* locus (yellow spike, Takahashi and Hayashi 1966) and the *ml-o* locus for mildew resistance, which is known to lie near the estimated position of *Height* (Søgaard and von Wettstein-Knowles 1987).

The method of maximum likelihood has been used in this study to estimate all the parameter values. The maximum of the likelihood surface was found numerically via the Nelder-Mead algorithm (Nelder and Mead 1965) thus enabling all seven parameter estimates to be found simultaneously. In each case valid results were obtained, i.e., the recombination fraction was less than 0.5 and the means and standard deviations were positive. Jensen (1989) also used maximum likelihood estimation, while Weller (1986) and Luo and Kearsey (1989, 1991) equated observed and expected means and variances of the trait distributions associated with each marker genotype to reduce the number of parameters to be estimated by maximum likelihood. However Luo and Kearsey (1991) suggest that these moment estimates may be biased due to skewness in the distributions associated with each marker genotype. The observed moments were not used to derive the parameter estimates reported in this paper but they have been compared to the estimated moments in Tables 3 and 5 and found to agree well. When the calculations were repeated using the method of moments the model fitted the data less well, although χ^2 goodness of fit tests did not indicate a significant lack of fit.

The estimates of the recombination fractions have quite large standard errors. These would be reduced if data were available from more lines. Alternatively, if the plants are scored for more genetic markers it should be possible to extend the model to a pair of flanking markers. Weller (1987) used a flanking-marker model to estimate recombination fractions for an interspecific cross in tomato from mean trait values of individuals homozygous for the marker alleles, and concluded that the estimates were more reliable than those from individual marker models. Lander and Botstein (1989) have used such models to calculate QTL likelihood maps showing how the LOD score varies along a chromosome. Jensen (1989) mapped a QTL for single-kernel weight in barley relative to two marker loci on chromosome 7. Knapp et al. (1990) have discussed two-marker models in more detail for doubled haploid and equivalent crosses, especially a model including a coefficient of coincidence to model interference. They have also proposed a no-double-crossover model for F_2 data, and have used linear, non-linear, and mixture models to estimate parameter values. These models could perhaps be adapted for use with a data set such as the present one, where the two linked markers lie on the same side of the QTL, thus allowing all the parameter values to be estimated simultaneously.

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