Theor Appl Genet (1994) 87:721–732

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1994

Genetic analysis of morphological variation in *Brassica oleracea* using molecular markers

W. C. Kennard[1,*], M. K. Slocum[2,], S. S. Figdore[2,***], T. C. Osborn[1]**

[1] Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA
[2] NPI, 417 Wakara Way, Salt Lake City, UT 84108, USA

Received: 15 September 1992 / Accepted: 16 June 1993

Abstract. A cross between the open-pollinated *Brassica oleracea* cabbage cultivar 'Wisconsin Golden Acre' and the hybrid broccoli cultivar 'Packman' was used with molecular markers to investigate the genetic control of morphological variation. Twenty-two traits derived from leaf, stem, and flowering measurements were analyzed in 90 F_2 individuals that were also classified for genotype by restriction fragment length polymorphism (RFLP) markers. Seventy-two RFLP loci, which covered the mapped genome at an average of 10 map-unit intervals on all nine linkage groups, were tested individually for associations to phenotypic measurements by single factor ANOVA, and markers with significant associations ($P < 0.05$) were used to develop multilocus models. These data were utilized to describe the location, parental contribution of alleles, magnitude of effect, and the gene action of trait loci. Single marker loci that were significantly associated ($P < 0.05$) with trait measurements accounted for 6.7–42.7% of the phenotypic variation. Multilocus models described as much as 60.1% of the phenotypic variation for a given trait. In some cases, different related traits had common marker-locus associations with similar gene action and genotypic class ranking. The numbers, action, and linkages, of genes controlling traits estimated with marker loci in this population corresponded to estimates based on classical genetic methods from other studies using similar, or similarly-wide, crosses. There was no evidence that genome duplication accounted for a significant portion of multiple genes controlling trait loci over the entire genome, but possible duplications of trait loci were identified for two regions with linked, duplicated marker loci.

Key words: Restriction fragment length polymorphism – *Brassica oleracea* – Quantitative trait loci – Morphological variation

Communicated by J. Beckmann

* *Present address:* Department of Agronomy, Colorado State University, Fort Collins, CO 80523, USA
** *Present address:* Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
*** *Present address:* Upper Iowa University, Fayette, IA 52142, USA
Correspondence to: T. C. Osborn

Introduction

Brassica oleracea is an important vegetable crop species which includes fully cross-fertile cultivars or form groups with widely-differing morphological characteristics (cabbage, broccoli, cauliflower, collards, Brussels sprouts, kohlrabi, and kale). Genetic studies of *B. oleracea* have been limited in part by the long generation time of the biennials, the complex inheritance patterns of some traits, and the difficulty in overcoming self-incompatibility. However, genetic analyses of progeny from crosses between cultivar groups of *B. oleracea* have provided information on the inheritance of some morphological traits, such as annual habit (Detjen 1926; Dickson 1968; Baggett and Walhert 1975; Pelofske and Baggett 1979), internode distance (Pease 1926; Dickson 1968; Pelofske and Baggett 1979), heading (Kristofferson 1924; Pelofske and Baggett 1979), and leaf characteristics (Kristofferson 1924; Pease 1926). Complex inheritance was often reported for these traits; and in some cases different studies reported conflicting results, probably due in part to

differences in the accessions or forms used as parents to determine inheritance. Although *B. oleracea* does not have an extensive genetic map of morphological markers, some linkage associations for genes controlling morphological traits have been detected in segregants of crosses between cultivar groups (Pease 1926; Pelofske and Baggett 1979).

The complex inheritance patterns of morphological traits in *B. oleracea* can be dissected by using molecular marker analyses. These types of analyses employ isozymes or DNA polymorphisms, such as restriction fragment length polymorphisms (RFLPs), to fully classify genotypes of individuals at marker loci. Using least-squares or maximum-likelihood methods, trait loci can be identified and localized by linkage to marker loci, and the magnitude of gene effects and types of gene action for trait loci can be estimated. Use of a common set of marker loci in different crosses allows for comparisons of trait loci from different sources. Molecular markers have been used to identify genes controlling various traits in tomato (Tanksley et al. 1982; Nienhuis et al. 1987; Osborn et al. 1987; Weller 1987; Paterson et al. 1988; Tanksley and Hewitt 1988; Young et al. 1988) maize (Edwards et al. 1987, 1992; Stuber et al. 1987, 1992; Doebly and Stec 1991) and soybean (Keim et al. 1990).

Accessions of *B. oleracea* are highly polymorphic, both within and between cultivar groups, for DNA restriction fragment lengths (Figdore et al. 1988; Song et al. 1988). This diversity has facilitated the construction of the first RFLP linkage map in *B. oleracea* using a segregating F_2 population from a cross between cabbage (cultivar group *capitata*) and broccoli (cultivar group *italica*) (Slocum et al. 1990). Cabbage and broccoli represent extremes for many traits. Cabbage is biennial, requires low temperatures for flower induction, forms short internodes, and has round, sessile leaves that clasp the plant apex; where as broccoli is an annual, does not require cold to induce flowering, forms long internodes, and has oblong, petiolate leaves that turn away from the apex of the plant. For this study, the same F_2 individuals used previously for RFLP linkage mapping by Slocum et al. (1990) were analyzed for variation in morphological traits, such as flower development, leaf dimensions, heading habit, and internode distance. Marker loci were used to localize and estimate the effects of genes controlling these traits.

Materials and methods

Plant materials

A single F_2 population was used for RFLP linkage mapping and morphological trait analysis. It was generated from a cross between the open-pollinated cabbage cultivar 'Wisconsin Golden Acré and the broccoli hybrid 'Packman', using cabbage as the maternal parent. F_1 plants were vernalized for 6 weeks and plants were bud-pollinated to produce F_2 seed. F_2 seed from a single F_1 plant, along with seeds from the same seed stock as the parents and F_1 seeds from crosses between the same parent plants, were sown in Madison, Wisconsin on July 14, 1987 (day 1). Seeds were sown in 12-pack units (Comp-pack D812) using autoclaved soil (1 peat moss:1 field soil:1 sand) transplanted to 30 cm wide by 25 cm deep posts on August 4 (day 21), and maintained outside in cold frames. Plants were transferred to a greenhouse on October 9 (day 87) so that flowering traits could be evaluated for up to 300 days. Plants were fed slow release fertilizer, Osmocote^R (10-10-10), and sprayed intermittently with Diazanon^R, Orthene^R, Thuricide^R and Pentac^R for pest control.

Trait measurements

Eighteen traits related to leaf, stem, whole plant, and flowering characteristics were scored on 70–90 F_2 plants (Table 1). Four plants from the same seed lots as the parent plants and three F_1 plants were measured concurrently with the F_2 population. Trait selection and measurement techniques were based on phenotypic differences observed among parents and F_2 plants at the same growth stage, using phenotypic descriptors of *B. oleracea* (IPBGR 1987) and methods from previous genetic linkage studies (Pease 1926; Yarnell 1956; Pelofske and Baggett 1979). The majority of measurements were taken between 37 and 97 days after sowing and before tissue harvest for RFLP analysis. Measurement of a given trait was performed on the same day for all individuals, except for traits associated with maturity. Leaf measurements were performed on the adaxial surface of the largest leaf of a plant except where noted. Some traits were measured or scored twice, either to obtain averages or to determine genetic control at two different stages in development (Table 1). Ratios or sums were calculated on a subset of component traits to generate four new variables (compiled traits) which were thought to have biological significance (Table 1). Many of the compiled traits and component traits, as well as other traits, were significantly correlated in the F_2 population. These traits were included in the marker analysis in order to determine the degree of common marker-locus associations among the traits.

RFLP marker analysis

The 90 random F_2 plants analyzed in this study were a subset of the 96 plants used previously by Slocum et al. (1990) to construct a detailed RFLP linkage map of *B. oleracea*. The source for DNA probes and the methods for detecting and mapping RFLPs were cited in that paper. Each plant was assigned to one of three genotypic classes for each of 72 marker loci: homozygous broccoli (B/B), homozygous cabbage (C/C), or heterozygous (B/C). These marker loci were selected to cover the mapped genome at intervals of no more than 21 map units with an average distance of 10 map units apart as determined by summing the map distances between all intervening marker loci (Slocum et al. 1990). Recombination frequencies were transformed with a mapping function developed by Kosambi (1944), so map distances reported herein are occasionally larger than the recombination frequencies reported by Slocum et al. (1990).

In order to detect potential duplicated trait loci, a second analysis was conducted using segregation data for all 116 replicated marker loci (two or more segregating loci detected per probe) that had been mapped previously in this population (Slocum et al. 1990). This analysis resulted in a comparison of 79 pairs of duplicated loci, since multiple comparisons could be made for replicated loci having three or more segregating copies.

Table 1. Designation of traits and description of trait measurements

Trait designation	Description
Leaf traits	
Lamina length	Distance (cm) from the base to the apex of the lamina (mean of measurements at 52 and 59 days)
Lamina width	Distance (cm) across the widest section of the lamina (mean of measurements at 52 and 59 days)
Petiole length	Distance (cm) from stem to lamina (mean of measurements at 52 and 59 days)
Lamina length/width	Ratio lamina length to lamina width
Lamina/petiole length	Ratio of lamina length to petiole length
Leaf length	Sum of lamina length and petiole length
Lamina petiole width	Width (cm) of petiole cross section at the junction of petiole and midrib at 89 days
Basal petiole width	Width (cm) of petiole cross section one cm from the stem taken at 89 days
Lamina petiole thickness	Thickness (cm) of petiole cross section at the junction of petiole and midrib at 89 days
Basal petiole thickness	Thickness (cm) of petiole cross section one cm from the stem at 89 days
Stem traits	
Distance of 20 internodes	Distance (cm) from soil to the twentieth internode measured at 97 days
Distance between second and third node	Distance (cm) from second node to third node measured at 52 days
Distance between third and fourth node	Distance (cm) from third node to fourth node measured at 52 days
Leaf and stem traits with discrete classification	
Leaf number	Number of open leaves at 59 days
Head-forming leaf overlap	Degree of head forming habit measured at 92 days on a scale of 0 (turning away from the apex at right angles) to 7 (tightly clasping the terminal bud)
Lamina curl	Degree of curling of the margins measured at 52 days on a scale of 0 (very wavy) to 3 (straight)
Lamina color	Gradation of lamina color measured at 52 days on a scale of 0 (blue green) to 5 (yellow green)
Flowering traits	
Annual vs biennial	Growth habit scored as annual (less than 300 days to flower with no vernalization) or biennial (more than 300 days to flower with vernalization)
Days-to-bud	Number of days to formation of a thumb sized bud cluster in the apical whorl of annual-type plants
Days-to-flower	Number of days to first open flower of annual-type plants
Days from bud to flower	Number of days from bud cluster formation to open flower of annual-type plants
Size of cluster	Estimation of number of buds in a thumb sized flower cluster of annual-type plants

Statistical analyses

Significant associations between the 72 marker loci and the 22 traits were detected initially using single factor ANOVA of the trait means for the three genotypic classes at marker loci (GLM procedure, SAS Institute 1982). The ANOVA model used to test for significant effects was:

$$\text{trait value} = \text{grand mean} + \text{genotypic class effect} + \text{error},$$

assuming errors were independent and equal among the three genotypic classes. Least significant differences among genotypic class means were determined by Scheffés test controlling the Type I erorr at $P < 0.05$ among classes (Snedecor and Cochran 1980). Genotypic class effects at a locus were partitioned into additive and dominance components and were estimated using the contrasts: B/B vs C/C (additive) and B/C vs (B/B + C/C)/2 (dominance). Additive, partial-dominance, dominance, and overdominance assignments were based on the magnitude of the ratio of the dominance component effect over the additive component effect (Stuber et al. 1987). Interlocus interactions (epistasis) for genotypic effects at selected pairs of marker loci were analyzed with the SAS GLM procedure, and the interaction was paritioned with the four orthogonal contrasts (additive × additive, dominant × additive, additive × dominant, and dominant × dominant). Precise trait locus map positions were not estimated in this study, but in most cases trait loci were assumed to be localized between the marker loci having the strongest associations (highest R^2 value and lowest P value).

The number of marker loci significantly associated with traits can underestimate the number of trait loci because tightly-linked genes affecting a given trait might not be distinguishable, or can overestimate the number of trait loci if multiple linked markers are associated with a single trait locus. To provide a minimum estimate of the number of trait loci, multiple marker loci associated with traits were further analyzed using the SAS GLM procedure. Approximately 4–12 unlinked or partially-linked marker loci that were the most significant as determined by one-way ANOVA were initially incorporated into the following multilocus model:

$$\text{trait value} = \text{grand mean} + \text{genotypic class effect}_1 + \text{genotypic class effect}_2 + \cdots + \text{error}.$$

Marker loci were excluded manually, one at a time, based on the criterion of least-significant (Type III sum of squares, $P < 0.05$) contribution to the model in a "backwards elimination" process analogous to stepwise regression. The genotypic data were slightly modified for multilocus models by estimating missing genotypes for particular plants using flanking marker loci. Heterozygotes were assigned for the few cases where missing data occurred in a crossover region and resulted in an 0.5 probability of choosing the correct marker locus genotype. This modification was necessary to maintain sufficient data set size.

Chi-square tests of marker associations were also performed for the trait annual vs biennial using cross-tabulation frequency tables with the SAS FREQ procedure. Normality of the F_2 population distribution for each trait was determined using the SAS UNIVARIATE procedure. Pearson correlation coefficients and simple statistics on the traits of the F_2 population were calculated with the SAS CORR procedure. Equality of variance among genotypic classes was checked through residual plot analysis using the SAS GLM procedure.

Results and discussion

Parental, F_1, and mapping-population phenotype

Significant differences between the parents, 'Wisconsin Golden Acre' cabbage and 'Packman' broccoli, were found for all traits except lamina width and basal petiole thickness (Table 2). The F_1 plants were intermediate to the parents for most traits, but for many leaf and stem traits, the F_1 values were closer to the broccoli parent values, suggesting dominance or partial dominance for broccoli alleles. The F_2 population included individuals with a wide range of phenotypes and transgressive segregants were observed for many traits (Table 2). Although the plants segregated widely for a number of morphological characteristics, many of which were analyzed in this study, no commericial broccoli or cabbage types were observed.

Highly-significant correlations ($P < 0.001$) were found between some traits measured in the F_2 population. Lamina length was positively correlated with lamina width ($R = 0.60$) and with petiole length ($R = 0.40$), and negatively correlated with lamina color ($R = -0.34$). Days-to-bud was strongly correlated with days-to-flower ($R = 0.92$) and with head-forming leaf-overlap ($R = 0.42$). Annual vs biennial was also correlated with head-forming leaf-overlap. All measurements of internode distance were positively correlated ($R = 0.20$ to 0.43).

The population was normally distributed for lamina width, petiole length, distance of 20 internodes, and stem diameter. However, for other traits, the population was not normally distributed. Deviations from normality might be expected for traits controlled by dominant or partially-dominant alleles having major effects.

Marker locus-trait associations

Of the 1,584 total marker locus-trait comparisons (22 traits × 72 marker loci), 192 (12.1%) were significant at $P < 05$, 88 (5.5%) were significant at $P < 0.01$, and 26

Table 2. Means (standard deviations) and ranges of parental, F_1 and F_2 plants for morphological traits

Trait[a]	Wisconsin Golden Acre mean (std. dev.)	Packman mean (std. dev)	F1 mean (std. dev.)	F2 mean (std. dev.)	F2 range
Lamina length (cm)	13.09 (0.59)	17.82 (0.69)	17.53 (0.65)	17.67 (2.36)	9.5–22.8
Lamina width (cm)	14.06 (0.51)	14.14 (1.22)	14.88 (0.28)	15.77 (2.13)	9.2–21.0
Petiole length (cm)	0.00 (0.00)	11.30 (0.83)	9.50 (1.08)	10.50 (2.28)	3.8–17.9
Lamina length/width	0.93 (0.06)	1.14 (0.02)	1.19 (0.04)	1.12 (0.14)	0.8–4.3
Lamina/petiole length	0.00 (0.00)	1.52 (0.43)	1.83 (0.73)	1.76 (0.47)	0.8–4.3
Leaf length (cm)	13.10 (0.07)	29.10 (1.31)	27.10 (0.94)	28.18 (3.80)	16.4–37.2
Lamina petiole width (cm)	2.18 (0.36)	0.80 (0.08)	1.17 (0.06)	1.16 (0.39)	0.6–2.9
Basal petiole width (cm)	2.18 (0.36)	1.63 (0.13)	2.13 (0.15)	1.99 (0.37)	1.1–2.9
Lamina petiole thickness (cm)	1.05 (0.06)	0.85 (0.10)	1.20 (0.17)	1.04 (0.19)	0.7–1.5
Basal petiole thickness (cm)	1.05 (0.06)	0.95 (0.10)	1.20 (0.17)	1.02 (0.26)	0.6–1.9
Distance of 20 internodes (cm)	5.12 (0.25)	17.40 (1.92)	12.40 (2.44)	16.46 (3.21)	8.5–25.5
Distance between second and third node (cm)	0.63 (0.30)	1.55 (0.92)	1.60 (0.10)	1.77 (0.73)	0.3–3.6
Distance between third and fourth node (cm)	0.55 (0.21)	1.45 (0.78)	1.53 (0.21)	1.66 (0.64)	0.5–3.6
Leaf number	10.5 (1.3)	13.5 (1.0)	13.0 (1.7)	12.0 (3.1)	6–25
Head-forming leaf overlap (0–7)	7.0 (0.0)	1.0 (0.0)	2.5 (0.6)	4.7 (1.2)	1–7
Lamina curl (0–3)	0.0 (0.0)	3.0 (0.0)	1.7 (0.6)	1.6 (0.9)	0–3
Lamina color (1–5)	5.0 (0.0)	1.0 (0.0)	3.0 (0.0)	2.9 (1.5)	1–5
Days-to-bud[b]	> 300.0 (0.0)	45.8 (4.4)	154.7 (15.8)	142.0 (48.9)	62–281
Annual (0) vs biennial (1)	1.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.3)	0–1
Days-to-flower[b]	> 300.0 (0.0)	79.0 (28.0)	171.0 (15.1)	179.5 (51.0)	83–295
Days from bud to flower[b]	13.0 (4.5)	33.2 (9.6)	16.3 (6.3)	30.8 (19.7)	11–70
Size of cluster (50–1000)[b]	50.0 (0.0)	1000.0 (0.0)	100.0 (0.0)	229.1 (117.1)	50–500

[a] See Table 1 for description of traits

[b] Measured only on annual type plants in the F_2 population

(1.6%) were significant at $P < 0.001$. Twenty-one of the twenty-two traits tested had at least one marker locus significantly associated at $P < 0.01$, and nine traits were significantly associated with at least one marker locus at $P < 0.001$. R^2 values ranged from 0.067 to 0.427 for significant ($P < 0.05$) comparisons. Thirteen marker loci had R^2 values of over 0.200 for some trait. These R^2 values are higher, but the percentage of significant comparisons are lower, than those found in studies of maize which utilized fewer marker loci and much larger populations (Edwards et al. 1987; Stuber et al. 1987). In a more recent study of maize which employed 114 marker loci and 187 F_2 plants (Edwards et al. 1992), the proportion of significant ($P < 0.05$) comparisons was closer to that observed in our study. Differences inherent in these species, the crosses used, the traits tested, population size, and the measurement precision, could account for variability in the proportion of significant comparisons observed.

With certain traits, unequal variances were evident among genotypic classes at some marker loci. This may be due in part to the non-normal distribution of the population for these traits. For two traits in which unequal variance was the most severe, log and square-root transformations reduced the nonconstant variance, but in both cases the transformations did not change the significance or magnitude of the trait loci detected. Thus, only the analysis of untransformed data are reported here.

The 192 significant marker locus-trait comparisons represent the total number irrespective of marker linkage. Marker loci significantly associated with a given trait were commonly found in clusters around a presumed trait locus. Generally, peaks in the magnitude of effects, reflected by R^2 values, were found in the center of a cluster and decreased on either side. Flanking marker loci may reflect effects of linkage to one trait locus or indicate multiple-linked trait loci conferring similar effects. In the multilocus models, generally all but one marker locus on a linkage group was eliminated from the model. Often marker loci on independent linkage groups were also excluded. One-to-four marker locus comparisons were maintained in a multilocus model for each of the traits. R^2 values for multilocus models ranged from 0.129 for leaf number to 0.601 for days-to-bud.

Flowering traits

The parents used in this study had diverse characteristics associated with floral development. The F_1 was intermediate to the parents and behaved as a late annual, forming buds at 155 days. The F_2 population segregated widely for flowering characteristics, several of which were evaluated in this study (Table 1). Bud development of the F_2 population spanned more than 300 days and included plants that did not flower by late summer or in the greenhouse during winter (Fig. 1).

Nine of eighty-three plants scored for this trait did not develop buds by 300 days from sowing. These plants required vernalization to flower and were designated as biennials. Plants were assigned indicator variables depending on whether they behaved as annuals (flowering by 300 days) or biennials (flowering after 300 days with vernalization). Significant comparisons ($P > 0.05$) were found for nine marker loci on linkage groups 2C, 5C, and 7C (Fig. 2). Peak R^2 values among linkage groups ranged from 0.119 to 0.236. The greatest effects were found on linkage groups 5C and 7C where significant associations were detected among contiguous markers spanning 27 and 35 map units, respectively. Gene action was determined to be partially dominant for marker loci on linkage groups 5C and 7C. Genotypic class rankings for the marker locus associations was always C/C > B/C > B/B. The multilocus model included marker loci *4a* on 5C and *43e* on 7C and had an R^2 of 0.368 for the trait. Analysis of the biennial plant genotypes at these two marker loci

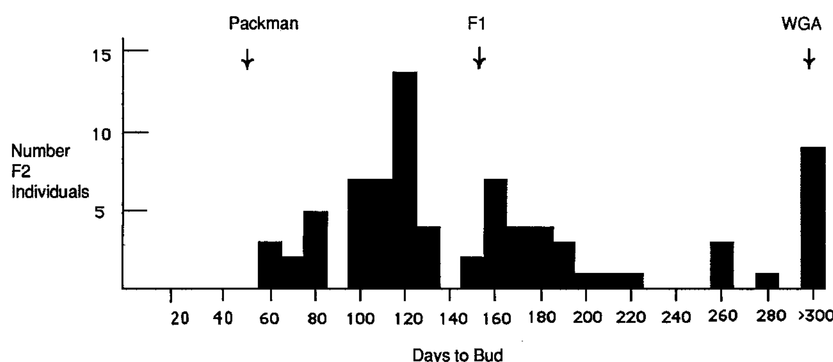

Fig. 1. Frequency distribution of the Wisconsin Golden Acre (WGA) × Packman F_2 population for days-to-bud. Nine individuals did not develop buds before 300 days without vernalization treatment. Days-to-bud for the parents and F_1 are shown with *arrows*

indicated that at least one of the loci was homozygous for cabbage alleles: four biennial plants were C/C at both marker loci *4a* and *43e*, four were C/C at one locus and B/C at the other, and one plant was C/C at marker locus *43e* and B/B at *4a*. No individuals were recovered that were annuals and were homozygous for cabbage alleles at both loci. A test of epistatic interaction between these two loci was highly significant ($P < 0.001$). Incorporation of the interaction term in the multilocus model increased the R^2 to 0.511. The effect was partitioned by gene action contrasts; additive-by-additive and dominant (marker locus *4a*)-by-additive (marker locus *43e*) interactions were found to be the most significant ($P < 0.001$).

Chi-square tests of marker associations with the categorical phenotype annual vs biennial were performed by using cross-tabulation frequency tables. This provided a confirmation of the GLM-derived associations, as ANOVA with categorical responses is not robust. The P values of associations derived with chi-square tests and ANOVA closely approximated each other in all cases tested. Thus, only results from ANOVA are reported.

To test for population homogeneity of the biennial and annual subsets of the F_2 population, genotypic class means for a set of traits (lamina length, lamina width, and distance of 20 internodes) were compared between biennial and annual classes. In general, the biennials and the annuals had similar means for these traits. In nearly all cases, results from analysis of annuals alone and results from analysis of annuals and biennials together provided similar magnitudes of associations with marker loci. Thus, both annuals and biennials were analyzed as one population for marker associations for all traits except days-to-bud, days-to-flower, days from bud to flower, and size of cluster.

F_2 plants within the annual groups were analyzed for days-to-bud and significant associations ($P < 0.05$) of 14 marker loci on five linkage groups were detected (Fig. 2). Maximum R^2 values ranged from 0.102 to 0.390 for marker loci among these linkage groups. The greatest effects were on linkage groups 2C and 7C where significant associations were detected for contiguous markers spanning over 40 map units on each linkage group. Gene action was estimated to be additive on linkage groups 1C, 2C, and 7C but dominant on

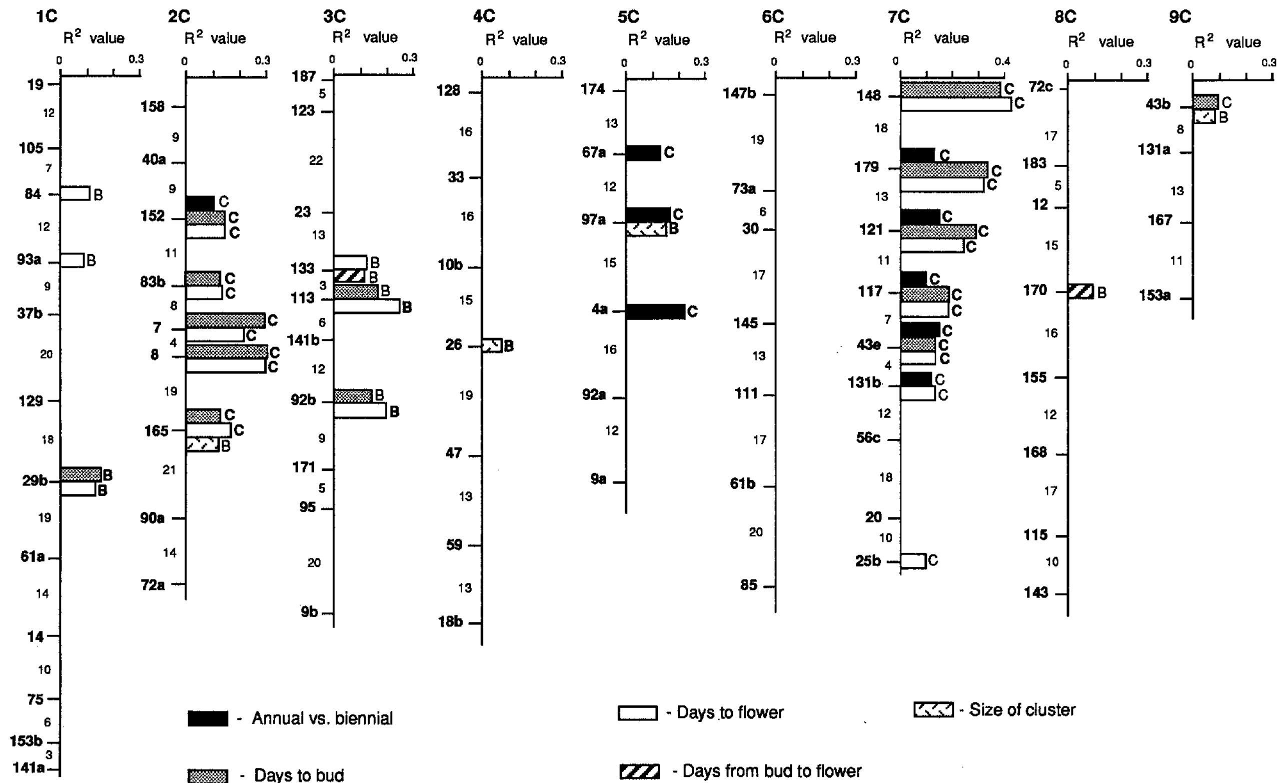

Fig. 2. Marker loci having significant associations with flowering-related traits. Marker locus positions and estimated map distances (cM) are indicated on the left-hand side of linkage groups 1C to 9C. Marker loci that were significantly associated ($P < 0.05$) with the traits days-to-bud, days-to-flower, days-from bud to flower, annual vs biennial, and size of cluster are identified by *bars*. Length of bars indicate R^2 values for the associations. Parental alleles, broccoli (*B*) or cabbage (*C*), contributing to the largest genotypic class mean are listed to the right of the bars. *Boldface type* of parental allele abbreviation indicates associations that are significant at $P < 0.01$

linkage groups 3C and 9C. The multilocus model included marker loci *29b* (1C), *8* (2C), *148* (7C), and *43b* (9C) and had an R^2 value of 0.601. Marker locus *113* on linkage group 3C was not significant in the multiple regression model and this locus may be associated with the trait as a result of a chance correlation of allelic segregation with other significant marker loci. The ranking of genotypic class means were C/C > B/C > B/B for significant marker loci on linkage groups 2C and 7C, as would be expected if cabbage provided alleles for delayed flowering. However, for significant marker loci on linkage groups, 1C and 3C, genotypic class means were ranked in the opposite order. Thus, marker analyses allowed us to uncover alleles for rapid flowering from the cabbage parent which normally may be masked by alleles for the biennial habit.

Most marker loci associated with days-to-bud were also associated with days-to-flower in the annual group of F_2 plants (Fig. 2). This result is not unexpected since many genes that affect time-to-bud formation also would affect time-to-flowering. The trait days from bud to flower was a measure of the interim between bud formation and floral opening. Two marker loci (*133* on linkage group 3C and *170* on linkage group 8C) were significantly ($P < 0.05$) associated with this trait. For both loci, the broccoli parent contributed alleles associated with a greater number of days from bud to flower. This is consistent with parental phenotypes in that broccoli bud clusters remain closed for a relatively long period of time after formation, while cabbage buds generally open more quickly. The significant marker locus associations indicate that both parents contribute alleles that delay flower development: primarily cabbage during early floral development and broccoli during late floral development.

The trait size of cluster was an estimate of the number of individual buds in the main floral stem of annual-type plants at the time of bud formation. Significant ($P < 0.05$) associations were observed for marker loci on linkage groups 2C, 4C, 5C, and 9C (Fig. 2). The expected ranking of genotypic class means was observed, as the B/B class had the largest clusters and the C/C and B/C classes had the smallest. Three of the four marker loci associated with size of cluster were also associated with days-to-bud or annual vs biennial, suggesting multiple related effects of single genes or close linkage among different genes controlling flowering traits in these regions.

Leaf traits

The F_2 population segregated visibly for leaf size and shape, including both lamina and petiole dimensions, and transgressive segregants were observed for all leaf traits (Table 2). Leaves were measured during active vegetative growth at 52 and 59 days and an average value was calculated. During the seven-day interval between the two sets of measurements, lamina growth averaged 2.9 cm for length and 1.2 cm for width. Petiole width and thickness were measured only once, and because of the small dimensions these measurements were less precise.

Highly-significant comparisons ($0.0001 < P < 0.0002$) were found for lamina length, lamina width, and petiole length, R^2 values for significant comparisons ($P < 0.05$) ranged for 0.067 to 0.179. Significant associations of marker loci were detected on linkage groups 4C, 5C, and 6C for lamina length, on linkage groups 1C, 4C, 5C, and 7C for lamina width, and on linkage groups 1C, 4C, 5C, and 9C for petiole length (Table 3). Multilocus models for these traits included a minimum of three marker loci, each positioned on a different linkage group, and R^2 values for these models were 0.256 for lamina length, 0.318 for lamina width, and 0.320 for petiole length. The small number of genomic regions with highly-significant associations suggests that a small number of genes with large effects controlled leaf dimensions.

Trait loci controlling different leaf traits were often localized to the same genomic regions (Table 3). Many of these trait loci with common positions also had the same genotypic class ranking and gene action. For example, lamina length, petiole length, and basal petiole thickness were associated with common marker loci on linkage group 4C which revealed the same type of gene action and genotypic class ranking for each trait. Common marker loci with the same gene action and genotypic class rankings also were observed for lamina length and basal petiole thickness on linkage group 6C and for lamina width and basal petiole width on linkage group 7C. Marker loci associated with basal petiole thickness were generally associated with lamina petiole thickness, and basal petiole width shared common marker locus associations with lamina petiole width (data not shown). Although significant associations were found on linkage groups 4C and 5C for both lamina length and lamina width, different markers within 4C and 5C were associated with the two traits and markers on other linkage groups were associated with only one trait. Thus, some regions of the genome appeared to control one leaf trait specifically whereas others had effects on several leaf traits.

Ratios and sums were taken for some of the leaf measurements to make the compiled traits leaf length, lamina length/width, and lamina/petiole length (Tables 1 and 3). Marker loci that were significantly associated with component traits were often, but not always, associated with a compiled trait. The magnitude of effects for significantly-associated markers were generally lower for complied traits than component traits, although some associations were larger (Table 3, petiole and lamina length vs leaf length on linkage

Table 3. Largest genotypic class mean, significance level, R^2 value, and gene action for 10 selected marker loci from five linkage groups that were significantly associated with leaf dimension traits

Traits[a]	Genetic information or statistic reported	Linkage group 1C	Linkage group 4C			Linkage group 5C			Linkage group 6C		Linkage group 7C
		61a	128	10b	26	174	67a	92a	30	111	25b
Lamina length	> Class and sig. level[b]			HB*	BH*		CB*		BH**	BH*	
	R^2 value	–[d]		0.120	0.102	–	0.080	–	0.179	0.195	–
	Gene action[c]			PDom	PDom		Dom		PDom	Add	
Lamina width	> Class and sig. level	CH**	HC*			CB*					BC**
	R^2 value	0.103	0.113	–	–	0.111	–	–	–	–	0.121
	Gene action	Add	ODom			ODom					ODom
Petiole length	> Class and sig. Level			HB**	BH**			BH**			
	R^2 value	–	–	0.156	0.161	–	–	0.121	–	–	–
	Gene action			Dom	PDom			Add			
Basal petiole width	> Class and sig. level										BC**
	R^2 value	–	–	–	–	–	–	–	–	–	0.135
	Gene action										ODom
Basal petiole thickness	> Class and sig. level			BH**	BH**					B**	
	R^2 value	–	–	0.116	0.108	–	–	–	–	0.169	–
	Gene action			PDom	PDom					Add	
Leaf length	> Class and sig. level			HB**	BH**		CB*	BCH*	BH**	BH**	
	R^2 value	–	–	0.194	0.181	–	0.067	0.074	0.139	0.112	–
	Gene action			Dom	PDom		PDom	ODom	PDom	PDom	
Lamina length/width	> Class and sig. level	B*	B*	BH***	HB**				BH*		
	R^2 value	0.095	0.142	0.154	0.115	–	–	–	0.071	–	–
	Gene action	Add	Add	PDom	Dom				PDom		
Lamina/petiole length	> Class and sig. level							BH*			
	R^2 value	–	–	–	–	–	–	0.081	–	–	–
	Gene action							Add			

[a] See Table 1 for description of traits
[b] C, H, and B denote cabbage homozygous, heterozygous, or broccoli homozygous genotypic class as having the largest mean. More than one assignment was used when classes could not be differentiated by Scheffe's test at $P < 0.05$. *,**, and *** denote levels of significance $P < 0.05$, 0.01, and 0.001, respectively, from single factor ANOVA
[c] Add, PDom, Dom, and ODom denote additive, partial dominant, dominant, and over (or under) dominant gene action, respectively
[d] –, denotes non-significant marker locus-trait association ($P > 0.05$)

group 4C). A fraction (6 of 27) of the significant associations for complied traits were found at marker loci with no detectable component trait association and these associations were small ($0.05 > P > 0.01$). In general, it appears there is little genetic variation for complied traits over and above that which was directly associated with component traits for leaf dimensions. That is, leaf shape appears to be determined by genetic effects on lateral or axial growth as opposed to an overall shape. Fundamental differences in genetic control of length and width is supported by anatomical observations of the two primordial leaf meristems (apical and marginal) that divide differentially to provide either length or width (Esau 1976).

The significant correlations observed between some pairs of traits are probably due to the same gene(s) controlling the traits. Evidence for this was obtained for the correlated traits petiole length and lamina petiole thickness ($R = 0.34$, $P = 0.0015$) where one half (7 of 14) of the marker loci with significant associations to petiole length had significant associations to lamina petiole thickness. These marker loci also showed similar gene action and genotypic class rankings. However, for the highly-correlated traits lamina length and lamina width ($R = 0.60$, $P < 0.0001$), only two out of the thirteen marker loci significantly associated with lamina length were also associated with lamina width. This result seems to suggest that these highly-correlated traits are controlled by different genes; however, they may be controlled by a common set of genes having only small effects on one or both traits which were not detected in this study.

Stem traits

Internode distance was measured by different methods (a single internode or 20 internodes) at different times (52 or 97 days, respectively). Of these, the best measure of overall plant compactness was the trait distance of 20 internodes. Significant comparisons ($P < 0.05$) for this trait were found in five genomic regions on linkage groups 1C, 2C, 3C, 4C and 9C (Fig. 3). Additive gene

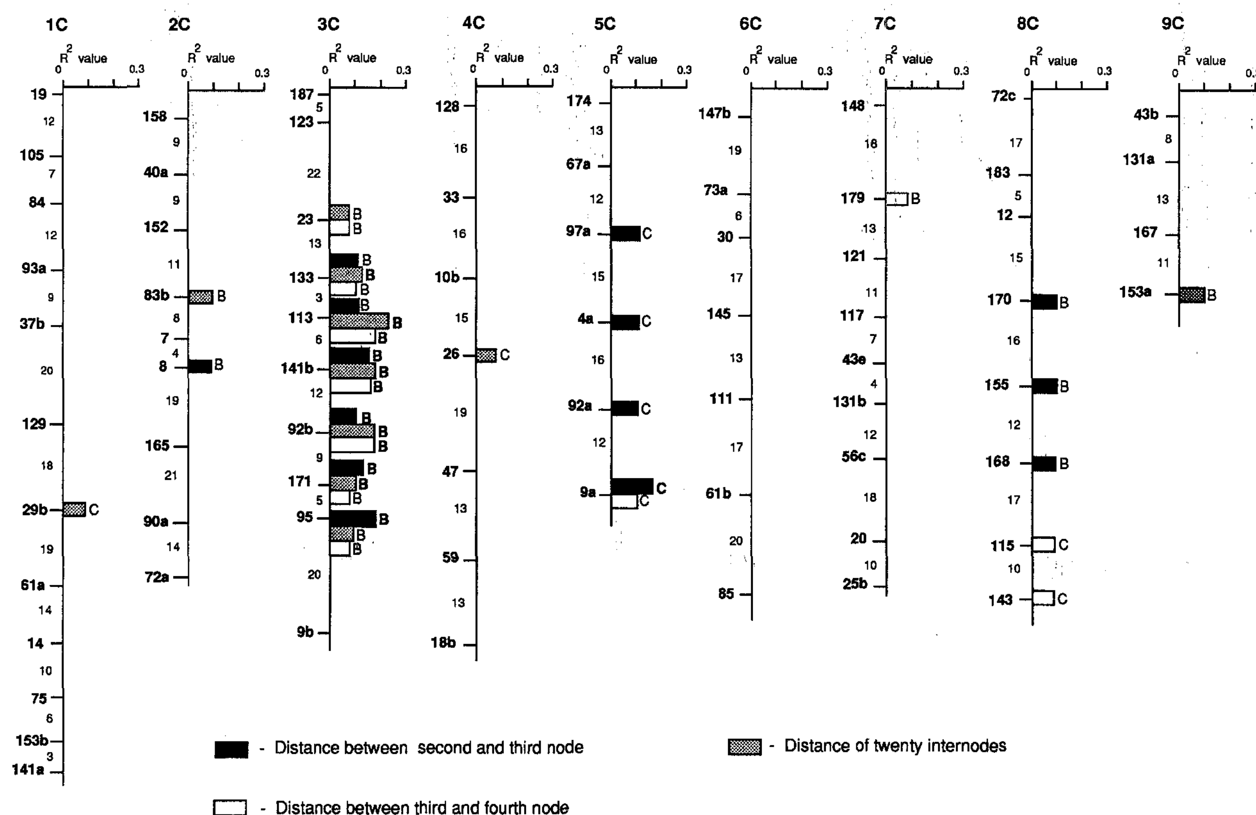

Fig. 3. Marker loci having significant associations to stem-related traits. Marker locus positions and estimated map distances (cM) are indicated on the left-hand side of linkage groups 1C to 9C. Marker loci that are significantly associated ($P < 0.05$) with the traits distance of 20 internodes, distance between second and third node, and distance between third and fourth node are indicated by *bars*. Length of bars indicate R^2 values for the associations. Parental alleles, broccoli (*B*) or cabbage (*C*), contributing to the largest genotypic class mean are listed to the right of the bars. *Boldface type* of parental allele abbreviation indicates associations that are significant at $P < 0.01$

action was observed for marker associations on linkage groups 1C, 3C, and 4C and overdominance was observed for those on 2C and 9C. The multilocus model included marker loci *29b* (1C), *113* (3C), and *153a* (9C) and had a cumulative $R^2 = 0.314$; however, most of the variation was explained by loci at the center of linkage group 3C (locus *113*, $R^2 = 0.213$).

The marker loci on 3C that were strongly associated with the distance of 20 internodes were also strongly associated with the single internode distances (Fig. 3). Maximum effects for distance between the third and fourth node were found at the same marker locus as for distance of 20 internodes (locus *113*). However, the greatest effect for the distance between the second and third internodes was found at marker locus *95*, 22 map units away from marker locus *113*. All associations of loci on linkage group 3C had additive gene action, with broccoli having the largest genotypic class mean. On other linkage groups, marker loci were associated exclusively with measurements of single internode distances. Highly-significant effects ($P < 0.001$) were found for the distance between second and third node on linkage group 5C where no marker locus associations were found for the distance of 20 internodes. Most interesting for this cluster of associations was the ranking of the genotypic class means, with cabbage contributing alleles to the larger mean value. Marker locus *9a* was also significantly associated with the distance between third and fourth nodes, and cabbage contributed alleles to the larger genotypic class mean. These associations are not unexpected since the first few internodes of a cabbage plant are not as compact as subsequent ones. The different marker loci associated with the distances at different internodes may reflect a developmental shift in the genetic control of internode distance.

Relationship to other inheritance studies

Using crosses among cultivar groups of *B. oleracea* (Detjen 1926; Dickson 1968) and using biennial mutants (Walkof 1963), previous genetic studies have provided evidence for single gene control of annual vs biennial habit. More recently, polygenic inheritance was observed for annual vs biennial habit in a cross between broccoli and cabbage (Pelofske and Baggett 1979). F_2 segregants and F_1 backcross progenies from this cross had dominant gene action for the annual habit. Multigenic additive inheritance was reported for the timing of bud development within the annual class (Pelofske and Baggett 1979). Our data are generally consistent with the same gene numbers and type of gene action as those estimated for the cabbage × broccoli population used by Pelofske and Baggett (1979).

Inheritance studies in F_2 progenies from crosses of *B. oleracea* indicated one major gene (*T*) controlling plant height (Pease 1926). However, multigenic additive inheritance for internode distance (measured from the average of 5–15 internodes of a center stem section) was found using backcross and F_2 segregation data from cabbage × broccoli (Pelofske and Baggett 1979). Marker analyses in our study indicated one linkage group (3C) contained a major gene for internode distances that is closely linked to marker locus *113*. While our population did not exhibit a discrete classification for this trait, this gene may correspond to the major gene *T* dentified by Pease (1926).

Crosses within *B. oleracea* have allowed some linkage associations to be established among genes controlling different morphological traits (Pease 1926; Yarnell 1956; Pelofske and Baggett 1979). Genes controlling heading habit, petiole length, and lamina width, were proposed as a linkage group in crosses of cabbage by kohlrabi (Pease 1926). Another linkage group for head-forming leaf overlap, plant height, and lamina curl has been reported for crosses of cabbage by curly kale (Pease 1926). Heading habit and biennial habit has been reported to be linked based on a cross of broccoli by cabbage (Pelofske and Baggett 1979). In our study, highly-significant ($P < 0.01$) marker locus-trait associations to head-forming leaf overlap, petiole length, lamina width, and annual vs biennial habit were found on linkage group 5C. The strongest marker locus-trait associations for lamina curl and the distance of 20 internodes were found on linkage group 3C; however, no association with heading habit was found for marker loci on this linkage group (data not shown). The similar linkages of genes controlling some traits reported in previous studies and in our study may reflect common genetic control of these morphological traits in different crosses of *B. oleracea* accessions.

Genome organization of trait loci

Many of the DNA clones used to construct the *B. oleracea* linkage map hybridized to multiple segregating loci, indicating extensive DNA sequence duplication in the genome (Slocum et al. 1990). Trait loci in the regions of these duplicated RFLP loci might also represent duplicated gene sequences. Detection of individual traits associated with both marker loci of a duplicated pair would provide evidence for the evolution of some polygene systems by genome duplication.

We investigated this possibility by comparing the number of traits observed to be associated significantly ($P < 0.05$) with both marker loci of a duplicated pair to the number of traits expected to be associated with both duplicated marker loci by chance. The expected number was calculated by multiplying the number of

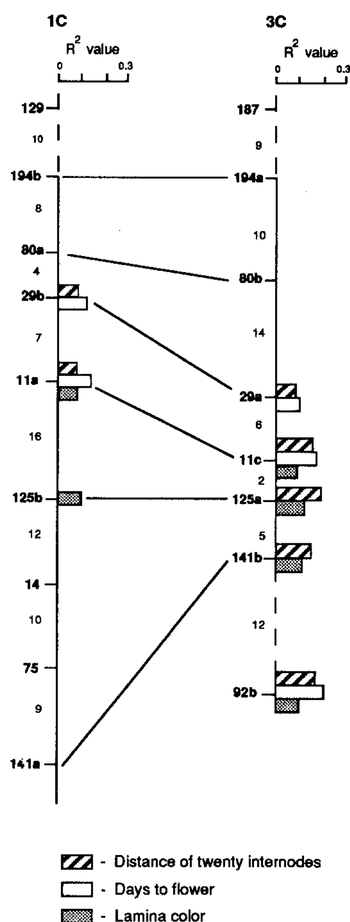

Fig. 4. Trait loci associated with duplicated marker loci on linkage groups 1C and 3C. Duplicated marker loci detected by the same set of probes on 1C and 3C are connected by *solid lines*. The traits distance of 20 internodes, days-to-flower and lamina color, which were associated with duplicated marker loci, are indicated by *bars* (on the right-hand side of the linkage group) next to all significantly associated marker loci (on the left-hand side of the linkage group). R^2 values of associations are indicated by the length of bars. Estimated map unit distances are listed between marker loci

traits associated with a marker locus to the number associated with its duplicated marker locus and dividing the product by 22, the total number of traits scored. These expected numbers of trait associations were summed for all 79 duplicated loci pairs observed (Slocum et al. 1990) and compared to the observed number of traits associated with both loci of the 79 marker locus pairs. The observed number of traits associated with both duplicated marker loci (32) was less than the number expected to be associated by chance (33.7). These results provide no evidence that pairs of loci controlling individual traits were preferentially associated with pairs of duplicated DNA sequences detected over the entire *B. oleracea* genome with our probes.

Although some of the duplicated RFLP loci previously mapped appeared to be distributed randomly throughout the genome, a large proportion were present as conserved duplicated linkage blocks (Slocum et al. 1990). These conserved duplicated regions might be expected to contain a higher frequency of duplicated trait loci than regions where duplicated linkage blocks were not detected. However, when only these conserved duplicated regions were considered, we observed no greater frequency of traits associated with both duplicated loci in the region than would be expected by chance. Thus, our data does not provide evidence that gene duplication is a major source of polygenes controlling morphological variation in this population as tested. However, one conserved duplicated region present on linkage groups 1C and 3C had pairs of duplicated marker loci associated with flowering, stem, and leaf traits (Fig. 4). These regions may represent a highly-conserved genome duplication containing homologous trait loci that were expressed in our population. Analysis of this population under different conditions, or of other segregating populations, using duplicated marker loci may reveal other regions where trait locus duplication possibly has occurred.

Conclusions

In this study, we report on the location, effects, and gene action of loci controlling morphological variation in a segregating population from a cross between cultivar groups of *B. oleracea.* Trait loci were detected by using the three genotypic classes as a variable in a single-factor ANOVA for each marker locus. We report all significant associations between marker loci and traits at $P < 0.05$, but include the significance levels so the reader can judge the importance of each association. By using RFLP marker loci distributed throughout the *B. oleracea* genome, we have probably detected most of the major genes and many of the minor genes controlling morphological variation in this population under these environmental conditions. Many of the trait loci we detected had large effects, and these may represent genes that were scored as qualitative factors in previous studies. Indeed, several of the linkages between trait loci we detected were also reported in previous studies, some of which involved crosses between different cultivar groups. It is possible that a common set of genetic loci control a major portion of the morphological variation among the various forms of *B. oleracea.* This could be tested by analyzing segregating populations from several inter-group crosses using a common set of molecular markers. Comparison of the map positions and effects of trait loci in various crosses would provide evidence for or against

common mechanisms for the genetic control of morphological variation in *B. oleracea*.

Acknowledgements. The authors are grateful to Paul Williams for assistance with growing plants, for helpful suggestions about trait measurements, and for reviewing the manuscript. They also thank Brian Yandell for reviewing the manuscript. This work was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin.

References

Baggett JR, Walhert WK (1975) Annual flowering and growth habit in cabbage-broccoli crosses Hort Science 10:170–172

Detjen LR (1926) A preliminary report on cabbage breeding. Proc Am Soc Hort Sci 23:325–332

Dickson MH (1968) Eight newly described genes in broccoli. Proc Am Soc Hort Sci 93:356

Doebly J, Stec A (1991) Genetic analysis of the morphological differences between maize and teosinte. Genetics 129: 285–295

Edwards MD, Stuber CW, Wendel JF (1987) Molecular-marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116:113–125

Edwards MD, Heletjaris T, Wright S, Stuber CW (1992) Molecular-marker facilitated investigations of quantitative trait loci in maize. IV. Analyses based on genome saturation with isozyme and restriction fragment length polymorphism loci. Theor Appl Genet 83:765–774

Esau K (1976) Anatomy of plants. John Wiley and Sons, New York

Figdore SS, Kennard WC, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica*. Theor Appl Genet 75:833–840

IBPGR (1987) Descriptors for *Brassica* and *Raphanus*. Int Board for Plant Genetic Resources, Rome

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: associations between marker loci and variation in quantitative traits. Genetics 126:735–742

Kristofferson KB (1924) Contributions to the genetics of *Brassica oleracea*. Hereditas 5:297–364

Kosombi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Nienhuis J, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci 27:797–803

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73:350–356

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Medelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Pease MA (1926) Genetic situation in *Brassica oleracea*. J. Genetics 16:363–385

Pelofske PJ, Baggett JR (1979) Inheritance of internode length, plant form, and annual habit in a cross of cabbage and broccoli (*Brassica oleracea* var. *capitata* and var. *italica* plenck). Euphytica 28:189–197

SAS Institute Inc. (1982) User's guide: basic and statistics. SAS Institute, Cary, North Carolina

Slocum MK, Figdore SS, Kennard WC, Suzuki J, Osborn TC (1990) Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor Appl Genet 80:57–64

Snedecor GW, Cochran WG (1980) Statistical methods. Iowa State University Press, Ames, Iowa

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLPs). II. Preliminary study on subspecies of *B. rapa* (syn. *campestris*) and *B. oleracea*. Theor Appl Genet 76:593–600

Stuber CW, Edwards MD, Wendel JF (1987) Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its components traits. Crop Sci 27:639–648

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Tanksley SD, Hewitt J (1988) Use of molecular markers in breeding for soluble solids content in tomato – a re-examination. Theor Appl Genet 75:811–823

Tanksley SD, Medina-Filho H, Rick CH (1982) Use of naturally-occurring variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49:11–25

Walkof C (1963) A mutant annual cabbage. Euphytica 12:77–80

Weller JI (1987) Mapping and analysis of qunatitative trait loci in *Lycopersicon* (tomato) with the aid of genetic markers using approximate maximum-likelihood methods. Heredity 59:413–421

Yarnell SH (1956) Cytogenetics of vegetable crops. II. Crucifers. Bot Rev 22:81–166

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. Genetics 120:579–585