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Molecular Markers Associated with Maize Kernel Oil Concentration in an Illinois High Protein × Illinois Low Protein Cross

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ABSTRACT

The Illinois Long Term Selection Strains offer a unique opportunity to investigate the quantitative genetic basis of kernel chemical traits. This study was conducted to determine the number and magnitude of quantitative trait loci (QTL) influencing kernel oil concentration and kernel weight in a maize (*Zea mays* L.) population derived from a cross of Illinois High Protein (IHP) × Illinois Low Protein (ILP). The parental strains had been divergently selected for protein concentration for 76 cycles, yet varied in oil concentration from 29 g kg^{-1} (ILP) to 54 g kg^{-1} (IHP) and in 300 kernel weight from 76.5 g (ILP) to 41.1 g (IHP). One hundred polymorphic RFLP loci spaced throughout the maize genome were scored in a segregating population of 100 S_1 families. Kernel oil concentration and kernel weight were obtained from replicated field trials grown during 1990 and 1991. Significant ($P < 0.05$ level) QTL associations of 25 marker loci on 13 chromosome arms with oil concentration and 18 marker loci on 10 chromosome arms with kernel weight were identified. These results demonstrate the effectiveness of identification of QTL for oil concentration in strains divergently selected for protein concentration for 76 cycles. Clusters of two or more marker loci in the same chromosomal region were significantly associated with oil concentration at four different chromosomal locations and with kernel weight at four chromosomal locations. The detection of relatively few clusters of marker loci associated with genomic regions controlling oil concentration suggests the development of high oil maize germplasm via RFLP marker-assisted selection may be feasible.

More than 50% of the maize grain crop produced in the USA is used for feeding animals (Perry, 1988). Maize grain with elevated oil concentration has a higher caloric content compared with standard maize grain and is advantageous as an animal feed. Feeding high oil maize grain instead of maize grain with standard levels of oil concentration to swine and poultry has resulted in accelerated weight gain (Han et al., 1987; Goss and Kerr, 1992). Thus, the development of high oil germplasm is an objective in some maize breeding programs. Substantial variation for kernel oil concentration has been detected among maize lines, and this variation has been shown to be quantitatively inherited (Dudley et al., 1974).

The recent development of molecular marker technology has enabled the association of DNA markers with important agronomic traits such as yield, plant height, and disease resistance (Beavis et al., 1991; Edwards et al., 1987; Paterson et al., 1988; Keim et al., 1990; Paran et al., 1991; Young et al., 1988; Edwards et al., 1992). Although there is limited published information on the association of molecular marker loci with genes controlling oil concentration in maize, both RFLP markers and allozyme frequency changes associated with long-term selection for oil concentration have been investigated. Brown (1971) suggested the allelic variation at six isozyme loci among the strains Illinois High Oil and Illinois Low Oil after 68 cycles of selection in the Illinois Long Term Selection Experiment could be largely accounted for by random genetic drift. Significant linear allozyme frequency changes at eight loci were associated with 25 cycles of selection for increased oil concentration in the Alexho maize synthetic (Kahler, 1985), suggesting the possible presence of genes controlling oil concentration at or near these allozyme loci. Tests for the presence of random genetic drift were significant at most of these loci, limiting conclusions regarding the effect of selection in changing allozyme frequency. Sughroue and Rocheford (1994) identified RFLP marker loci whose changes in frequency among oil strains in the Illinois Long Term Selection Experiment were consistent with selection response.

Phenotypic extremes for kernel oil and protein concentration exist among maize strains from the Illinois Long Term Selection Experiment. This experiment was initiated in 1896 to investigate changes in maize kernel chemical composition resulting from selection for protein and oil concentration (Hopkins, 1899). The Illinois Long Term Selection strains are ideally suited for studies designed to analyze quantitative variation in kernel composition at the molecular level because of the phenotypic (Dudley and Lambert, 1992) and restriction fragment length variation (Sughroue and Rocheford, 1994) among them.

Selection for high protein concentration has increased kernel oil concentration, whereas selection for low protein concentration has decreased oil concentration in the Illinois Long Term Selection protein strains (Dudley and Lambert, 1992). This correlated response resulted in an approximate two-fold difference in oil concentration between Illinois High Protein (IHP) and Illinois Low Protein (ILP) at Cycle 76. Kernel weight of ILP was nearly twice as great as kernel weight of IHP at Cycle 77 (Dudley and Lambert, 1992). Associations of RFLP marker loci with quantitative trait loci (QTL) controlling protein and starch concentration in segregating cycles from the cross of Cycle 76 of IHP × ILP have been reported (Goldman et al., 1993). As part of that study, data were obtained on oil concentration in the grain and kernel weight. The objectives of this paper are to report the RFLP-QTL associations identified for oil and kernel weight from this cross and to relate these findings to those for protein and starch.

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Abbreviations: cM, centimorgan; RFLP, restriction fragment length polymorphism; QTL, quantitative trait locus or loci; IHP, Illinois High Protein; ILP, Illinois Low Protein.

MATERIALS AND METHODS

The Illinois Long Term Selection experiment was initiated in 1896 with the open pollinated variety Burr's White. Details of specific selection procedures have been reported (Dudley and Lambert, 1992). IHP and ILP plants from Cycle 76 were used as parents of the segregating population used in this investigation. Mean protein concentrations in the IHP and ILP strains from Cycle 76 were 250 g kg^{-1} and 40 g kg^{-1}, respectively. Mean oil concentrations in the IHP and ILP strains from Cycle 76 were 54 g kg^{-1} and 29 g kg^{-1}, respectively. Corn belt germplasm typically has a kernel oil concentration of approximately 45 g kg^{-1}. Mean 300 kernel weight for Cycle 77 was 39.0 g for IHP and 76.5 g for ILP (J.W. Dudley, 1992, unpublished data).

F_1 plants were created from crosses of five to seven IHP plants × five to seven ILP plants, with each plant used only once as a pollinator. F_1 plants were selfed to obtain an F_2 population. The F_2 seeds were bulked and F_2 plants were self-pollinated to produce S_1 families. One-hundred S_1 families formed the segregating populations used in QTL analyses. Each S_1 family was represented by two replications of single row plots 5 m long by 0.76 m wide. The experiment was planted in a replicates-in-blocks design with four blocks of 25 families each. The experiment was grown in 1990 and 1991 at the Agronomy South Farm, Urbana, IL. Five to seven plants per plot were self-pollinated and equal numbers of seeds from each selfed ear within a plot were bulked for chemical analysis. Protein, starch, and oil concentrations in the kernel were measured on a sample of the bulked seed from each family using a Dickey-john GAC III near-infrared analyzer (Hymowitz et al., 1974) as outlined by Dudley and Lambert (1992). Results for protein and starch were reported (Goldman et al., 1993). Weight of 300 kernels (in contrast to typical measures of 100 kernels) was measured for each S_1 family in 1990 and 1991 in an effort to obtain a more precise estimate of kernel weight.

DNA was isolated from 600 mg of finely-ground freeze-dried leaf tissue by a modified CTAB procedure (Saghai-Maroof et al., 1984; Hoisington, 1991). The tissue sample was composed of a bulk sample of 25 randomly selected plants from each family. Ten micrograms of DNA were digested with 30 units of restriction endonuclease *Eco*RI and loaded into 8 g L^{-1} agarose gels. Southern blotting was conducted according to Hoisington (1991). DNA was transferred to 0.45 μm nylon filters and bound to membranes by ultra-violet linking and baking at 90 °C for 2 h.

Genomic clones were selected from collections of mapped maize clones developed and provided by University of Missouri (Columbia, MO, hereafter UMC). Brookhaven National Laboratory (Long Island, NY, hereafter BNL, obtained from University of Missouri), and Pioneer Hi-Bred International (Johnston, IA, hereafter P). Clones from the Pioneer Hi-Bred source also included a set of genomic clones from Native Plants, Inc., Salt Lake City, hereafter NPI. cDNA clones of four structural genes, *Shrunken-1*, (*Sh1*, Sheldon et al., 1983), *Shrunken-2*, (*Sh2*, Bhave et al., 1990), *Brittle-2*, (*Bt2*, Bae et al., 1990), and *opaque-2*, (*o2*, Schmidt et al., 1990) were also used. Genomic and cDNA clones were oligolabeled (Feinburg and Vogelstein, 1983) and hybridized to membranes according to Hoisington (1991).

More than 175 maize genomic and cDNA clones were tested for detection of RFLP between IHP and ILP on survey blots composed of DNA from bulked samples of randomly-chosen plants from IHP and ILP from Cycles 65 and 90. Approximately 60% of these clones revealed RFLP between IHP and ILP. A set of 92 clones comprised of 88 genomic clones and 4 cDNA clones spaced approximately every 20 cM throughout the maize genome (based on map distances given by Coe, 1992) were chosen. IHP and ILP from cycle 76 were not RFLP genotyped in this investigation. Ten clones (UMC 17, UMC 16, UMC 72, NPI 593, UMC 104, BNL 5.47, BNL 8.39, NPI 455, NPI 253, NPI 266) revealed two polymorphic loci, thus 100 polymorphic loci were scored in this investigation. Two of these clones (UMC 72 and UMC 104) have revealed two RFLP loci in another maize cross and each locus has been placed on an RFLP map (Coe, 1992). Chromosomal locations of the remaining eight additional loci are unknown. Linkage relationships among RFLP loci in this segregating population could not be calculated because of the unknown RFLP allelic constitution of the specific IHP and ILP parental plants. Therefore, all chromosomal locations of RFLP loci used in this study were taken from Coe (1992) and unpublished information from Native Plants, Inc. (provided by Pioneer Hi-Bred International). Significant correlations between alleles at known linked RFLP loci, such as those on the long arm of Chromosome 3, were detected, likely indicating linkage between those marker loci. Genetic mapping studies have revealed a general pattern of stability for gene order of RFLP marker loci in different maize crosses (B. Burr, 1992, personal communication), however map distance of marker loci have been shown to vary. It is therefore possible some RFLP marker loci may reside in different chromosomal locations in these genetic stocks than described in other mapping efforts. However, genetic mapping investigations in the Illinois Long Term Selection Oil Strains (T. Berke and T. Rocheford, 1993, unpublished data) have revealed location of RFLP marker loci in these strains is consistent with locations reported by Coé (1992). Restriction fragment length polymorphism alleles at a locus were assigned letter designations *A*: highest molecular weight fragment and *B*: next highest molecular weight fragment. Additional RFLP alleles at a locus (when present) were designated *C*, *D*, etc., by descending molecular weight. Genotypes were assigned accordingly.

Individual marker loci were tested for linkage to QTL by analysis of variance of mean performance of marker classes (Edwards et al., 1987). Single factor analysis of variance was performed on all loci for oil concentration and kernel weight separately using the GLM procedure of Statistical Analysis Systems (SAS Inst., 1988). In certain cases, marker classes had widely differing numbers of observations, especially when multiple alleles were present. The GLM procedure is deemed more appropriate than ANOVA for hypothesis testing under these circumstances. In addition, data were visually inspected and genotypes of families comprising rare marker classes (i.e., fewer than six) were not included in the analysis due to the lack of balance for marker class comparisons. Significance of the among marker genotypes mean square was tested using the among-families-within-marker-genotypes mean square as the denominator in an *F*-test. Associations of marker loci with QTL were considered to be significant when the *F*-test exceeded a value necessary for a probability value less than 0.05. Significant differences in marker class means were interpreted as linkage of marker loci to QTL influencing oil concentration or kernel weight. No attempt was made to identify markers associated with the QTL peak, rather associations were reported based on their probability value resulting from *F*-tests. Linear and quadratic contrasts were performed for loci with two RFLP alleles segregating which exhibited a significant *F*-test ($P < 0.05$). Linear effects measured average effect of substituting one marker allele for another. Quadratic effects measure the deviation of the heterozygous class from the linear model (Stuber et al., 1987).

Multiple linear regression models for these traits were constructed in an effort to approximate the quantity of variation

explained by significant marker loci. Initially, a single significant marker locus from each chromosome arm where QTL were detected was chosen and added as a main effect into a regression model in a stepwise fashion. Only those loci that were significant at $P < 0.05$ in the multiple regression model were retained in the model.

RESULTS AND DISCUSSION

Phenotypic Evaluation of S_1 Families for Oil Concentration and Kernel Weight

Mean oil concentration of S_1 families ranged from 37 to 58 g kg^{-1} with an overall mean of 46 g kg^{-1} (Fig. 1). Kernel weight means varied from 43 to 88 g per 300 kernels with an overall mean of 65.4 g (Fig. 1). Combined over years, the coefficients of variation for oil concentrations and kernel weight were 6.63% and 9.21%, respectively. The analysis of variance combined over years revealed highly significant differences ($P < 0.01$) among S_1 families for oil concentration and kernel weight (data not shown). The year × family interaction was highly significant for both traits, however this interaction was due to changes in magnitude rather than rank of means, thus data were combined over years for QTL mapping analyses. Genetic variance estimates were calculated for oil concentration (0.17) and kernel weight (73.58). Heritability estimates for these traits in this population of S_1 families were calculated as 0.74 and 0.80, respectively.

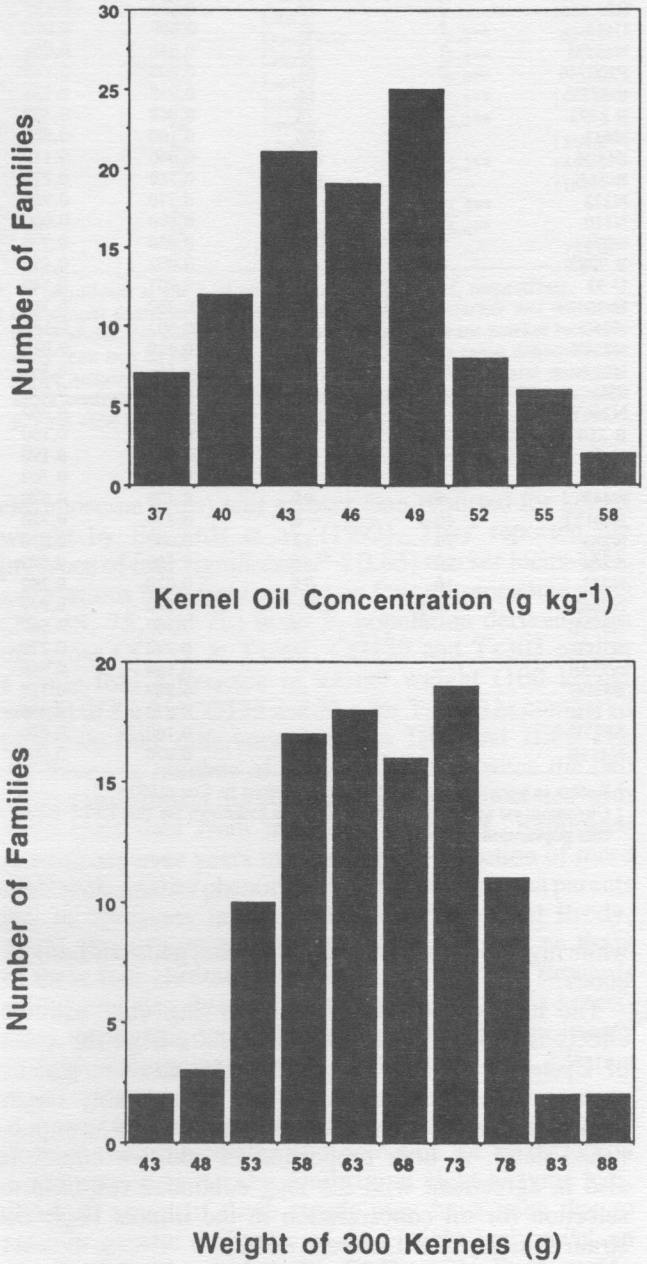


Fig. 1. Distribution of kernel oil concentration (top) and 300 kernel weight (bottom) among S_1 families grown in replicated trials in 1990 and 1991.

Of the 25 marker loci associated with oil concentration, 14 were detected in both years according to single factor analysis of variance. Of the 18 marker loci significantly associated with kernel weight, 9 were significant in both years. The majority of these interactions were due to changes in magnitude of marker class means between the two years rather than changes in rank, thus general trends of rank between marker class means were similar for both years.

QTL Associations with Oil Concentration and Kernel Weight

Significant associations with oil concentration were detected for 25 marker loci on 13 different chromosome arms (Table 1). Eighteen marker loci on 10 different chromosome arms were significantly associated with kernel weight. The true type I error rate in this investigation is approximately 0.99 (Lander and Botstein, 1989) suggesting the possibility one or more QTL might be incorrectly identified for each of these traits. Chromosomal regions exhibiting significant associations between two or more marker loci in a similar chromosomal region (as determined by Coe, 1992) for oil concentration were detected on Chromosome Arms 2L, 4L, 6L, and 8L. Clusters of two or more linked marker loci significantly associated with kernel weight were detected on 2L, 4L, 5S, and 6L. Linkage between RFLP alleles and multiple QTL alleles may have been detected in this study, however these linkages would only serve to reduce our ability to detect significant marker-QTL associations and thus emphasize the significance of the associations reported here.

Marker loci significantly associated with either oil concentration or kernel weight tended to exhibit nearly significant *F*-values for the other trait, and six RFLP loci were significantly associated with both traits. The finding of several marker loci showing significant associations with both oil concentration and kernel weight is in agreement with a general pattern of significant correlations between oil and kernel weight detected in the long-term selection oil strains (Dudley and Lambert, 1992). Despite this pattern, no significant genotypic or phenotypic correlation between oil concentration and kernel weight was detected in this investigation, indicating differences in kernel chemical trait relationships among the Illinois Protein and Illinois Oil strains.

Table 1. RFLP locus, chromosome, approximate location, and probability values based upon *F*-test for oil concentration and kernel weight.

RFLP locus	Chromosome†	Approximate chromosomal location†	Prob. value for oil concentration	Prob.value for kernel weight
U 94	1	−25	0.460	0.020
P200689	1	−14	0.690	0.120
N234	1	11	0.580	0.560
N286	1	26	0.090	0.800
N401	1	45	0.990	0.440
U 67	1	51	0.740	0.150
N272	1	56	0.590	0.150
N262	1	74	0.052	0.240
U 23	1	85	0.570	0.530
N447	1	97	0.005	0.001
U 72L	1	129	0.340	0.710
N239	2	4	0.390	0.630
U 6	2	39	0.440	0.580
N269	2	54	0.910	0.090
U134	2	73	0.00	0.090
N456	2	107	0.006	0.002
U 5	2	132	0.060	0.004
N123	2	133	0.460	0.020
U125	2	140	0.001	0.200
U 36	2	177	0.850	0.020
P200905	3	−5	0.960	0.250
N249	3	51	0.720	0.950
U 10	3	62	0.001	0.090
P200511	3	84	0.100	0.230
B1024	3	93	0.540	0.110
U 39	3	112	0.280	0.660
U 17T	3	118	0.680	0.250
U 16T	3	120	0.090	0.070
N432	3	125	0.410	0.020
P100080	3	147	0.330	0.090
U 63A	3	149	0.440	0.500
SH2	3	149	0.550	0.140
U 96	3	160	0.200	0.230
N457	3	161	0.390	0.600
N420	3	164	0.00	0.260
U123	4	20	0.030	0.150
BT2	4	67	0.190	0.040
N270	4	110	0.00	0.170
N410	4	118	0.003	0.017
P100025	4	137	0.540	0.940
U 66	4	143	0.760	0.007
U104T	4	146	0.005	0.090
N593T	4	165	0.520	0.560
N451	4	170	0.060	0.002
N409	5	−32	0.730	0.030
U 72U	5	−4	0.009	0.001
U 90	5	−3	0.490	0.620
U 27	5	14	0.360	0.390
U 43	5	22	0.070	0.280
U 1	5	24	0.090	0.490
N213	5	61	0.370	0.630
P100017	5	116	0.250	0.490
U104B	5	120	0.120	0.500
N237	5	130	0.540	0.090
U 85	6	5	0.002	0.160
N377	6	27	0.014	0.290
U 21	6	55	0.200	0.470
U137	6	72	0.230	0.330
N252	6	73	0.420	0.220
B 547U	6	81	0.00	0.010
U 38A	6	90	0.020	0.120
U 62	6	112	0.180	0.040
N280	6	118	0.00	0.00
N277	7	1	0.170	0.020
O2	7	16	0.650	0.170
U116	7	41	0.009	0.070
N455M	7	53	0.510	0.004
P200746	7	61	0.400	0.180
B 832T	7	66	0.035	0.350
B 839T	7	78	0.060	0.330
N433	7	106	0.100	0.620
B1606	7	115	0.060	0.110
B 844	7	119	0.160	0.270
N222	8	−49	0.270	0.960
N110	8	−24	0.490	0.060
N276	8	−11	0.050	0.770
B 908T	8	4	0.002	0.450
U 93	8	26	0.007	0.140
U 30	8	34	0.480	0.380
N260	8	70	0.003	0.150
N253T	9	14	0.410	0.040
U113	9	23	0.550	0.560
SH1	9	29	0.510	0.680
N266T	9	44	0.110	0.200
B 510	9	60	0.890	0.150
U 20	9	63	0.370	0.190
N427	9	92	0.300	0.760
N443	9	95	0.470	0.670
B1017T	10	−6	0.230	0.220
N285	10	12	0.680	0.970
N264	10	44	0.560	0.580
N232	10	75	0.020	0.240
N266B	−‡	–	0.190	0.990
N593B	–	–	0.360	0.540
U 16B	–	–	0.790	0.270
N253B	–	–	0.160	0.940
B839B	–	–	0.320	0.710
U 17B	–	–	0.670	0.680
B547B	–	–	0.160	0.620
N455B	–	–	0.070	0.200

† Refers to genetic map position as described in Coe (1992).
‡ Chromosome and chromosomal location unknown for this RFLP locus in this population.

Of the 25 marker loci showing significant associations with oil concentration, 14 had only two alleles segregating in the population. Of these 14 loci, significant additive effects were found at 11 loci and significant dominance effects at four loci (Table 2). At one locus both additive and dominance effects were significant. Of the 18 loci showing significant effects for kernel weight, 14 had only two alleles. Twelve of the 14 showed significant additive effects only, one had both additive and dominance effects, and for one locus only the dominance effect was significant (Table 2). The finding of multiple alleles at marker loci was expected because multiple parents were used from each strain and IHP and ILP, while highly inbred, are neither homozygous nor homogenous.

The high proportion of loci showing only additive effects is in agreement with the results from the cross of Cycle 70 of IHP × ILP in which additive genetic variance for oil concentration was considerably larger than dominance variance (J.W. Dudley, 1991, unpublished data). A high proportion of additive effects is also in agreement with the long continued response to selection for oil concentration in the Illinois High Oil strain (Dudley and Lambert, 1992).

The number of significant marker locus-QTL associations for kernel weight in this study (18 loci on 10

Table 2. RFLP locus, chromosome arm, orthogonal contrast, and contrast mean square for marker loci exhibiting a significant *F*-test and a significant linear or quadratic contrast from the analysis of variance for oil concentration and kernel weight.

RFLP locus	Chromosome arm	Contrast†	Mean squares	
			Oil concentration	Kernel weight
U134	2L	Linear	6.8**	–
N456	2L	Linear	3.6**	1420.2**
N123	2L	Linear	–	1225.5**
U 10	3L	Linear	3.1**	–
		Quadratic	2.5**	
N432	3L	Linear	–	661.8**
Bt2	4S	Linear	–	958.7*
N270	4L	Linear	6.0**	–
N410	4L	Linear	4.9**	1334.1**
U 66	4L	Linear	–	960.0*
U104T	4L	Quadratic	3.5**	–
N451	4L	Linear	–	2167.5**
N409	5S	Quadratic	–	1011.7**
U72U	5S	Linear	–	3016.9**
		Quadratic	3.2**	821.7*
U 85	6S	Linear	5.4**	–
N377	6S	Linear	3.9**	–
B5.47	6L	Linear	12.1**	1577.8**
U 38A	6L	Linear	3.2**	–
U 62	6L	Linear	–	1140.6**
N280	6L	Linear	5.2**	1441.1**
N277	7L	Linear	–	1162.7**
N260	8L	Linear	5.5**	–
B9.08	8L	Quadratic	5.4**	–
N253T	9S	Linear	–	753.5*

*, ** Significant at the 0.05 and 0.01 probability level, respectively.

† Orthogonal contrasts performed on all significant marker loci with two RFLP alleles. No contrast was performed on significant marker loci with more than two RFLP alleles. Linear effect contrasts mean difference between homozygous marker classes (additive gene action) and quadratic effect contrasts mean of homozygous marker classes with heterozygous marker class (dominant gene action).

chromosome arms) was greater than reported for kernel weight by Edwards et al. (1992). They reported the presence of four significant ($P < 0.05$) marker locus-QTL associations for kernel weight on four chromosome arms (2L, 4S, 7S, and 7L) in an F_2 population derived from the cross CO159 × Tx303. CO159 and Tx303 exhibit a three-fold difference in kernel weight (100 kernel weight of 8 g for CO159 and 24 g for Tx303) as compared to a two-fold difference between IHP and ILP. The difference in number of QTL detected between the two studies may reflect the potential for error associated with phenotypic data from individual F_2 plants. Replicated evaluations over years may enable the detection of more QTL with smaller phenotypic differences between parents due to decreases in error variance (Soller and Brody, 1976). Significant associations of marker loci on three of these four chromosome arms detected in the Edwards study were also detected for kernel weight in this experiment, suggesting some chromosomal position consistency of QTL influencing kernel weight in two very different maize crosses.

Kahler (1985) detected significant linear allozyme frequency trends associated with selection for increased oil concentration in Alexho synthetic at eight enzyme loci, although many of these trends may have been due to random genetic drift. Six of these eight loci, *Adh1* on 1L, *Prx1* on 2L, *Est4* on 3S, *Pgd2* on 3L, Pgd1 on 6L, and *Est1* on 7L are located on the same chromosome arms and within 25 cM (Coe, 1992) of marker loci associated with significant effects for oil concentration. The remaining two loci are also located on chromosome arms found to contain marker loci with significant effects on oil concentration, but are not <25 cM from significant loci in this experiment. RFLP allelic frequency trends consistent with selection for oil concentration have been detected among the Illinois Oil Strains at the RFLP marker locus UMC 10, located close to the centromere of chromosome 3 (Sughroue and Rocheford, 1994). A significant association for oil concentration was detected at the UMC 10 locus in our investigation. *Pgd2*, one of the six enzyme loci Kahler found to be significantly associated with selection for oil concentration is also located near the centromere on Chromosome 3. The agreement of results involving Chromosome 3 in our investigation, the Kahler study, and by Sughroue and Rocheford suggests the same general genomic region may be influencing oil concentration in different genetic backgrounds. Our results provide an example of QTL mapping data which is consistent with molecular marker population genetic data.

Edwards et al. (1992) reported the presence of a highly significant association between marker locus 49 (*Adh2*, T. Helentjaris, 1992, personal communication), located on 4S, and kernel weight in the CO159 × Tx303 mapping population. A similar significant association with kernel weight was detected for *Bt2* in our study. Based upon genetic linkage maps developed by Coe (1992), the *Bt2* locus resides on the same chromosome arm (approximately 20 cM) from the *Adh2* locus. The *Bt2* locus is responsible for production of the smaller subunit of the enzyme ADP-glucose pyrophosphorylase, a major starch synthetic enzyme (Bae et al., 1990). Kernels carrying the *bt2* mutant have a brittle and greatly-reduced endosperm. The general finding that endosperm effects conditioned by the *bt2* mutant affect kernel weight may be consistent with the detection of a significant association between *Bt2* and kernel weight in our study. A QTL at or near a locus that has a known qualitative kernel mutant effect may signify the existence of important wild-type allelic variants with quantitative effects on kernel chemical and physical composition. Our results at the *Bt2* locus may be consistent with the hypothesis that minor allelic variants at known qualitative loci may be responsible for quantitative effects (Thompson, 1975; Robertson, 1985). Associations of RFLP marker loci at or near major qualitative loci with QTL controlling relevant traits has also been documented in maize for plant height (Beavis et al., 1991; Edwards et al., 1992) and starch concentration (Goldman et al., 1993). Quantitative trait loci for anther culturability, androgenesis, and plant regeneration in maize have been found in close proximity to the *viviparous-1* mutant locus in three separate studies using different genetic materials (Armstrong et al., 1992; Cowen et al., 1992; Wan et al., 1992), suggesting the broad applicability of RFLP-QTL associations for certain traits in maize.

Due to the large agronomic differences between Burr's White and much of modern Corn Belt germplasm, identification of some marker loci linked to QTL in this study

may be unique to the Illinois Long Term Selection strains. However, the positional similarity of a significant marker locus located near *Bt2* for kernel weight in the Edwards et al. (1992) study and our study as well as between significant isozyme loci in Alexho in the Kahler study and RFLP marker loci (UMC 10) associated with QTL for oil concentration in our investigation suggests there may be some consistency for loci controlling kernel oil concentration and kernel weight across *Z. mays* germplasm. However, introgression and evaluation of the marker locus-QTL associations reported here is necessary in additional and more elite genetic backgrounds before these associations could be considered for use in the context of marker facilitated selection for oil concentration in the development of commercial high oil hybrids.

Relationship of QTL for Oil and Kernel versus Protein and Starch

Goldman et al. (1993) detected QTL influencing protein and starch concentration in IHP × ILP at 23 marker loci distributed throughout the genome. Three of these marker loci (NPI 447, UMC 72A, BNL 5.47A) were among the six marker loci exhibiting significant associations with both oil concentration and kernel weight in this study and thus were significant for all four kernel traits. The three marker loci in common, located on 1L, 5S, and 6L, respectively, may reflect genomic regions involved in allocation of photoassimilates for starch, protein, and/or oil deposition in the maize kernel. As the Illinois Long Term Selection Experiment has demonstrated, changes in one major kernel constituent such as oil, protein, or starch concentration can result in corresponding changes in other major chemical components as well as changes in kernel weight. Phenotypic correlations were calculated for protein, oil, and starch concentration and kernel weight from family means. Significant ($P < 0.01$) negative correlations were detected between protein and starch (-0.897) and between protein and kernel weight (-0.307). These high negative correlations are expected based upon results reported by Dudley and Lambert (1992). A significant positive correlation was detected between starch and kernel weight (0.316).

The two-fold difference in oil concentration between IHP and ILP families was approximately the same magnitude as the difference in protein concentration between S_1 families of IHP and ILP (Goldman et al., 1993), thus the identification of a similar number of marker locus-QTL associations may be related to the amount of phenotypic variation for the two traits in IHP and ILP.

Only five marker loci were significantly associated with both protein and oil concentration in these two studies. Given this relatively small number, it is possible effective selection for either oil or protein concentration might be accomplished while holding the other trait relatively constant. This type of selection may facilitate the development of specialty maize hybrids with elevated levels of one of these kernel constituents. Only two loci were significant for both protein and kernel weight in these studies, reflecting the small, though significant, correlation between these traits. Our findings of significant negative correlation between protein and kernel weight and positive correlation between starch and kernel weight are consistent with large differences in kernel weight between IHP and ILP.

Multiple Regression Models Predictive of Oil Concentration and Kernel Weight

Significant marker loci were used to develop a predictive model for genetic control of oil concentration and kernel weight. A significant marker locus with only two RFLP alleles was chosen from each chromosome to represent the effects of that chromosome. Initially, these selected significant marker loci were entered into a multiple linear regression model in a stepwise fashion. Marker loci remained in the model if the locus was significant at $P < 0.05$. Four marker loci on four chromosomes (4, 5, 6, 8) accounted for 43% (R^2) of the total phenotypic variation for oil concentration in this experiment (Table 3). Six marker loci located on Chromosomes 2, 4, 5, 6, 7, and 9 explained 47% (R^2) of the total phenotypic variation for kernel weight. The R^2 value for oil concentration (43%) reported in this study is comparable to those reported for similar multiple regression models describing QTL variation in corn for traits such as grain yield and plant height (Edwards et al., 1992). The a priori decision to include only those marker loci with two RFLP alleles in the multiple regression model limited the proportion of variation the model could explain. Higher R^2 values were obtained for both oil concentration and kernel weight upon inclusion of marker loci with multiple RFLP alleles (data not shown). In general, these models suggest nearly one-half of the total phenotypic variation for both traits can be explained by relatively few marker loci.

CONCLUSIONS

Results from this investigation demonstrate the effectiveness of identification of QTL for oil concentration and kernel weight in a population descended from a cross of two strains divergently selected for protein concentration. Quantitative trait loci for oil concentration were detected despite a relatively narrow range of oil concen-

Table 3. Multiple regression models and percentage of total phenotypic variation explained by marker loci for oil concentration and kernel weight in the Illinois Long Term Selection strains.

Model	Source of variation	df	Mean Square	$P > f$
A. Oil concentration: $R^2 = 0.43$				
	N410	2	212.0	0.000
	U 72A	2	179.0	0.000
	U 85	2	271.0	0.000
	B908A	2	122.0	0.000
B. Kernel weight: $R^2 = 0.47$				
	N123	2	184.6	0.0366
	U 66	2	627.7	0.000
	N409	2	280.0	0.007
	U62	2	392.0	0.001
	N455B	2	683.1	0.000
	N253A	2	325.6	0.003

tration. Chromosomal positions of some QTL influencing oil concentration and kernel weight are supported by finding from other investigators (Kahler, 1985; Edwards et al., 1992; Sughroue and Rocheford, 1994). Thus, there is evidence for the idea that some QTL controlling oil concentration and kernel weight may reside in similar chromosomal locations in different genetic backgrounds. Detection of relatively few clusters of marker loci associated with genomic regions influencing oil concentration suggests the development of high oil maize germplasm via RFLP marker-assisted selection is feasible. Because most QTL identified showing favorable effects for oil also had unfavorable effects for kernel weight, care will need to be taken when using marker assisted or conventional selection for oil concentration to assure maintenance of kernel weight. Since alleles at several RFLP marker loci were associated with increases of as much as 0.5% kernel oil concentration, a selection index of several significant marker loci could be employed in a marker-assisted selection scheme for the development of high oil maize.

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