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# Restriction fragment length polymorphism differences among Illinois long-Term selection oil strains

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**Abstract.** Restriction fragment length polymorphism (RFLP) analysis was used to characterize variability in the Illinois Long-Term Selection Experiment oil strains. Considerable polymorphism was detected within each oil strain and among oil strains. Fifty-two individual plants from each of the Illinois High Oil (IHO), Illinois Low Oil (ILO), Reverse High Oil (RHO) and Reverse Low Oil (RLO) strains were sampled to determine RFLP allele/variant frequencies. Generation 90 was sampled for IHO, RHO, and RLO whereas generation 87 was sampled for ILO. Forty-nine RFLP probes distributed throughout the maize genome were used. Chi-square analysis was performed to determine if RFLP genotypes at each of the 49 RFLP loci were significantly different among strains. Oil strains that have been separated for 90 generations showed high levels of significantly-different RFLP genotypic frequencies. The comparison of ILO vs RHO gave only significant chi-square values while the comparisons of IHO vs RLO and RHO vs RLO had 11:1 ratios of significant to non-significant chi-square values. Strains that have been separated for only 42 generations showed a lower level of significantly-different RFLP genotypic frequencies. The comparisons of IHO vs RHO and ILO vs RLO both had only a 3:2 ratio of significant to non-significant chi-squares values. Detection of multiple RFLP alleles/variants among the oil strains was common with 59% of the RFLP loci examined exhibiting multiple variants. A number of RFLP loci in RHO (3) and RLO (11) were associated with a trend in RFLP allele/variant frequencies consistent with a response to reverse selection for oil concentration.

**Key words:** Long-term selection – Oil concentration – *Zea mays* L. – Restriction fragment length polymorphism (RFLP)

## Introduction

Increased oil concentration in maize (*Zea mays* L.) grain can increase animal feeding efficiency, particularly in animals requiring a high caloric diet, since oil contains 2.25 times more calories per gram of dry matter than starch or protein (Han et al. 1987; Adams and Jensen 1988; Atwell et al. 1988; Goss and Kerr 1992). Consequently, there have been plant breeding efforts with the objective of developing higher oil maize hybrids (Alexander 1988). The first selection experiment on the chemical composition of the maize kernel was started in 1896 by C. G. Hopkins at the University of Illinois. Selection was performed for oil and protein concentration in kernels from the open-pollinated maize cultivar 'Burr's White' (Hopkins 1899). Ninety generations of selection increased oil concentration from 4.7% in the original population to 19.3% in IHO (Fig. 1). In contrast, 87 generations of selection for low oil concentration reduced percent oil from 4.7% to < 1.0% in ILO (Dudley and Lambert 1992). Reverse selection, initiated in IHO and ILO after 48 generations of selection, created two additional strains: Reverse High Oil (RHO) and Reverse Low Oil (RLO) (Leng 1962). Forty-two generations of selection changed the oil concentration in RHO from 13.4% to 4.8% and in RLO from 0.8% to 4.2%. Details of specific

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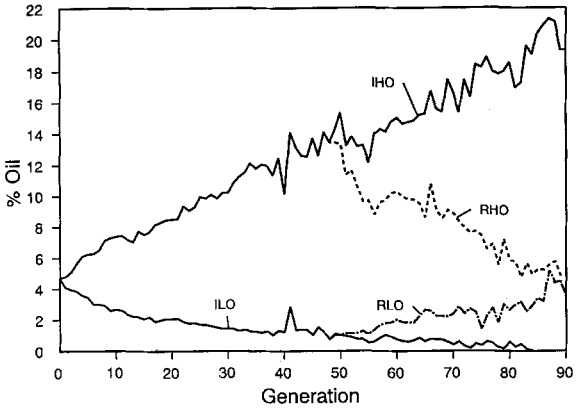


**Fig. 1.** Mean percentage of oil by generation for the Illinois oil strains

selection procedures, chemical analyses, and statistical evaluations have been reported elsewhere (Dudley et al. 1974; Dudley and Lambert 1992).

Selection for oil concentration has affected several other agronomic traits in the Illinois Long-Term Selection strains. IHO has small ears and small kernels with large embryos. In comparison ILO has relatively larger ears and larger kernels but the kernels have smaller embryos. IHO flowers earlier than ILO, and IHO has lower plant and ear heights than ILO (Dudley et al. 1977).

Before the availability of restriction fragment length polymorphism (RFLP) technology, researchers performing molecular investigations on maize populations were limited to the use of isozymes. Frequency changes of alleles at enzyme loci in maize populations which have undergone selection have been well documented (Brown 1971; Brown and Allard 1971; Stuber and Moll 1972; Stuber et al. 1980; Kahler 1985). These studies attempted to determine if the changes in the estimated allozyme frequencies were associated with selection for specific traits such as yield and oil concentration.

Brown (1971) examined generation 68 of the Illinois Long-Term Selection Experiment strains and found that the observed allozyme variation at six enzyme loci could be accounted for by neutral genetic drift but that selection could not be entirely ruled out. Brown's study had limited generality because only six enzyme loci were assayed. Kahler (1985) examined two other maize populations to detemine whether the frequency of certain allozymes changed during selection for increased oil concentration. The first study involved the population Reid Yellow Dent in which seven cycles of selection for increased oil concentration changed the percent oil from 4.0% to 9.1% (Miller et al. 1981). Kahler (1985) monitored ten enzyme loci over seven cycles and concluded allozyme frequencies did not change linearly with selection for increased oil concentration. The second study involved the synthetic population Alexho in which 25 cycles of selection resulted in a change in percent oil from 4.6% to 19.1% (Misevic et al. 1985). At eight of thirteen enzyme loci assayed allozyme frequencies exhibited a significant linear trend with selection for increased oil concentration (Kahler 1985). The results suggest that selection for increased oil concentration in the Alexho population affected allozyme frequencies at certain loci. However, allozyme frequencies at the same loci were not affected by selection for increased oil concentration in the Reid Yellow Dent population.

Little research has been reported on the evaluation of RFLP frequencies in maize populations that have undergone long-term selection for agronomic traits. In maize, the availability of a very large number of RFLP probes distributed throughout the genome enables investigators to evaluate a much greater portion of the genome than with isozymes. The overall objective of this study was to characterize genetic variability at the RFLP level in IHO, ILO, RHO, and RLO strains. Specific objectives include: (1) characterization of RFLP frequencies, (2) determination of the percent of RFLP loci examined which have the same RFLP allele/variant present in a homozygous state in all plants sampled from a strain, and (3) identification of RFLP alleles/variants whose frequencies have changed in the reverse selection strains in a manner consistent with response to selection. The identification of RFLP loci that may be associated with selection for oil concentration provides information complementary to RFLP mapping studies designed to identify quantitative trait loci (QTLs) for oil concentration. The identification of RFLPs associated with oil concentration may be useful in molecular-marker-facilitated breeding programs designed to develop high-yielding, high-oil maize hybrids.

## Materials and methods

### *Genetic stocks*

The following strains were used in this study: generation 90 of IHO, RHO, and RLO strains, and generation 87 of the ILO strain. Generation 90 of the reverse-selection strains represents 48 generations of forward selection followed by 42 generations of reverse selection. These generations were selected because they were the most recent ones available. DNA was isolated from 52 random plants sampled from each of the strains.

### *RFLP laboratory procedures*

DNA isolation, restriction endonuclease digestion with *Eco*RI, gel electrophoresis, DNA transfer to nylon membranes, oligo-labeling with $^{32}P$, and hybridizations were all accomplished using standard procedures (Saghai-Maroof et al. 1984; Hoisington 1989; Lee 1991; Sambrook et al. 1989). The genomic DNA clones

selected for use as RFLP probes were from sets of mapped maize clones provided by the University of Missouri-Columbia (umc), Brookhaven National Laboratory (bnl), and Pioneer Hi-Bred International (php). Some of the php probes are the former Native Plants Incorporated (NPI), probes.

Lambda digested with *Hin*dIII was used as a molecular weight standard on each gel in lanes 1, 15 and 30 of both the upper and lower set of lanes. DNA samples isolated from the maize inbreds B73 and Mo17 and digested with *Eco*RI were also used as standards on all gels. The B73 sample was loaded in lane 14 of the upper set of lanes and the Mo17 sample in lane 14 of the lower set of lanes. The digested DNA from individual plant samples of IHO and ILO were alternated in lanes 2–13 and 16–29 of both the upper and lower part of the gels and transferred to one set of nylon membranes. Individual samples of RHO and RLO were arranged in the same manner on a different set of gels and transferred to a different set of nylon membranes.

*Analysis of RFLP data*

Forty-nine RFLP probes distributed throughout the maize genome were used (Table 1). Chromosomal locations of the RFLP probes employed were taken from the map in the 1992 Maize Genetics Newsletter, the January 26, 1992, incorporated maize RFLP map provided at the 1992 Maize genetics meetings, and unpublished NPI information. RFLP probes were selected on the basis of detecting a polymorphism between a bulk DNA sample of 50 plants of IHO and ILO digested with *Eco*RI. One hundred-and-fifty-five RFLP probes were screened and over 60% of the probes detected a polymorphism between IHO and ILO (Sughroue et al. 1992).

Consistent with established terminology (Maize Genetics Nomenclature Subcommittee Report, 1993 Maize Genetics Newsletter), the chromosomal region where each RFLP probe primarily hybridizes is defined as an RFLP locus. Each distinct hybridzation fragment detected by a probe is defined as an RFLP variant (except in the case of two-banded variants in which the two bands comprise the RFLP variant). For 20 of the RFLP loci examined in this study, allelism tests have been performed for two variants at each locus and allelism was demonstrated in all cases. The allelism tests were performed on 200 S1 families developed from a cross of an IHO plant × an ILO plant (T. Berke, unpublished results). However, we have not demonstrated allelism for all RFLP variants detected in this study. Therefore, for consistency and clarity, we will only use the term RFLP variant, and not the term RFLP allele, in the remainder of this report and simply acknowledge that many variants have been demonstrated to be allelic.

Each different RFLP variant at an RFLP locus was assigned a letter designation with the highest molecular weight fragment designated 'A' and additional fragments at a locus (if present) designated 'B', 'C', etc., in descending order according to molecular weight. The variant composition at each RFLP locus of a plant DNA sample determined the RFLP genotype; for example AA, AB, BB. We use the term RFLP genotype but acknowledge that in the case where a variant is demonstrated to be nonallelic the term RFLP phenotype would be appropriate.

Chi-square analysis (Cochran 1954) was used to determine whether strains differed significantly in RFLP genotypic frequencies at individual RFLP loci (Tsumura et al. 1992). The standard chi-square calculation for a 2 × n (row × column) contingency table was used (SAS 1988). Two strains were compared at a time and comprised the two rows, with the number of columns (n) varied to reflect the number of genotypic classes for each RFLP locus. Because the RFLP probes used were preselected on the basis of being polymorphic between IHO and ILO, genetic drift calculations were not performed since a random set of probes was not used to determine RFLP genotypic frequencies.

**Table 1.** Chromsomal location of the 49 DNA probes used in this study

| Chromosome | Clone designation[a] |
|---|---|
| 1 | umc 94, umc 157, php 9234, php 9286, umc 67, php 9272, php 9447, umc 107 |
| 2 | php 9239, php 9421, umc 5, umc 137 |
| 3 | php 200905, umc 10, umc 102, bnl 10.24, umc 16, php 9457 |
| 4 | php 9259, umc 47, php 9270, umc 66, php 9451 |
| 5 | bnl 6.25, umc 147, umc 51, umc 68 |
| 6 | umc 85, umc 65, umc 21, umc 132, php 9280, umc 133 |
| 7 | php 9277, umc 116, php 9240, bnl 16.06, umc 168 |
| 8 | umc 103, php 9276, php 9268, php 9438 |
| 9 | umc 113, umc 81, php 9209 |
| 10 | php 9285, umc 64, php 9264, umc 44 |

[a] Clone designations according to 1992 and 1993 Maize Genetics Newsletter

## Results and discussion

The four Illinois Long-Term Selection oil strains differed in RFLP genotypic frequencies. Observations contributing to the variability detected include RFLP loci with one variant present in a homozygous state among all the plants sampled from one oil strain (fixed) and a different variant fixed in another oil strain, the presence of multiple RFLP variants among the strains at many RFLP loci, and RFLP loci with variants segregating at differing frequencies among the strains. Examples of among-strain variation in RFLP genotypic frequencies are shown in Table 2. The number of significant and non-significant chi-square values for each of the comparisons was determined (Table 3). The 0.05 probability level was used to declare differences significant. The comparison of IHO vs ILO was not included in Table 2 because all 49 probes were selected on the basis of being polymorphic between bulked DNA samples of IHO and ILO and all comparisons were significantly different (data not shown). ILO vs RHO had significant genotypic frequency differences at all 49 RFLP loci. The IHO vs RLO and RHO vs RLO comparisons had similar percentages (92% and 94%, respectively) of RFLP loci with significant genotypic frequency differences. The remaining two comparisons, IHO vs RHO (65%) and ILO vs RLO (55%) also had similar percentages of loci with significantly-different genotypic frequencies.

The relative percentage of significant chi-square values for the RFLP genotypic frequency comparisons might be related to the number of generations for

**Table 2.** Genotypic frequencies of a sample of the 49 RFLP loci evaluated for generation 90 of IHO, RHO, RLO and generation 87 of ILO

| Chromosome location | RFLP locus | Genotypic class | Strain | | | |
|---|---|---|---|---|---|---|
| | | | IHO | ILO | RHO | RLO |
| 1S | umc 157 | AA | 52 | 0 | 14 | 13 |
| | | BB | 0 | 5 | 17 | 19 |
| | | BB | 0 | 47 | 21 | 14 |
| 3L | bnl 10.24 | AA | 0 | 47 | 35 | 0 |
| | | AC | 0 | 0 | 12 | 0 |
| | | BB | 51 | 0 | 1 | 49 |
| | | CC | 0 | 0 | 4 | 0 |
| 5S | umc 147 | AA | 0 | 40 | 0 | 8 |
| | | AB | 0 | 3 | 0 | 28 |
| | | BB | 48 | 1 | 52 | 16 |
| 7S | php9277 | AA | 51 | 1 | 0 | 36 |
| | | AB | 0 | 0 | 0 | 14 |
| | | BB | 0 | 43 | 0 | 0 |
| | | BC | 0 | 7 | 0 | 0 |
| | | CC | 0 | 1 | 52 | 0 |
| 9L | php 9209 | AA | 46 | 1 | 46 | 52 |
| | | AB | 0 | 12 | 0 | 0 |
| | | AC | 5 | 1 | 2 | 0 |
| | | BB | 1 | 31 | 0 | 0 |
| | | CC | 0 | 0 | 3 | 0 |
| 10L | umc 44 | AA | 52 | 0 | 52 | 0 |
| | | BB | 0 | 52 | 0 | 52 |

**Table 3.** Number of significant and non-significant chi-square values within each of the comparisons made among the Illinois oil strains

| Significance | Strain comparison | | | | |
|---|---|---|---|---|---|
| | IHO vs RHO | IHO vs RLO | ILO vs RHO | ILO vs RLO | RHO vs RLO |
| ** | 30 | 44 | 49 | 27 | 46 |
| * | 2 | 1 | 0 | 0 | 0 |
| ns | 17 | 4 | 0 | 22 | 3 |

ns, *, ** = not significant, significant at the 0.05 probability level, and significant at the 0.01 probability level, respectively

which the strains under comparison have been separated. The comparisons of IHO vs RHO and ILO vs RLO identified genotypic frequencies which were frequently not significantly different (35% and 45%, respectively). This may be due to RHO originating from IHO generation 48 and RLO originating from ILO generation 48. The IHO and ILO strains had each undergone 48 generations of isolation and selection before the reverse strains were derived, possibly resulting in many RFLP variants being fixed before reverse selection occurred. Lack of variability at RFLP loci would have reduced the number of loci where differences among the forward strain and respective reverse strain could develop.

RFLP variant frequencies for each strain are reported in Table 4. Within each strain from 18 to 28 of the 49 RFLP loci had segregating variants. RHO had the highest number of RFLP loci (28) with segregating variants and ILO had the lowest number (18). IHO had 23 and RLO 21 RFLP loci with segregating variants. Fourteen RFLP loci had the same RFLP variant fixed in both IHO and RHO and 18 RFLP loci in ILO and RLO had the same variant fixed. For all four strains considered collectively, the percentage of RFLP loci with a variant fixed was 54%. This shows that nearly half of the RFLP loci examined in the oil strains have RFLP variants that are still segregating after approximately 90 generations of selection. Similarly, Wilson (1992) reported only 60% fixation for zein proteins of the Illinois oil and protein strains considered collectively. These results indicate that molecular-level analyses have found higher levels of variability within the Illinois Long-Term Selection oil strains than would be predicted if the estimated inbreeding coefficient of 88% (Dudley 1992, personal communication; calculations according to those outlined in Dudley 1977) was used to estimate molecular-level variability.

Forty percent (20/49) of the RFLP loci in RHO had a RFLP variant not detected in IHO and 35% (17/49)

**Table 4.** RFLP variant frequencies at 49 loci within each of the Illinois oil strains

| Chromosome location | RFLP locus | Allele | Strain | | | |
|---|---|---|---|---|---|---|
| | | | IHO | ILO | RHO | RLO |
| 1S | umc 94 | A | 1.00 | 0.00 | 0.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| | | C | 0.00 | 0.00 | 1.00 | 0.00 |
| | umc 157 | A | 1.00 | 0.05 | 0.43 | 0.49 |
| | | B | 0.00 | 0.95 | 0.57 | 0.51 |
| | php 9234 | A | 0.48 | 0.00 | 0.66 | 0.00 |
| | | B | 0.52 | 0.05 | 0.32 | 0.85 |
| | | C | 0.00 | 0.95 | 0.02 | 0.15 |
| | php 9286 | A | 1.00 | 0.00 | 1.00 | 0.00 |
| | | B | 0.00 | 0.00 | 0.00 | 0.87 |
| | | N | 0.00 | 1.00 | 0.00 | 0.13 |
| | umc 67 | A | 0.04 | 1.00 | 0.07 | 1.00 |
| | | B | 0.01 | 0.00 | 0.93 | 0.00 |
| | | C | 0.95 | 0.00 | 0.00 | 0.00 |
| 1CE | php 9272 | A | 1.00 | 0.00 | 0.36 | 0.00 |
| | | B | 0.00 | 1.00 | 0.64 | 1.00 |
| 1L | php 9447 | A | 0.00 | 0.62 | 0.00 | 0.45 |
| | | B | 1.00 | 0.17 | 0.05 | 0.54 |
| | | C | 0.00 | 0.21 | 0.95 | 0.01 |
| | umc 107 | A | 1.00 | 0.00 | 1.00 | 0.33 |
| | | B | 0.00 | 1.00 | 0.00 | 0.67 |
| 2S | php 9239 | A | 1.00 | 0.19 | 0.00 | 1.00 |
| | | B | 0.00 | 0.81 | 0.26 | 0.00 |
| | | C | 0.00 | 0.00 | 0.74 | 0.00 |
| | php 9421 | A | 0.00 | 1.00 | 0.68 | 0.00 |
| | | B | 1.00 | 0.00 | 0.00 | 0.00 |
| | | C | 0.00 | 0.00 | 0.32 | 0.67 |
| | | D | 0.00 | 0.00 | 0.00 | 0.33 |
| 2L | umc 5 | A | 0.98 | 0.94 | 0.00 | 0.00 |
| | | B | 0.02 | 0.06 | 0.49 | 0.00 |
| | | C | 0.00 | 0.00 | 0.51 | 0.56 |
| | | D | 0.00 | 0.00 | 0.00 | 0.44 |
| | umc 137 | A | 0.00 | 1.00 | 0.34 | 0.00 |
| | | B | 0.67 | 0.00 | 0.00 | 1.00 |
| | | C | 0.33 | 0.00 | 0.66 | 0.00 |
| 3S | php 200905 | A | 0.00 | 1.00 | 0.00 | 1.00 |
| | | B | 0.18 | 0.00 | 0.07 | 0.00 |
| | | C | 0.82 | 0.00 | 0.93 | 0.00 |
| 3CE | umc 10 | A | 1.00 | 0.00 | 0.90 | 0.55 |
| | | B | 0.00 | 1.00 | 0.10 | 0.45 |
| | umc 102 | A | 1.00 | 0.82 | 1.00 | 0.05 |
| | | B | 0.00 | 0.18 | 0.00 | 0.95 |
| 3L | bnl 10.24 | A | 0.00 | 1.00 | 0.79 | 0.00 |
| | | B | 1.00 | 0.00 | 0.02 | 1.00 |
| | | C | 0.00 | 0.00 | 0.19 | 0.00 |
| | umc 16 | A | 0.78 | 0.00 | 1.00 | 0.00 |
| | | B | 0.22 | 1.00 | 0.00 | 1.00 |
| | php 9457 | A | 1.00 | 0.00 | 1.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| 4S | php 9259 | A | 0.45 | 1.00 | 0.30 | 1.00 |
| | | B | 0.38 | 0.00 | 0.00 | 0.00 |
| | | C | 0.17 | 0.00 | 0.70 | 0.00 |
| | umc 47 | A | 0.29 | 0.33 | 0.70 | 0.00 |
| | | B | 0.71 | 0.67 | 0.30 | 1.00 |
| 4L | php 9270 | A | 0.92 | 0.00 | 0.09 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| | | C | 0.08 | 0.00 | 0.91 | 0.00 |
| | umc 66 | A | 0.87 | 0.00 | 0.00 | 0.00 |
| | | B | 0.13 | 0.00 | 1.00 | 0.00 |
| | | N | 0.00 | 1.00 | 0.00 | 1.00 |

**Table 4.** (*Continued*)

| Chromosome location | RFLP locus | Allele | Strain | | | |
|---|---|---|---|---|---|---|
| | | | IHO | ILO | RHO | RLO |
| | php 9451 | A | 0.00 | 0.64 | 0.00 | 0.46 |
| | | B | 1.00 | 0.16 | 0.12 | 0.54 |
| | | C | 0.00 | 0.20 | 0.88 | 0.00 |
| 5S | bnl 6.25 | A | 0.00 | 0.95 | 0.00 | 0.00 |
| | | B | 0.33 | 0.00 | 0.58 | 0.16 |
| | | C | 0.59 | 0.05 | 0.42 | 0.84 |
| | | D | 0.08 | 0.00 | 0.00 | 0.00 |
| | umc 147 | A | 0.00 | 0.94 | 0.00 | 0.42 |
| | | B | 1.00 | 0.06 | 1.00 | 0.58 |
| 5 L | umc 51 | A | 0.68 | 0.01 | 0.00 | 0.00 |
| | | B | 0.32 | 0.99 | 0.20 | 1.00 |
| | | C | 0.00 | 0.00 | 0.80 | 0.00 |
| | umc 68 | A | 1.00 | 0.00 | 1.00 | 1.00 |
| | | B | 0.00 | 1.00 | 0.00 | 0.00 |
| 6S | umc 85 | A | 1.00 | 0.51 | 1.00 | 0.08 |
| | | B | 0.00 | 0.49 | 0.00 | 0.90 |
| | | C | 0.00 | 0.00 | 0.00 | 0.02 |
| 6 L | umc 65 | A | 0.88 | 0.00 | 0.04 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| | | C | 0.12 | 0.00 | 0.00 | 0.00 |
| | | D | 0.00 | 0.00 | 0.96 | 0.00 |
| | umc 21 | A | 0.00 | 0.76 | 0.00 | 0.00 |
| | | B | 0.17 | 0.00 | 0.00 | 0.00 |
| | | C | 0.00 | 0.24 | 0.00 | 1.00 |
| | | D | 0.83 | 0.00 | 1.00 | 0.00 |
| | umc 132 | A | 0.54 | 0.00 | 0.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| | | C | 0.46 | 0.00 | 1.00 | 0.00 |
| | php 9280 | A | 0.00 | 0.99 | 0.08 | 0.00 |
| | | B | 1.00 | 0.00 | 0.92 | 1.00 |
| | | C | 0.00 | 0.01 | 0.00 | 0.00 |
| | umc 133 | A | 1.00 | 0.00 | 1.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| 7S | php 9277 | A | 1.00 | 0.02 | 0.00 | 0.86 |
| | | B | 0.00 | 0.89 | 0.00 | 0.14 |
| | | C | 0.00 | 0.09 | 1.00 | 0.00 |
| 7L | umc 116 | A | 0.96 | 0.00 | 0.67 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| | | C | 0.04 | 0.00 | 0.00 | 0.00 |
| | | D | 0.00 | 0.00 | 0.33 | 0.00 |
| | php 9240 | A | 0.83 | 0.00 | 0.95 | 0.00 |
| | | B | 0.17 | 0.00 | 0.05 | 0.01 |
| | | C | 0.00 | 1.00 | 0.00 | 0.99 |
| | bnl 16.06 | A | 1.00 | 0.00 | 1.00 | 0.07 |
| | | B | 0.00 | 1.00 | 0.00 | 0.93 |
| | umc 168 | A | 1.00 | 0.00 | 1.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| 8S | umc 103 | A | 0.44 | 0.80 | 0.00 | 1.00 |
| | | B | 0.56 | 0.20 | 1.00 | 0.00 |
| | php9276 | A | 0.00 | 1.00 | 0.49 | 0.89 |
| | | C | 1.00 | 0.00 | 0.51 | 0.11 |
| 8L | umc 113 | A | 0.00 | 1.00 | 0.00 | 1.00 |
| | | B | 1.00 | 0.00 | 1.00 | 0.00 |
| | php 9268 | A | 0.00 | 0.99 | 0.41 | 0.99 |
| | | B | 0.39 | 0.00 | 0.00 | 0.00 |
| | | C | 0.61 | 0.01 | 0.59 | 0.01 |
| | php 9438 | A | 0.83 | 0.00 | 0.84 | 0.00 |
| | | B | 0.17 | 0.00 | 0.15 | 0.99 |
| | | C | 0.00 | 1.00 | 0.00 | 0.01 |
| | | D | 0.00 | 0.00 | 0.01 | 0.00 |

**Table 4.** *(Continued)*

| Chromosome location | RFLP locus | Allele | Strain | | | |
|---|---|---|---|---|---|---|
| | | | IHO | ILO | RHO | RLO |
| 9S | umc 81 | A | 0.62 | 0.00 | 0.57 | 0.00 |
| | | B | 0.38 | 0.00 | 0.43 | 0.00 |
| | | C | 0.00 | 1.00 | 0.00 | 0.76 |
| | | D | 0.00 | 0.00 | 0.00 | 0.24 |
| 9L | php 9209 | A | 0.94 | 0.17 | 0.92 | 1.00 |
| | | B | 0.01 | 0.82 | 0.00 | 0.00 |
| | | C | 0.05 | 0.01 | 0.08 | 0.00 |
| 10S | php 9285 | A | 0.00 | 1.00 | 0.00 | 0.94 |
| | | B | 1.00 | 0.00 | 1.00 | 0.00 |
| | | C | 0.00 | 0.00 | 0.00 | 0.06 |
| 10L | umc 64 | A | 1.00 | 0.00 | 1.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |
| | php 9264 | A | 0.52 | 1.00 | 0.34 | 1.00 |
| | | B | 0.48 | 0.00 | 0.66 | 0.00 |
| | umc 44 | A | 1.00 | 0.00 | 1.00 | 0.00 |
| | | B | 0.00 | 1.00 | 0.00 | 1.00 |

N, null RFLP variant

of the RFLP loci in RLO had a RFLP variant not detected in ILO. RHO had 13 RFLP loci with a variant not present in IHO but present in ILO. RLO had ten RFLP loci with a RFLP variant not present in ILO but present in IHO. Six RFLP loci in RHO had variants which were not observed in IHO, ILO, or RLO. Similarly, five RFLP loci in RLO had variants which were not observed in IHO, ILO, or RHO. These data suggest that some of these RFLP variants may have been present at a low frequency in generation 48 of IHO and/or ILO before reverse selection was initiated, but have since been lost in IHO and/or ILO.

Several RFLP variants among the strains were present at frequencies below $q < 0.10$. These relatively-rare RFLP variants were usually detected only in the heterozygous condition. Detecting the presence or absence of a rare RFLP variant may have been affected by sampling error. There is an 0.36 probability of missing an allele in a sample of 52 plants taken from a population with a frequency of 0.01 for that allele (Steel and Torrie 1980). However, there is only an 0.006 probability of missing an allele in a sample of 52 plants taken from a population with a frequency of 0.05 for that allele. Another factor that should be considered in the evaluation of rare variants is that recombination, mutation, or transposable element movements, may have created RFLP variants.

Fifty-nine percent (29/49) of the RFLP probes detected multiple RFLP variants among the strains. Forty-three percent (21/49) of the probes detected three RFLP variants among the strains and 16% (8/49) of the probes detected four RFLP variants among the strains. Within individual strains, IHO and ILO had four loci, RHO had three loci, and RLO had two loci with multiple RFLP variants. Therefore, the majority of the multiple RFLP variants at a given locus were detected among, as opposed to within, the individual oil strains.

The comparison of IHO vs RHO indicated more loci with significantly different genotypic frequencies (32) than loci with similar genotypic frequencies (17). There is an approximate 14.5% difference in oil concentration between IHO and RHO, which is 76% of the approximate 19% oil difference between IHO and ILO. The comparison of ILO vs RLO also found more loci with significantly-different genotypic frequencies (27) than loci with similar genotypic frequencies (22). However, the percentage oil difference between ILO and RLO is only approximately 3.6%. In this case the difference in oil for ILO vs RLO was only 24% of that for ILO vs IHO yet 55% of the loci between ILO and RLO had significant RFLP genotypic frequency differences, similar to the 65% of loci that had significant RFLP genotypic frequency differences between IHO and RHO. This would suggest that the number of RFLP loci with significantly-different RFLP genotypic frequencies among these two strain comparisons is not closely related to differences in oil concentration. However, the change in oil concentration due to reverse selection for 42 generations in RLO shows about the same magnitude of response as 48 generations of forward selection in ILO (Fig. 1). Similarly the magnitude of response due to 42 generations of reverse selection in RHO is comparable to the response from 48 generations of forward selection in IHO. Therefore, the changes in oil concentration in the two reverse strains may be biologically similar and the corresponding

**Table 5.** Location of RFLP loci associated with a possible transition in RFLP variant frequencies in RHO and RLO strains

| RHO | | RLO | |
|---|---|---|---|
| Chromosome location | RFLP locus | Chromosome location | RFLP locus |
| 1S | umc 157 | 1S | php 9234 |
| 2S | php 9421 | 1L | php 9447 |
| 3L | bnl 10.24 | 2S | php 9239 |
| | | 3CE | umc 10 |
| | | 3L | bnl 10.24 |
| | | 4L | php 9451 |
| | | 5S | umc 147 |
| | | 5L | umc 68 |
| | | 6L | php 9280 |
| | | 7S | php 9277 |
| | | 9L | php 9209 |

RFLP genotypic frequency differences might be related to these changes.

The comparison of RLO vs RHO detected only three loci with RFLP genotypic frequencies that were not significantly different. The extent of the genotypic differences observed between RLO and RHO is noteworthy since these strains are similar in oil concentration. This suggests that different sets of genes or alleles controlling oil concentration may be under selection in RLO versus RHO. Some loci may have been fixed in IHO and ILO during forward selection and consequently genetic variation at these loci was not present for reverse selection to act upon in RHO and RLO, resulting in reverse selection influencing other RFLP loci. Genetic drift effects may also have contributed to the genotypic differences between RLO and RHO since these two strains have been isolated for 90 generations.

The selection of probes that only detect a polymorphism between IHO and ILO may have increased the probability of detecting significantly different genotypic frequencies between IHO and RLO and between ILO and RHO (and RHO vs RLO). These RFLP loci were therefore good candidates to look for possible effects of reverse selection resulting in directional changes in variant frequencies. There were RFLP variants at 11 loci which were the most frequent in the RLO and IHO strains only (Table 5). This suggests there may have been a transition in the frequency of these RFLP variants in RLO. The frequency of these RFLP alleles in RLO are suggestive of a response to reverse selection since these same RFLP variants were also the most frequent in IHO but not in ILO or RHO. There were RFLP variants at three loci which were the most frequent for RHO and ILO only, suggesting there may have been a transition in the frequency of these RFLP alleles in RHO. Only one RFLP locus (bnl 10.24) was associated with a possible transition of variant frequencies in both RHO and RLO. RFLP variants which exhibited a transition in frequency may have a higher probability of being linked to genes influencing oil concentration. Noteworthy is the fact that chromosomal regions important in the control of oil concentration in which associated RFLP loci were fixed before reverse selection was imposed do not have the genetic variability necessary for a transition in allelic frequencies to occur.

Probe umc 10 was identified in RLO as possibly identifying a chromosomal region associated with selection for higher oil concentration (Table 5). The umc 10 RFLP locus is near the chromosome 3 centromere region and is approximately 5 cM from the *Pgd2* isozyme locus and approximately 12 cM from the *Est4* isozyme locus that Kahler (1985) associated with selection for increased oil concentration in the Alexho population. These findings support a hypothesis that a gene(s) influencing oil concentration may be present near the centromere region of chromosome 3.

Comparisons with common RFLP loci evaluated in a QTL mapping study involving a cross of Illinois High Protein × Illinois Low Protein indicate that three RFLP loci (php 9447, umc 10, php 9280) associated with putative QTLs for oil concentration (Goldman et al. 1992) were also associated with a possible response to reverse selection for oil concentration (Table 5). These findings, notably for umc 10, provide evidence of how RFLP population genetic studies and QTL mapping studies may provide complementary and supportive data towards the identification of chromosomal regions influencing oil concentration in maize. The other RFLP loci associated with a possible transition in RFLP variant frequencies in response to reverse selection for oil concentration are under evaluation for associations with QTLs controlling oil concentration in a study involving a cross of Illinois High oil × Illinois Low oil.

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