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QTL Mapping in Tropical Maize: III. Genomic Regions for Resistance to *Diatraea* spp. and Associated Traits in Two RIL Populations

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ABSTRACT

The southwestern corn borer (SWCB, *Diatraea grandiosella* Dyar) and sugarcane borer (SCB, *Diatraea saccharalis* Fabricius) are two related insect species that cause serious damage in maize production in subtropical and tropical regions of Central and Latin America. We analyzed quantitative trait loci (QTL) involved in resistance to the first generation of both borer species in two recombinant inbred line (RIL) populations from crosses CML131 (susceptible) × CML67 (resistant) and Ki3 (susceptible) × CML139 (resistant). Resistance was evaluated as leaf feeding damage (LFD) in replicated field trials across several environments under artificial infestation. Leaf protein concentration and leaf toughness were evaluated in one environment as putative components of resistance. The method of composite interval mapping was employed for QTL detection with RFLP linkage maps derived for each population of RIL. Estimates of the genotypic and genotype × environment interaction variances for SWCB LFD and SCB LFD were highly significant in both populations. Heritabilities ranged from 0.50 to 0.75. In Population CML131 × CML67, nine and eight mostly identical QTL were found for SWCB LFD and SCB LFD, respectively, explaining about 52% of the phenotypic variance ($\hat{\sigma}^2_p$) for each trait. In Population Ki3 × CML139, five QTL for SWCB LFD were detected, explaining 35.5% of $\hat{\sigma}^2_p$. Several of these QTL were found in regions containing QTL for leaf protein concentration or leaf toughness. A low number of QTL in common between the two RIL populations and between RIL and corresponding populations of $F_{2:3}$ indicated that the detection of QTL depended highly on the germplasm and population type. Consequently, chances of successful application of marker-based selection (MBS) for corn borer resistance are reduced when QTL are not identified in the germplasm in which the final selection will be carried out.

THE RELATED insect species southwestern corn borer and sugarcane borer are among the most important lepidopteran pests affecting maize production in Central and South America. First generation larvae of both insects affect plant growth by leaf feeding during the whorl stage, causing direct yield losses. Later generations damage the plant mainly by stalk boring and tunneling, resulting in indirect yield losses as a consequence of lodging. Breeding for multiple borer resistance (MBR) has been a major research objective at the International Maize and Wheat Improvement Center (CIMMYT). A MBR population has been developed with Caribbean germplasm being the primary source of resistance (Smith et al., 1989). Resistance in this material was found to be quantitative with mainly additive gene action (Hinderliter, 1983; Thome et al., 1992).

Mechanisms involved in resistance against SWCB and SCB include non-preference, antibiosis, and tolerance (Davis et al., 1989; Kumar and Mihm, 1995; Williams and Davis, 1987). Most breeding programs have concentrated on the antibiosis type of resistance by assessing the degree of leaf feeding damage under artificial infestation. Resistant maize lines were found to have higher physical strength of the cell wall because of higher concentrations of crude fiber and phenolic acid, while susceptible lines contained higher levels of nutritional components such as crude protein, lipid, and total sugar content (Bergvinson, 1993; Hedin et al., 1984).

The mapping of quantitative trait loci (QTL) by means of molecular markers such as RFLPs allows the detection, localization, and characterization of genetic factors contributing to the variation of a polygenically inherited trait. QTL have been mapped in populations of $F_{2:3}$ lines in U.S. maize for resistance to the second generation European corn borer (Schön et al., 1993) and in tropical maize to the first generation SWCB and SCB (Bohn et al., 1996, 1997). Several QTL have been found for corn borer resistance in tropical maize with mostly additive or partial dominant gene effects, confirming the assumptions of quantitative resistance with mainly additive gene action. Comparison of these QTL with results from a second population of $F_{2:3}$ lines analyzed at CIMMYT (Khairallah et al., 1997) revealed a poor consistency across different germplasm (Bohn et al., 1997).

Most genetic studies employed populations of $F_{2:3}$ lines or backcross progenies for the identification of QTL as a first step for marker-based selection (MBS). Recombinant inbred line (RIL) populations developed from F_2 populations by single seed descent have been recommended as an alternative population type for QTL mapping (Burr et al., 1988). They are expected to have an increased power of QTL detection because of almost complete homozygosity at QTL and marker loci (Moreno-Gonzalez, 1993). Furthermore, RIL should allow a better resolution of linked QTL because of additional recombination during line development. RIL have been used for QTL mapping in maize for several traits, including yield and yield components (Austin and Lee, 1996). These authors found a greater number of QTL, including those with smaller effects, for RIL than

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Abbreviations: CIM, composite interval mapping; CIMMYT, International Maize and Wheat Improvement Center; cM, centimorgan; CML, CIMMYT maize line; LFD, leaf damage rating; LOD, log odds ratio; LR, likelihood ratio; LT, leaf toughness; MBS, marker-based selection; MBR, multiple borer resistance; PC, protein concentration; QTL, quantitative trait locus (or loci); *RE*, relative efficiency; RIL, recombinant inbred line (or lines); SCB, sugarcane borer; SWCB, southwestern corn borer.

for $F_{2:3}$ lines. Comparisons of QTL results from different generations are not only important for evaluating the efficiency of QTL mapping, but are also of great interest with respect to MBS. Only if QTL determined in early generations can largely be recovered in later generations, can they be used effectively for MBS during the process of line development.

In this study, we mapped QTL for resistance against the first generation SWCB and SCB using two RIL populations derived from the same crosses as two previously mapped populations of $F_{2:3}$ lines (Bohn et al., 1996, 1997; Khairallah et al., 1997). Our objectives were to (i) detect and characterize QTL responsible for leaf feeding resistance to first generation SWCB and SCB in these two RIL populations, (ii) determine the presence of common QTL for resistance to both insects, (iii) investigate putative components of resistance and identify their underlying QTL, (iv) determine the consistency of QTL for leaf feeding resistance and resistance components across both RIL populations, and (v) compare the QTL obtained for the RIL with those previously mapped by using $F_{2:3}$ lines from the same two crosses.

MATERIALS AND METHODS

Mapping Populations

Four maize inbred lines were selected as parents to produce two RIL populations. CML131, a highly susceptible, subtropical white dent line out of CIMMYT's population 42, was crossed to CML67, a highly resistant, tropical red-yellow semident line from Antigua Group 2 to develop the first population. For the second population, Ki3, a tropical yellow flint line from Suwan 1 with susceptibility to corn borers, was crossed to CML139, a resistant, subtropical yellow semi-flint line selected out of Dominican Republic Group 1 and Antigua Group 2. A total of 215 $F_{2:3}$ lines from cross CML131 × CML67 and 475 $F_{2:3}$ lines from the cross Ki3 × CML139 have been used for QTL mapping in previous studies (Bohn et al., 1996, 1997; Khairallah et al., 1997). RIL were developed by selfing a random subsample of F_2 plants by single seed descent until the generation of $F_{6:7}$ lines in CML131 × CML67 and $F_{7:8}$ lines in Ki3 × CML139. Each line was then sib-mated for seed increase. Population size was 187 RIL for CML131 × CML67 and 158 RIL for Ki3 × CML139.

RFLP Assays

Leaf samples were bulked, lyophilized, and ground to a fine powder from 10 field grown plants per RIL to determine the RFLP genotype of F_6 plants in Population CML131 × CML67 and F_7 plants in Population Ki3 × CML139. Otherwise, we followed the same procedures for DNA extractions and RFLP assays as described by Bohn et al. (1996). Maize probes were chosen from collections of the University of Missouri (UMC), California State University (CSU), Brookhaven National Laboratory (BNL), and Native Plants Inc. (NPI); probes from other species (CDO, RZ) were obtained from the Cornell University core set. A total of 108 probes detecting single or low copy sequences were used in Population CML131 × CML67 and 122 probes were employed in Population Ki3 × CML139.

Field Trials

Population CML131 × CML67 was tested in four successive seasons from the summer season of 1994 (1994B, June through October) to the winter season of 1996 (1996A, December through May) at CIMMYT's experimental station in Tlaltizapán, Morelos, Mexico (18°N lat, 940 m elevation). A total of 198 entries, including 183 RIL, CML131 and Ki3 as susceptible, and CML67 and CML139 as resistant checks, were grown in a 18 by 11 alpha lattice design with two replicates. Rows were 2.5 m long with 10 plants and 75 cm between rows. Resistance to SWCB was evaluated during three seasons (1994B, 1995A, 1995B), and resistance to SCB was measured during two seasons (1995B, 1996A). In 1994B and 1995A, plots consisted of two rows with one under SWCB infestation and one under insecticide protection to allow for comparisons between infested and protected plants. In the 1995B trial, plots consisted of three rows, with one under SWCB infestation, one under SCB infestation, and one under insecticide protection. In 1996A, only SCB resistance was evaluated in single-row plots.

Population Ki3 × CML139 was evaluated for SWCB resistance during the summer season 1994B and winter season 1995A. We used a 15 by 11 alpha lattice design with two replicates that included 158 RIL and the parental lines of both populations as checks with single-row plots under artificial SWCB infestation.

For both insects, resistance was measured as leaf feeding damage (LFD) under artificial infestation. All 10 plants per row were infested 4 to 5 wk after planting at the six- to seven-leaf stage with 25 to 30 neonate SWCB or SCB larvae per plant with a mechanical dispenser (Mihm, 1983). We used larvae from insects that were reared in CIMMYT's entomology laboratory. Three to 4 wk after infestation, two independent ratings at different dates were taken on each individual infested plant by means of a 1 (no visible damage) to 10 (dead plant) rating scale.

Leaf protein concentration was analyzed only in Population CML131 × CML67 from leaf samples collected in Tlaltizapán in the 1996A trial prior to infestation. Two leaf sections from the middle part of the third youngest leaf from five plants per plot were sampled, dried at 65°C for 3 d and ground to a fine powder. An automatic micro-Kjeldahl analyzer was used for nitrogen determination, and crude protein concentration ($g\ kg^{-1}$) was calculated by multiplying with the conversion factor 6.25.

Leaf toughness was evaluated in 1994 at the Plant Research Centre of Agriculture Canada, Ottawa, ON. Twenty plants of each RIL of both populations and the parental lines were planted in the field in single-row plots. Because of germination and growth problems of the unadapted tropical and subtropical materials, only 145 RIL per population could be used for the evaluation. Germination for parental lines CML131 and CML67 was very poor, therefore, only Ki3 and CML139 could be evaluated. Leaf toughness readings were taken before tasseling from the second fully exposed leaf from the top. Two sections per leaf were sampled from 15 plants per plot and kept wet until analyzed. A standard instron technique (Model TM-M, Instron, Canton, MA) was used for readings of the peak force (in Newton, N) required to penetrate the lower epidermis of the leaves (Bergvinson et al., 1994).

Data Analysis

Plot means of LFD were calculated from the ratings of individual plants and averaged across both ratings for further computations. Analyses of variance were performed for each trial. Each year-season combination was considered as one environment. Adjusted entry means and effective error mean squares were used to compute combined analyses of variance across environments. The assumption of homogeneity of the error variances was tested with a modified Levene test (Brown and Forsythe, 1974). Estimates of the genotypic variance

(σ_g^2) and genotype × environment interaction variance (σ_{ge}^2) in the case of multiple environments, estimates of error variance (σ_e^2) and phenotypic variance (σ_p^2) as well as heritabilities (h^2) and their exact 90% confidence intervals were calculated as described in detail by Bohn et al. (1996). Phenotypic correlation coefficients ($\hat{r}_p$) were calculated from adjusted entry means across environments for LFD, adjusted entry means for protein concentration, and single-plot means for leaf toughness. T-tests [t_1 = (RIL − high parent)/2(MQ_{GxE}/i), t_2 − (RIL − low parent)/2(MQ_{GxE}/i)] were used on adjusted entry means across environments for testing the significance of transgressive segregation for LFD and protein concentration. Because multiple tests were performed (corresponding to the number of RIL), appropriate Type I error rates were determined by the sequentially rejective Bonferroni procedure described by Holm (1979).

A χ^2 analysis was performed for each RFLP marker locus to test for deviations from the expected gene frequencies of 0.5. Because multiple tests were performed for each population, corresponding to the number of RFLP markers assayed, appropriate Type I error rates were determined by the sequentially rejective Bonferroni procedure. The range and average level of heterozygosity at codominant markers was calculated for all RIL. The proportion of the genome from the resistant parent was determined for each RIL in both populations by dividing the sum of all marker alleles from the resistant parent by twice the number of scorable marker loci in the respective RIL. Correlations were calculated between the percentage of CML67 and CML139 genome and LFD.

Linkage maps were constructed from 187 RIL and 136 marker loci for Population CML131 × CML67 and from 143 RIL and 146 marker loci for Population Ki3 × CML139, applying MAPMAKER with the 'RI self' setting (Lander et al., 1987). By this setting, the recombination fraction observed in the RIL (R) is related to the proportion of recombinants in a single meiosis (r) by the equation $r = R/2(1-R)$ (Haldane and Waddington, 1931). A LOD ($\log_{10}$ of the likelihood odds ratio) value of 3.0 was used as critical threshold to declare linkage between two markers. Recombination frequencies between adjacent markers were estimated by multi-point analyses and transformed into centimorgan (cM) by Haldane's mapping function. A combined linkage map was constructed from the merged data set of both RIL populations to allow for a comparison of the position of QTL between them.

QTL Mapping

QTL analyses were performed on a subset of 170 RIL in Population CML131 × CML67 and 135 RIL in Population Ki3 × CML139 for which both phenotypic and molecular data were available. QTL mapping for leaf toughness in Population CML131 × CML67 was based on a subset of only 145 RIL. The composite interval mapping procedures of Zeng (1994) and Jiang and Zeng (1995) were used to combine data based on adjusted entry means from each environment in a joint analysis for LFD. Leaf toughness and protein concentration were analyzed with (adjusted) entry means from one environment only. A special software developed by C. Jiang (1996, personal communication) was used for computations.

Model. The statistical model for the QTL analysis was

$$y_{ij} = b_i + b_i^* x_j^* + \sum_k b_{ik} x_{jk} + e_{ij},$$

where y_{ij} = the phenotypic value of RIL j in Environment i; b_i = the mean phenotypic value of RIL with Genotype qq at the putative QTL and mm at the markers used as cofactors in Environment i; b_i^* = the additive effect of a putative QTL in Environment i; x_j^* = the number of alleles from the resistant parent at the putative QTL, taking Values 0, 1, and 2 with probabilities depending on the genotype at the flanking markers in the interval under search (1 only if RIL were heterozygous); b_{ik}= the partial regression coefficient of the phenotype on the Marker k; x_{jk} = number of alleles from the resistant parent at the selected Marker k; and e_{ij} = the residual variable of RIL j in Environment i.

The analysis was performed by applying three different models (Zeng, 1994). Model III, which corresponds to simple interval mapping (Lander and Botstein, 1989) extended to the analysis of multiple environments, was used for the selection of cofactors. Putative QTL were identified in the joint analyses across environments and markers closely linked to these QTL were selected as cofactors. When several putative QTL were detected on a chromosome, only the marker near the highest peak was chosen as cofactor. Model II was subsequently fitted with the selected markers as cofactors as long they were unlinked to the genomic regions under search. Finally, Model I was used to confirm the QTL detected with Model II by including markers as cofactors linked to the tested regions in addition to the preselected ones. Model I was applied in two separate analyses with markers selected as cofactors flanking the target region at a minimal distance (window size) of 30 and 20 cM. The closest markers at both sides outside the chosen distance from the target region were employed as cofactors.

Hypotheses. The following hypotheses were tested: (1) QTL detection (H_0: $b_i^* = 0$ for all i vs. H_1: $b_i^* \neq 0$ for at least one i); (2) QTL-by-environment (QTL × E) interactions (H_0: $b_i^* = b^*$ for all i vs. H_1: $b_i^* \neq b^*$ for at least one i).

Thresholds. The threshold used for QTL detection with data from one environment was set to a likelihood ratio (LR) of 11.5 (equivalent to LOD = 2.5). This critical value is equivalent to a significance level $\alpha' = 0.0032$ in a distribution with df = 2 in the analysis of one trait (or environment) for a single interval. To keep the same level of significance in a joint analysis across two or three environments, the degrees of freedom changed to 3 and 4, and the corresponding values of the LR were 13.8 and 15.9, respectively (LOD = 3.00 and 3.45). The same thresholds were employed in Model III for the selection of markers used as cofactors. QTL × E interactions were tested with a significance level of 0.05 and 0.01, because tests were only performed at positions where a QTL was detected. The corresponding LR thresholds for QTL × E interactions were 3.8 ($P < 0.05$) and 6.6 ($P < 0.01$) for two environments, and 6.0 ($P < 0.05$) and 9.2 ($P < 0.01$) for three environments.

QTL Detection. Presence of a QTL was declared when the LR exceeded the threshold in Model II and a peak was also detected in Model I with both window sizes. If the LR was significant only under Model I but a peak could not be detected in Model II (e.g., because of linked QTL), a QTL was also declared to be present. Two peaks for the same trait on one chromosome were accepted as two different QTL, when they were separated by at least two markers and a minimum distance of 20 cM. Otherwise, the higher peak was chosen to represent the QTL.

QTL Effects. The additive effect of a QTL for data from one environment ($\hat{b}_i^*$) was obtained under the assumption of Model I (window size 30 cM). For traits with multiple environments, an overall additive effect ($\hat{b}^*$) was estimated by mean values across environments. The phenotypic variance explained by QTL k was calculated from its estimated effect as $R_k^2 = b^{*2}/\hat{\sigma}_p^2$ across environments. The proportion of σ_p^2 explained by all QTL (R^2) was obtained from a multiple regression of the phenotypic values from one environment or overall means across environments on markers closely linked to all detected QTL. The proportion of σ_g^2 explained by all QTL was calculated as $Q^2 = R^2/\hat{h}^2$. The presence of digenic epistatic interactions between the detected QTL was tested applying

the regression approach of Haley and Knott (1992) based on stepwise regression adding epistatic effects to the main effects in the model as described by Lübberstedt et al. (1997).

Comparison of QTL. QTL for different traits within a population were declared as "common" QTL when they were located within the same 20 cM interval. Comparisons between QTL across the two populations were performed with the combined linkage map with the common marker loci as reference points to locate the QTL. If two QTL were separated at their highest peak by more than 20 cM, they were not considered as common QTL. The same criterion was used for comparing the QTL in RIL populations and their corresponding populations of $F_{2:3}$ lines. However, these comparisons were approximate, because linkage maps in different generations had different interval lengths and testing for common QTL was based on the linkage maps from RIL populations only.

RESULTS

Segregation and Linkage of RFLPs

The linkage map for the RIL of Population CML131 × CML67 with 136 markers had a total length of 1564 cM and an average interval length of 11.5 cM. The linkage map for the RIL of Population Ki3 × CML139 with 146 marker loci had a total length of 2117 cM and an average spacing of 14.4 cM between markers. Both linkage maps are available from the internet (http://www.cimmyt.mx), or upon request from the corresponding author. The linear order of RFLP markers in both RIL populations was consistent with the linkage maps obtained for the corresponding F_2 populations except for some minor inversions of adjacent markers. In total, 23 loci in CML131 × CML67 and 17 in Ki3 × CML139 had to be scored as dominant markers. A few markers (*bnl6.06*, *bnl7.13*, *cdo202*, *csu11*, *npi386*, and *umc146*) did not show the same RFLP pattern in the RIL as in a previous screening of the parents or in the F_2 populations and mapped to unpredicted positions. Nevertheless, these markers were used for map construction and QTL detection in order to avoid larger gaps between loci even though contamination or probe mix-up in the laboratory cannot be ruled out. In order to avoid confusion with other maps, cmt (for CIMMYT) was added to the locus name. Two RFLP loci (*bnl6.32*, *npi386*) in Population CML131 × CML67 and one (*csucmt11a*) in Population Ki3 × CML139 showed significant distortion from the expected gene frequencies of 0.5.

The proportion of CML67 genome in RIL of Population CML131 × CML67 ranged from 18.9 to 78.8% with a mean of 50.3%. By comparison, the proportion of CML139 genome in RIL of Population Ki3 × CML139 ranged from 30.8 to 70.4% with a mean of 50.5%. The average heterozygosity at codominant markers in CML131 × CML67 (3.5%) and Ki3 × CML139 (1.9%) was in close agreement with the theoretically expected proportion of 3.1% for F_6 plants and 1.6% for F_7 plants, calculated as 0.5^n, where n is the number of selfing generations.

Phenotypic Data

Temperatures, solar radiation, and rainfall were lower during the winter seasons than during the summer seasons. As a consequence, plant development until infestation (midwhorl stage) was delayed by one week in the winter seasons, and flowering was delayed by two to three weeks. The winter season 1996A was cooler than average and resulted in a high mortality rate of SCB larvae. As a consequence, the infestation level in 1996A in Population CML131 × CML67 was lower in comparison to 1996B, but the range and ranking of the RIL were similar in both seasons. For SWCB LFD, the three seasons were comparable in both populations for their infestation levels and the resulting LFD.

The parental lines of both crosses differed significantly ($P < 0.01$) from each other for all traits (Table 1). The resistant parents had lower LFD ratings, tougher leaves (data available only for parental lines Ki3 and CML139), and lower leaf protein concentration. Transgressive segregation was observed for protein concentration. The most resistant genotypes had SWCB LFD of 4.5 in Population Ki3 × CML139 and 4.2 in Population CML131 × CML67, which corresponds to small holes on only a few leaves. The most susceptible RIL had LFD around 7.8 in both populations, which corresponds to long lesions on the majority of the leaves. On the average, SCB LFD were lower, reflecting that SCB is a less aggressive feeder than SWCB in Tlaltizapán.

The assumption of homogeneity of the error variances across seasons was not met for SWCB LFD because of an error variance of only half the size in 1995B compared to 1994B and 1995A. As a consequence, in the combined analysis of variance across three seasons the F value for testing genotype × environment interactions and the heritability might be overestimated (Cochran and Cox, 1957).

Genotypic variances ($\hat{\sigma}^2_g$) were highly significant for all traits. Estimates of σ^2_{ge} were also significant for SWCB and SCB LFD but smaller than σ^2_g (Table 1). Heritabilities for SWCB LFD and SCB LFD across environments ranged from 0.50 to 0.75. Heritability for protein concentration in CML131 × CML67 measured in one environment was also relatively high (0.76). Variance components and heritabilities could not be calculated for leaf toughness, because a non-replicated trial was used.

Phenotypic correlations were significant but only of intermediate magnitude for most trait combinations in Population CML131 × CML67 (Table 2). The highest correlation was found between SWCB LFD and SCB LFD ($\hat{r}_p = 0.76$). Leaf toughness showed negative associations with SWCB LFD, SCB LFD, and protein concentration. In Population Ki3 × CML139, the correlation between SWCB LFD and leaf toughness was significant but low. The percentage of CML67 genome in RIL of Population CML131 × CML67 had a significant negative correlation with SWCB LFD ($\hat{r}_p = -0.57$) and SCB LFD ($\hat{r}_p = -0.56$). The percentage of CML139 genome in RIL of Population Ki3 × CML139 showed a lower correlation with SWCB LFD ($\hat{r}_p = -0.38$).

QTL Analyses

SWCB Leaf Feeding Damage

In RIL of Population CML131 × CML67, nine QTL located on Chromosomes 1 (4 QTL), 5, 7, 8 (2 QTL),

Table 1. Means of susceptible (S) and resistant (R) parents (P), midparent ($\bar{P}$), and RIL populations from crosses CML131 × CML67 and Ki3 × CML139, range of RIL populations, and estimates of variance components and heritabilities for SWCB and SCB leaf feeding damage (LFD), protein concentration (PC), and leaf toughness (LT).

Parameter	CML131 × CML67 (n = 183, 145†)				Ki3 × CML139 (n = 158, 145†)	
	SWCB LFD	SCB LFD	PC	LT	SWCB LFD	LT
	1–10 scale		g kg^{-1}	N‡	1–10 scale	N
Means§						
P(S)	7.3 ± 0.30	6.0 ± 0.23	206 ± 0.85	-¶	7.3 ± 0.28	0.59
P(R)	4.7 ± 0.36	3.3 ± 0.24	178 ± 0.93	-	4.8 ± 0.28	0.70
$\bar{P}$	6.0 ± 0.27	4.7 ± 0.16	192 ± 0.63	-	6.1 ± 0.19	0.65
RIL	6.3 ± 0.04	4.9 ± 0.05	191 ± 0.30	0.65 ± 0.004	5.9 ± 0.04	0.70 ± 0.005
Range	4.2–7.8	2.9–6.6	153–224	0.50–0.81	4.5–7.9	0.54–0.85
σ_g^2	0.22 ± 0.033**	0.36 ± 0.051**	13.8 ± 1.9**	-	0.11 ± 0.028**	-
σ_{gc}^2	0.06 ± 0.023**	0.08 ± 0.027**	-	-	0.09 ± 0.028**	-
h^2	0.72	0.75	0.76	-	0.50	-
90% C.I. of h^2	(0.65, 0.77)	(0.69, 0.81)	(0.71, 0.82)	-	(0.31, 0.63)	-

** Variance component was significant at the 0.01 probability level.
† Number of RILs evaluated for LT was 145.
‡ N = Newton.
§ Standard errors are attached.
¶ Data not available.

and 9 were detected in the joint analysis across three environments, with five unlinked markers as cofactors (Table 3). The first QTL located on Chromosome 1 was only found in 1995B, while all other QTL were detected in at least two environments (results for individual environments not shown). Four QTL showed significant QTL × E interactions. The QTL on Chromosome 9 was detected in all environments but estimated gene effects differed in their magnitude, resulting in significant QTL × E interactions. The most important regions with highest values and consistently expressed across environments were detected on Chromosomes 7, 8, and 9. For all QTL, the alleles from the resistant parent CML67 contributed to reduced LFD. The proportion $\hat{\sigma}_p^2$ and $\hat{\sigma}_g^2$ explained by all QTL in a simultaneous fit was 52.4 and 72.8%, respectively.

In Population Ki3 × CML139, five QTL were detected on Chromosomes 1, 6, 8, and 9 (2 QTL) with the aid of four cofactors (Table 4). The two QTL on Chromosomes 1 and 6 showed significant QTL × E interactions and were detected in only one environment each, while the QTL on Chromosomes 8 and 9 were stable across both environments. For all but one QTL on Chromosome 6, the resistance alleles originated from the resistant parent CML139. A simultaneous fit with all five QTL explained 35.5% of $\hat{\sigma}_p^2$ and 57.3% of $\hat{\sigma}_g^2$.

SCB Leaf Feeding Damage

In Population CML131 × CML67, eight QTL were detected on Chromosomes 1 (3 QTL), 5, 7, 8 (2 QTL), and 9 with the same five markers used as cofactors as for SWCB LFD (Table 3). The QTL on Chromosome 9 represented the most important region, explaining 25.8% of $\hat{\sigma}_p^2$. Only the two QTL on Chromosome 8 showed significant QTL × E interaction; all other QTL were consistent across environments. All alleles contributing to reduced LFD originated from the resistant parent and explained 52.8% of $\hat{\sigma}_p^2$ and 70.4% of $\hat{\sigma}_g^2$ in a simultaneous fit.

Table 2. Phenotypic correlations between SWCB and SCB leaf feeding damage (LFD), protein concentration (PC), and leaf toughness (LT) for RIL Populations CML131 × CML67 and Ki3 × CML139.

Population	Trait	LT	SWCB LFD	SCB LFD
CML131 × CML67	SWCB LFD	−0.47**		
	SCB LFD	−0.46**	0.76**	
	PC	−0.39**	0.43**	0.47**
Ki3 × CML139	SWCB LFD	−0.24**		

** Significant at the 0.01 probability level.

Leaf Protein Concentration

In CML131 × CML67, five QTL were found on Chromosomes 1 (2 QTL), 5, 8, and 9. The first QTL on Chromosome 1 and the QTL on Chromosome 9 had the largest effects, each explaining more than 15% of $\hat{\sigma}_p^2$. For all QTL, the allele reducing protein concentration came from the resistant parent. A simultaneous fit with all five QTL explained 38.5% of $\hat{\sigma}_p^2$ and 50.7% of $\hat{\sigma}_g^2$.

Leaf Toughness

A total of five QTL on Chromosomes 1 (2 QTL), 4, 7, and 8 were detected for leaf toughness in Population CML131 × CML67. The most important QTL were located on Chromosome 1 (2 QTL), and 4, each explaining more than 11% of $\hat{\sigma}_p^2$. At four QTL the allele from the resistant parent increased leaf toughness, while the reverse was true for the QTL on Chromosome 4. A simultaneous fit with all five QTL explained 39.0% of $\hat{\sigma}_p^2$.

In Population Ki3 × CML139, only two QTL were detected on Chromosomes 5 and 8, each explaining about 14% of $\hat{\sigma}_p^2$. In both cases, the allele from the resistant parent increased leaf toughness. A simultaneous fit with both QTL explained 23.9% of $\hat{\sigma}_p^2$.

Epistatic Effects

No significant ($P < 0.05$) digenic epistatic effects were found between the detected QTL for all traits in both populations of RIL.

Table 3. Parameters associated with QTL for SWCB and SCB leaf feeding damage (LFD), protein concentration (PC), and leaf toughness (LT), estimated from phenotypic data of 170 RIL (145 for LT) for Population CML131 × CML67.

Trait	Chromosome	QTL position	Marker interval	Likelihood ratio (LR)†	QTL effect‡	LR for QTL × E interactions	Phenotypic variance explained
SWCB LFD		cM			1–10 scale		%
(E = 3, R = 2)§	1	14	*npi97a-umc157*	22.0	0.09	12.7**	3.2
	1	91	*npi286-csu95c*	18.8	0.12	3.1	5.5
	1	123	*csu92-csucmt11a*	37.8	0.12	10.0**	5.3
	1	192	*umc72b-npi97c*	25.9	0.13	3.4	5.7
	5	109	*bnl5.40-umc51a*	28.1	0.10	5.3	3.6
	7	36	*bnl15.21-umc110a*	31.1	0.17	2.5	10.3
	8	95	*cdo580a-csu31*	47.1	0.20	4.8*	14.0
	8	138	*umc150a-csu38b*	21.6	0.12	8.6*	5.4
	9	50	*csu158-csu147*	39.5	0.20	10.0**	13.7
	Total¶						52.4
SCB LFD							
(E = 2, R = 2)	1	90	*npi286-csu95c*	27.3	0.20	0.1	9.2
	1	125	*csu92-csucmt11a*	28.3	0.14	3.8	4.5
	1	207	*bnl8.29a-bn16.32*	14.4	0.10	3.0	2.3
	5	111	*bnl5.40-umc51a*	24.5	0.19	2.5	7.8
	7	49	*umc110a-csu36d*	20.4	0.16	0.0	6.0
	8	94	*cdo580a-csu31*	30.6	0.16	11.0**	6.1
	8	136	*umc150a-csu38b*	15.0	0.10	7.4*	2.1
	9	61	*csu147-bnlcmt6.06a*	62.8	0.34	0.8	25.8
	Total						52.8
PC					g kg^{-1}		
(E = 1, R = 2)	1	121	*csu91-csucmt11a*	34.4	5.1	–	15.7
	1	208	*bnl8.29a-bnl6.32*	21.0	3.3	–	6.6
	5	125	*umc51a-umc127*	21.2	4.0	–	9.6
	8	79	*csu75d-cdo580a*	23.3	4.7	–	13.3
	9	51	*csu158-csu147*	16.5	5.4	–	17.6
	Total						38.5
LT					N¶		
(E = 1, R = 1)	1	129	*csu92-csucmt11a*	23.9	−0.019	–	13.2
	1	155	*umc83a-umc49c*	29.7	−0.018	–	11.9
	4	60	*umc31a-umc49d*	15.2	0.018	–	11.9
	7	94	*bnl14.07-umc151*	12.1	−0.012	–	5.3
	8	121	*umc150a-csu38b*	14.5	−0.013	–	6.2
	Total						39.0

*,** QTL × E interactions were significant at the 0.05 and 0.01 probability levels, respectively.
† Likelihood ratio was estimated under Model II using unlinked markers as cofactors (SWCB, SCB: *csu92*, *bnl5.40*, bnl15.21, *cdo580a*, *csu147*; PC: *csu92*, *umc51a*, *csu75d*, *csu147*; LT: *umc83a*, *umc49d*, *umc151*, *csu38b*).
‡ QTL effects were estimated in Model I; a positive value implies that the allele from the susceptible parent increases the numeric value of the trait.
§ E = number of environments, R = number of replicates per environment.
¶ Estimates were obtained from a simultaneous fit of all putative QTL affecting the trait.
¶ N = Newton.

Table 4. Parameters associated with QTL for SWCB leaf feeding damage (LFD) and leaf toughness (LT), estimated from phenotypic data of 135 RIL for Population Ki3 × CML139.

Trait	Chromosome	QTL position	Marker interval	Likelihood ratio (LR)†	QTL effect‡	LR for QTL × E interactions	Phenotypic variance explained
SWCB LFD		cM			1–10 scale		%
(E = 2, R = 2)§	1	87	*npi286-npi262*	15.4	0.14	5.3*	8.9
	6	106	*umc38a-umc140c*	17.0	−0.08	12.2**	2.6
	8	184	*umc150a-csu165a*	13.9	0.11	2.4	5.4
	9	96	*csu56d-umc95*	25.3¶	0.18	1.5	14.6
	9	132	*csu59-csu93a*	13.8¶	0.16	1.0	10.4
	Total						35.5
LT					N††		
(E = 1, R = 1)	5	164	*umc126a-umc51a*	17.3	−0.021	–	13.8
	8	192	*umc150a-csu165a*	18.8	−0.022	–	14.7
	Total						23.9

*,** QTL × E interactions were significant at the 0.05 and 0.01 probability levels, respectively.
† Likelihood ratio was estimated under Model II using unlinked markers as cofactors (SWCB: *npi286*, *umc38a*, *umc150a*, *umc95*; LT: *csu26a*, *umc30a*).
‡ QTL effects were estimated in Model I; a positive value implies that the allele from the susceptible parent increases the numeric value of the trait.
§ E = number of environments, R = number of replicates per environment.
¶ Likelihood ratio estimated in Model I (window size 30 cM).
# Estimates were obtained from a simultaneous fit of all putative QTL affecting the trait.
†† N = Newton.

DISCUSSION

QTL Detection Method

A major problem in QTL mapping is the detection of linked QTL. Under Model I, markers linked to a QTL used as cofactors make the test statistic inside the window independent of linked QTL outside the window and thus may allow the resolution of a single QTL detected under Model II into two QTL in coupling phase linkage. Furthermore, two QTL linked in repulsion phase may be detected only under Model I but remain undetected under Model II because their effects cancel each other. In the present study, the second QTL for SWCB LFD on Chromosome 9 in Population Ki3 × CML139 was detected under Model I but not under Model II. When only unlinked markers as cofactors were included in the model, a "ghost" QTL (Martinez and Curnow, 1992) was detected at position 107 cM. With the use of linked markers (window size 30 cM), this QTL shifted to 96 cM and a second QTL at 132 cM became significant. In Population CML131 × CML67, we also detected a large region affecting SWCB LFD and SCB LFD on Chromosome 9, which seems to contain two linked QTL, but we were not able to resolve this region into multiple QTL.

Model I also has the advantage that it reduces the bias in the estimates of QTL effects caused by linked QTL. However, its statistical power of QTL detection is smaller than in Model II (Zeng, 1994). In the present study, the estimated QTL effects for SWCB LFD and SCB LFD were generally greater under Model II than under Model I because of QTL in coupling phase linkage detected on several chromosomes. This should be considered when comparing our values with those from other studies employing different statistical models.

With the joint CIM method of Jiang and Zeng (1995), a QTL is not only detected when it is consistently expressed across environments but also when it shows a pronounced effect in only one environment. In the latter case, the values of a QTL can be small, because environments in which the QTL have no effect decrease the overall mean. As a result, we detected QTL explaining as little as 2.1% of $\hat{\sigma}^2_p$, whereas in a previous study, Bohn et al. (1996) revealed only QTL with larger effects employing a QTL detection method based on means across environments. Using the same approach as ours, Bohn et al. (1997) detected QTL with similar small effects.

Our criteria to declare QTL for different traits or different populations as "common" was their presence within the same 20 cM interval. This conservative rule was employed in order to avoid too many positive results that would be obtained if the decision was based on the large confidence intervals of QTL positions (Mangin et al., 1994).

The control of Type I error in a genome-wide search for QTL remains to be a problem because no information is yet available on the distribution of the test statistic under the null hypothesis. In this study, a LR threshold of 11.5 was employed which corresponds to a genome-wise significance level of $\alpha \approx 0.45$, assuming independency of tests in different intervals applying the Bonferroni correction. However, the total number of independent tests is lower than supposed in the Bonferroni correction because of correlations between tests caused by small marker intervals with rare recombination and the inability to separate linked QTL. Therefore, the actual genome-wise significance level in the present study can be expected to be lower than $\alpha = 0.45$. In contrast to this parametric approach, a permutation-based method for estimating empirical thresholds for experimental data was recently proposed (Churchill and Doerge, 1994; Doerge and Churchill, 1996). However, the application of this method to CIM requires further research and development of appropriate software.

QTL × E Interactions

Less than half of the QTL detected for SWCB LFD displayed significant QTL × E interactions in the joint analysis across three environments in Population CML131 × CML67 and two environments in Population Ki3 × CML139. For SCB LFD, only two out of eight QTL showed significant QTL × E interactions across two environments in Population CML131 × CML67. These results were in good agreement with estimates of $\hat{\sigma}^2_{ge}$ for the corresponding traits and populations. In contrast, Bohn et al. (1997) found fewer QTL with significant QTL × E interactions even though estimates of $\hat{\sigma}^2_{ge}$ were greater than in our study.

Environments were represented by different seasons because only one location was used for the field trials. Changes in climatic conditions between the summer and winter seasons in Tlaltizapán affected plant growth as well as insect development. Low temperatures during the winter months delayed larval growth and seemed to affect their feeding behavior. Resistance mechanisms were probably also altered, because plant growth was affected by differences in temperature and solar radiation. These factors could explain the differences in the number and magnitude of QTL for SWCB LFD and SCB LFD detected in Population CML131 × CML67 in the winter and the summer season. The greatest number of QTL for SWCB LFD and SCB LFD was found in 1995B which had the highest repeatability of all seasons. This underlines that reliable phenotypic data are of crucial importance in QTL mapping studies.

In Population Ki3 × CML139, the QTL for SWCB LFD on Chromosome 6 showed the highest QTL × E interaction and was only detected in 1995A with a positive effect for resistance from the allele of the susceptible parent. Ki3 is a highly vigorous line and was less affected by the unfavorable climatic conditions during the winter season, suggesting that this QTL for insect resistance is actually a QTL region contributing to improved plant vigor.

Comparison across Traits

In Population CML131 × CML67, eight out of nine QTL for SWCB LFD and all eight QTL for SCB LFD mapped within common genomic regions (Fig. 1) and the phenotypic correlation between both traits was high. This is in good agreement with the results of Bohn et

al. (1997) for $F_{2:3}$ lines from the same cross, who found that seven out of 10 QTL had pleiotropic effects on both SWCB LFD and SCB LFD. As opposed to these authors, we did not test the hypothesis of pleiotropy versus linkage in our study, because the two traits were evaluated in different environments and the test for pleiotropy could be confounded by QTL × E interactions. Summarizing, the results from both studies suggest that antibiosis to both *Diatraea* spp. has largely the same genetic foundation, as was expected from their similar life cycle and feeding behavior. Therefore, improvement of resistance to one insect species should result in a high genetic gain for the other.

We investigated leaf protein concentration and leaf toughness as putative components of resistance because these two traits were closely associated with leaf feeding resistance to the European corn borer in CIMMYT's MBR germplasm (Bergvinson, 1993). Plant nitrogen is a major factor contributing to larval growth (Scriber and Slansky, 1981). Genotypes with low nitrogen levels

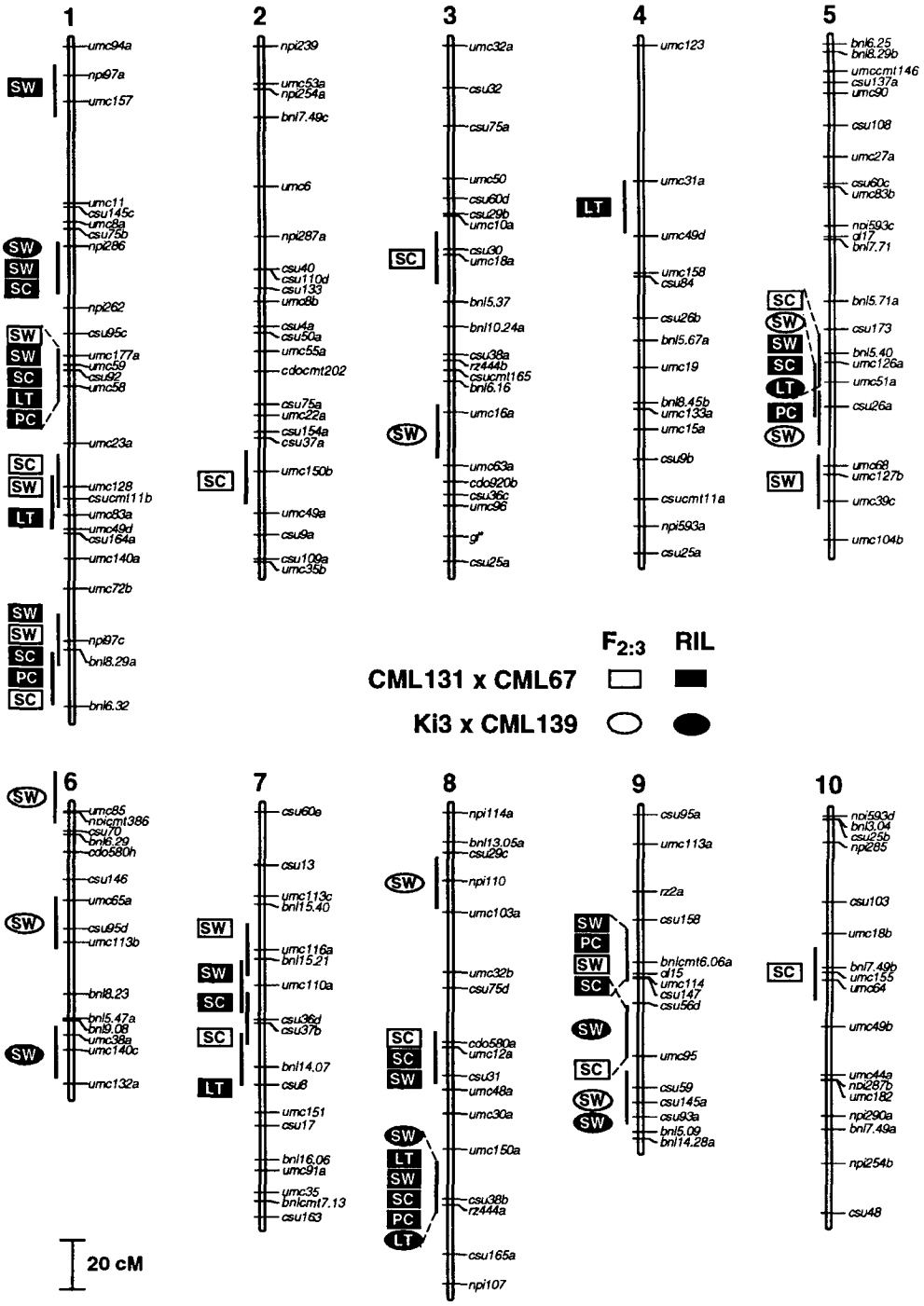

Fig. 1. Combined RFLP linkage map based on RIL from crosses CML131 × CML67 and Ki3 × CML139. Approximate QTL positions for RIL of leaf feeding damage by SWCB (SW) and SCB (SC), protein concentration (PC), and leaf toughness (LT), and relative positions of QTL detected in $F_{2:3}$ lines (Bohn el al., 1997; Khairallah et al., 1997) are shown within common 20 cM intervals indicated by bars.

may not provide sufficient protein for larval development. Bergvinson et al. (1994) also found higher levels of crude fiber and cell wall phenolic acids in resistant maize genotypes. They hypothesized that leaf toughness, caused by cell wall fortification through cross linking of hemicellulose by dehydrodiphenolic acids contributes to antibiosis by inhibiting neonate larvae to penetrate the tissue.

In the present study, correlations of SWCB LFD and SCB LFD with protein concentration and leaf toughness were only of intermediate magnitude in Population CML131 × CML67. Surprisingly, all five QTL for protein concentration were located at the same position or close to QTL for SWCB LFD and SCB LFD. For leaf toughness, two out of five QTL on Chromosomes 1 and 8 were in common with QTL for SWCB LFD and SCB LFD. This consistency suggests a common genetic basis of these traits, implying that QTL were the better indicator of genetic associations between traits than phenotypic correlations. In Population Ki3 × CML139, the correlation between SWCB LFD and leaf toughness was low, but of the two QTL for leaf toughness and five QTL for SWCB LFD, the one on Chromosome 8 was in common. The comparison between QTL for LFD and leaf toughness may be confounded by environmental effects because leaf toughness was measured in Canada under completely different climatic conditions. Thus, the evaluation of leaf toughness should be repeated under more appropriate conditions and both protein concentration and leaf toughness should be measured in additional trials in order to obtain reliable estimates of QTL positions as well as genotypic correlations. If the association of both traits with insect resistance can be confirmed in future experiments, these traits could be used for indirect selection, thus eliminating the need for costly mass rearing of insects.

Comparison of QTL across RIL Populations

For SWCB LFD, only two QTL on Chromosomes 1 and 8 were in common between the nine QTL found in Population CML131 × CML67 and the five QTL in Population Ki3 × CML139 (Fig. 1). Chromosome 9 carried large overlapping QTL for SWCB LFD in both populations, but peaks were separated by more than 20 cM. Therefore, they were not regarded as common QTL. This constitutes a further indication that Chromosome 9 in Population CML131 × CML67 actually carried two unresolved QTL, one of which coincides with the QTL in Population Ki3 × CML139. For leaf toughness, the one QTL on Chromosome 8 was in common between the five and two QTL detected in both populations.

The smaller number of QTL detected in Ki3 × CML139 and the inconsistency of QTL positions across populations can be explained by several reasons. First, the population sizes were rather small for both crosses, particularly for Ki3 × CML139. Regarding the low power of QTL detection for small sample sizes ($n < 300$) found in simulation studies (Utz and Melchinger, 1994), chances are high that because of sampling effects a QTL is detected in only one population, even though it is actually present in both (Beavis, 1994). According to the proportions of $\hat{\sigma}_g^2$ explained by all QTL, several small QTL could not be detected in both populations, especially in Ki3 × CML139.

Second, the resistant and susceptible parents of the two crosses differ in their level of resistance and susceptibility, respectively, even though they had similar LFD ratings in the present study. According to previous observations at CIMMYT, CML67 is more resistant to LFD than CML139 and CML131 is more susceptible than Ki3, suggesting a smaller number of segregating QTL in cross Ki3 × CML139. In accord with these observations, we detected a QTL for SWCB LFD in Ki3 × CML139, where the allele from the susceptible parent Ki3 contributed to resistance. The differences between the two crosses could be the result of different resistance components in each population. We did not map QTL for protein concentration in Population Ki3 × CML139 but evaluated its values for the parental lines Ki3 and CML139. Protein concentration was high for Ki3 and low for CML139, suggesting that it was also a component of resistance in this cross. Further studies would be necessary to investigate whether other germplasm specific components are involved in resistance to *Diatraea* spp.

Third, Population CML131 × CML67 was evaluated in three environments, while Ki3 × CML139 was evaluated in only two of them, not including the environment in which the greatest number of QTL was detected in the first population. While this may account for the smaller number of QTL detected in Ki3 × CML139, it does not explain the differences in QTL positions between both populations.

A poor consistency of QTL positions for SWCB LFD across populations was also reported by Bohn et al. (1997) for $F_{2:3}$ lines from the same crosses. Their results in combination with the findings from our study suggest that QTL for resistance to *Diatraea* spp. are partly germplasm specific. If these QTL were based on different biological components, insect resistance in subtropical and tropical maize germplasm could be improved by pyramiding QTL from different sources.

Comparison between RIL and $F_{2:3}$ Lines

By comparing our results with those previously reported for $F_{2:3}$ lines, we were able to contrast QTL detected in early and late selfing generations. For $F_{2:3}$ lines of Population CML131 × CML67, Bohn et al. (1997) found six QTL for SWCB LFD and nine QTL for SCB LFD of which four and five were in common with QTL detected in the RIL, respectively. In Population Ki3 × CML139, seven QTL were detected for SWCB LFD in the $F_{2:3}$ lines (Khairallah et al., 1997) with only one in common with the RIL.

Different reasons may explain why several QTL were consistent across generations for CML131 × CML67 but not for Ki3 × CML139. First and probably of primary importance, sampling effects because of small population sizes can lead to the detection of different sets of

QTL as discussed above. In CML131 × CML67, a similar sample size ($n \approx 170$) was employed in both generations, which may explain the higher consistency in this cross. By contrast, 472 $F_{2:3}$ lines but only 134 RIL were used in Ki3 × CML139, leading to a considerable imbalance in the power of QTL detection.

Second, only additive effects can be determined with RIL, while both dominance and additive effects can be estimated in $F_{2:3}$ lines. Therefore, QTL with small additive and large dominance effects can be detected in $F_{2:3}$ lines but not in RIL. In CML131 × CML67, only the first QTL for SWCB LFD on Chromosome 1 displayed purely dominance effects but was also detected in the RIL. In both populations, there was no clear association between the importance of dominance effects at a particular QTL in $F_{2:3}$ lines and their absence in the RIL.

According to theory (Moreno-Gonzalez, 1993), RIL are expected to be superior over $F_{2:3}$ lines with regard to the power of QTL detection and the resolution of linked QTL. Contrary to a previous report on QTL for grain yield and yield components (Austin and Lee, 1996), we were not able to demonstrate a clear advantage of RIL over $F_{2:3}$ lines with regard to the number and resolution of QTL. In Population CML131 × CML67, we detected more QTL and greater estimates of $\hat{\sigma}_g^2$ for SWCB LFD and fewer QTL and smaller estimates of $\hat{\sigma}_g^2$ for SCB LFD in the RIL than in the $F_{2:3}$ lines. We did not observe the separation of a single QTL detected in $F_{2:3}$ lines into two closely linked QTL using RIL in this cross. In Population Ki3 × CML139, resolution of a single QTL into two QTL linked in coupling phase was observed in only one instance (QTL on Chromosome 9). In conclusion, we suspect that the advantage of RIL over $F_{2:3}$ lines in resolving linked QTL is only minor when CIM instead of simple interval mapping is employed for QTL detection.

Perspectives for Marker-Based Selection

The relative efficiency (RE) of MBS in comparison to phenotypic selection depends on the proportion of the additive variance explained by the markers and the heritability and can be estimated as $RE = \sqrt{Q^2/h^2}$ (Lande and Thompson, 1990). For both RIL populations, estimates of RE for LFD were close to 1, indicating that both selection methods would have about the same efficiency under identical selection intensities. The choice of the selection method depends therefore on the cost and feasibility of each method.

The consistency of QTL for leaf feeding resistance across germplasm was poor for RIL populations and also for $F_{2:3}$ lines (Bohn et al., 1997) with only few regions in common. This implies that separate QTL mapping experiments must be conducted in each population in order to identify germplasm specific QTL-marker associations for MBS. The high costs and the time required for the genotypic and phenotypic evaluation of each new mapping population is a clear disadvantage for MBS compared to conventional selection, which only requires phenotypic evaluation and allows testing of more crosses each with a smaller sample size at the same time.

A high agreement between QTL positions across generations is essential for MBS, because QTL are usually identified in early generations and their flanking markers are used for selecting lines during the selfing generations. The consistency of QTL for SWCB LFD and SCB LFD between $F_{2:3}$ lines and RIL was intermediate for Population CML131 × CML67. Thus, only part of the QTL regions selected in early generations would contribute to improved insect resistance of homozygous lines in case the lack of consistency was caused by biological reasons. If the lack of consistency was caused by the low power of QTL detection, a lower efficiency of MBS than estimated from a single population type would be expected. Several QTL detected in the $F_{2:3}$ lines mapped to the same chromosomes in the RIL but were located in adjacent regions possibly due to the large confidence intervals of QTL positions. This shows that a large section of a chromosome must be introgressed by MBS to ensure that it actually contains the target QTL. In Population Ki3 × CML139, only one QTL was in common across generations, indicating that MBS would not be successful for line improvement of SWCB resistance in this cross.

Only QTL consistently expressed across a wide range of environments can be recommended for use in MBS. Several QTL in our study displayed QTL × E interactions when evaluated at the same location in different seasons, reducing the number of useful QTL for MBS. Additional field trials would be necessary to determine the stability of QTL across different locations before initiating a MBS experiment.

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