Genetic Components of Resistance to Stalk Tunneling by the European Corn Borer in Maize

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ABSTRACT

Identification of the genes conferring resistance to European corn borer (ECB) [*Ostrinia nubilalis* (Hübner)] is an important step in understanding how resistance is expressed and whether different sources of maize (*Zea mays* L.) germplasm can be combined to enhance protection. The locations of genes for resistance to ECB tunneling have been reported but are inconsistent across studies. The objectives of this study were to map and characterize quantitative trait loci (QTL) for resistance to tunneling in De811 and compare these with related studies and with QTL for anthesis and ear height. Inbred De811 (resistant) was crossed to susceptible inbred B73 to produce a population of 147 F_3 lines. The population was artificially infested and evaluated in three environments. The F_3 lines were genotyped at 88 restriction fragment length polymorphism (RFLP) loci to facilitate QTL mapping with composite interval mapping (CIM). Seven QTL for ECB tunneling were detected on chromosomes 1, 3, 4, 5, and 8, associated with 42% of the phenotypic variation. The F_1 exhibits partial dominance for resistance but only one QTL with dominant gene action was observed. An F_3 population of B73 × B52 that was evaluated in the same environments facilitated comparisons of genetic heterogeneity between inbreds De811 and B52. Only one QTL for tunneling was common between the populations, indicating that the two inbreds may contribute different genes for resistance in crosses with B73. This information could be useful for combining the favorable alleles of De811 and B52.

ECB IS A MAJOR PEST of temperate maize, with yield losses and control measures exceeding $1000 million annually (Mason et al., 1996). In temperate zones, the larvae of two or more sexual generations feed on leaf, sheath, and collar tissues and pollen and tunnel into the stalk and ear shank (Pesho et al., 1965; Guthrie et al., 1970; Mason et al., 1996). The stalk tunneling reduces grain yield (Pesho et al., 1965; Klenke et al., 1986b; Mason et al., 1996) by interfering with physiological processes, physically weakening the stalk and ear shoot (Lynch, 1980; Klenke et al., 1986b) and by providing points of entry for pathogens associated with stalk rot (Mason et al., 1996).

Adapted inbred lines with elevated levels of resistance to stalk tunneling by ECB have been identified (e.g., B52, De811, Mo47; Russell et al., 1971; Hawk, 1985; Barry et al., 1995). Knowledge of the inheritance and the genetic basis of resistance to tunneling could facilitate development of germplasm with enhanced levels of resistance and desired agronomic traits. High heritabilities (0.63–0.78) for resistance to tunneling have been reported (Schön et al., 1993; Jampatong, 1999; Cardinal et al., 2001). Identification of genetic components of resistance to tunneling has been hindered by environmental variation, a laborious and lengthy screening process, and the polygenic nature of the trait; however, linkage analysis has provided estimates of gene locations for inbred lines B52 and Mo47 (Onukogu et al., 1978; Schön et al., 1993; Jampatong, 1999; Cardinal et al., 2001).

The inbred line De811 is resistant to ECB stalk tunneling (Hawk, 1985) and shows partial dominance for resistance in the F_1 of crosses to susceptible inbreds (e.g., A619, B73, C131A; Guthrie et al., 1989). The effects and positions of genes for resistance to tunneling in De811 have not been previously reported. Such information could be useful for breeding with De811 and other germplasm. In this study, 147 F_3 lines of B73 × De811 were genotyped at RFLP loci and evaluated for tunneling and two other traits that could potentially confound assessment of tunneling: anthesis and ear height (Dicke, 1954; Coors, 1987). This population and F_3 lines of B73 × B52 (Schön et al., 1993) were grown in the same environments. The common environments and susceptible parent provide an opportunity to assess genetic heterogeneity for resistance. The objectives of this study were (i) to assess genetic and environmental components of resistance to ECB tunneling in the F_3 generation of B73 × De811; (ii) to determine the genotypic correlations between ECB tunneling and ear height and anthesis; (iii) to map genetic factors for resistance, anthesis, and ear height; and (iv) to evaluate the relative importance of additive and dominance gene effects on resistance.

MATERIALS AND METHODS

Plant Materials

Random F_2 plants from a cross between inbred lines B73 and De811 were self-pollinated to produce 150 F_3 lines. Inbred B73 is widely used in temperate maize breeding programs but is highly susceptible to stalk tunneling by ECB (Table 1). Inbred De811 and the F_1 (B73 × De811) exhibit high levels of resistance to ECB tunneling in the stalk (Table 1).

Field Experiments

The experiments were planted at two locations: the Agronomy and Agricultural Engineering Research Center (AAERC) near Ames, IA, and the Iowa State University Research Farm near Ankeny, IA, on 11 May and 25 April, respectively, in

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Abbreviations: AIC, Aikake information criteria; CI, confidence interval; CIM, composite interval mapping; cM, centimorgan; ECB, European corn borer; GDD, growing-degree days; LOD, log of the odds ratio; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; SCB, sugarcane borer; SWCB, Southwestern corn borer.

Table 1. Phenotypic data for the parents, F_1, and F_3 lines of B73 × De811.

Trait	Environment	B73 mean	De811 mean	F_1 mean	F_3 lines					
					Mean	Range	σ_g^2	95% CI	σ_e^2	95% CI
Stalk tunneling†		cm								
	Ames 1989	80	35	38	50	20–86	103	75–152	86	68–113
	Ankeny 1989	65	25	40	43	22–78	40	24–82	87	68–116
	Ames 1990	29	19	9	16	4–36	11	6–29	37	29–48
	Mean	58	26	31	37	21–59	36	25–54	85	74–97
Anthesis‡		GDD								
	Ames 1989	1017	1058	1008	1018	983–1057	211	159–292	113	91–145
	Ames 1990	892	919	893	890	833–946	223	139–413	434	345–564
	Mean	957	985	951	954	910–998	197	141–292	362	308–431
Ear height§		cm								
	Mean	91	93	102	91	59–113	87	67–116	68	60–79

† LSD for Stalk Tunneling: Ames, 1989: A = 19, B = 19, C = 14; Ankeny, 1989: A = 27, B = 22, C = 15; Ames 1990: A = 13, B = 11, C = 9; Across Locations: A = 14, B = 12, C = 10.
‡ LSD for Anthesis: Ames, 1989: A = 23, B = 20, C = 16; Ames 1990: A = 44, B = 38, C = 30; Across Locations: A = 36, B = 31, C = 25.
§ LSD for Ear Height: A = 11, B = 11, C = 10.
A = comparison among F_3 lines.
B = comparison between F_3 lines and parental inbreds.
C = comparison among parental inbreds.

1989, and at the AAERC on 29 May 1990. Each location–year was considered a separate environment. Soil fertilization, weed control, and cultivation practices were consistent with optimum maize production for this region. The entries in each experiment consisted of the 150 F_3 lines and two entries each of B73, De811, and the F_1. Entries were evaluated in hill plots consisting of two hills spaced on centers of 0.76 m at Ames and 1.02 m at Ankeny, and were arranged in a 12 × 13 simple lattice design with two replications per environment. Plots were overplanted and thinned to three plants per hill (i.e., six plants per plot; Guthrie et al., 1985).

Trait Evaluation

All plants in each plot were artificially infested with ECB larvae when 50% of the entries in the experiment had reached anthesis. Anthesis was defined as three of the six plants in a plot shedding pollen. Newly hatched larvae were obtained from the USDA Corn Insect Laboratory, Ames, IA. The larvae were applied at four infestation points: the primary leaf axil, the first and second leaf axils above the primary ear, and the first leaf axil below the primary ear. Larvae were applied to each plant for six consecutive days for a total of 650 larvae per plant. Approximately 60 d after infestation, the plants were split from the soil level to the first node above the primary ears and stalk tunneling was recorded to the nearest centimeter for each of the six plants. Parallel tunnels were recorded only once.

Plots were also evaluated for growing degree-days (GDD) to anthesis and ear height to assess their correlation with ECB tunneling. GDD were calculated for each day from planting to anthesis, according to the formula [(max.°C + min.°C)/2] − 10°C, where 10°C was used for the minimum temperature and 30°C was used for the maximum temperature if the actual temperatures exceeded those limits (Cross and Zuber, 1972). Ear height was measured on all plants in the plot as the distance (to the nearest 5 cm) from the soil level to the highest ear-bearing node. Anthesis was only measured at the Ames environments, while ear height was measured at all environments.

Analysis of Phenotypic Data

For each trait and entry, least square means (lsmeans) were calculated with complete and incomplete blocks as random effects and entries as fixed effects for each environment (Cardinal et al., 2001). Environments were also treated as random effects when calculating lsmeans for the mean environment. These means were used for the QTL analysis. Means of the two parental lines and the F_1 were calculated as the average of the lsmeans of the two entries in each environment. Genotype, genotype × environment, and error variance were calculated with environments, complete and incomplete blocks, and entries and the entry × environment interaction as random effects (Cardinal et al., 2001). Broad-sense heritabilities on an entry-mean basis and their exact confidence intervals were calculated according to established procedures (Knapp et al., 1985; Fehr, 1987). Genetic correlations (r_g) were calculated by means of PROC GLM considering entries and environments as random effects (SAS Institute, Inc., 1990). Box's test (Milliken and Johnson, 1992) was used to test for homogeneity of error variances between environments. The error variances were significantly different for tunneling ($P = 0.001$), GDD to anthesis ($P = 0.015$), and ear height ($P = 0.043$). Therefore, a separate analysis was performed for each environment.

Detection of QTL

The protocols for DNA isolation, Southern hybridization, and collection of segregation data at RFLP loci have been described (Veldboom et al., 1994). Ninety-four genomic and cDNA probes detected 103 RFLP loci. One hundred forty-seven F_3 lines were used for linkage mapping and QTL analysis. Three F_3 lines were excluded from all analyses because of technical difficulties during the collection of RFLP data or the detection of non-parental alleles.

Linkage analysis was performed by MAPMAKER/EXP v. 3.0 (Lander et al., 1987). Loci were assigned to linkage groups using the program's default settings [minimum $\log_{10}$ of the likelihood odds ratio (LOD) score 3.0, maximum distance between loci of 50 centimorgans (cM)]. Multipoint analysis was performed by means of the "order" command (informativeness criteria of 120 individuals, 2 cM between loci). In cases where a "best order" could not be determined because of close linkage, the least informative locus was excluded and the order command was used for the subset. Fifteen of the initial 103 loci were excluded from the study because they could not be mapped to a unique location with a LOD value of at least 2.0 or because they exhibited dominant banding patterns. The remaining 88 loci were mapped to unique positions and comprised the genetic map of 996 cM with an average

distance of 12.8 cM between loci for QTL analysis. A recombination frequency of 0.10 to 0.15 (11–18 cM) between loci is sufficient for QTL detection (Darvasi et al., 1993; Darvasi and Soller, 1995). Large intervals between marker loci can result in detection of "false" QTL (a type I error; Lincoln et al., 1993). A QTL was detected on chromosome 1 (*umc157*) in a 36-cM interval. That QTL was reported because it is not possible to determine whether it is the result of actual genetic effects or a type I error. Because of a very large gap (>75 cM) between loci *bnl12.06a* and *bnl7.08*, chromosome 1 consists of two linkage groups. The chi-square test for segregation distortion was not significant for any locus.

QTL were detected by PlabQTL (Utz and Melchinger, 1996), which employs CIM (Jansen, 1993; Jansen and Stam, 1994; Zeng, 1994). Cofactor selection was performed as described (Utz and Melchinger, 1996, Austin et al., 2000). First the "cov select" option was used to select cofactors by means of stepwise regression. Cofactors not associated with QTL effects were eliminated from the model (Zeng, 1994). The LOD threshold value of 2.5 (default value) was used to declare the presence of a QTL. Previous reports suggest a LOD threshold value between 2 and 3 (Lander and Botstein, 1989) or a permutation test to calculate the LOD threshold value for a specified Type I error rate (Churchill and Doerge, 1994). The LOD threshold value of 2.5 has been used in similar studies of QTL in maize (Lübberstedt et al., 1998; Cardinal et al., 2001) and (i) allows for comparisons with the B73 × B52 F_3 population (LOD > 2.2; Schön et al., 1993) and (ii) minimizes the risk of a Type II error (i.e., missing a QTL). Then, the "cov/+ select" option was used to detect closely linked QTL of opposite effects. All QTL were then integrated into a model by means of the "seq/s" option in PlabQTL. Model selection was performed by means of forward and backward stepwise selection. If the Aikake Information Criteria (AIC) values of two models differed by less than 2.0, the model with the fewest parameters was chosen (Jansen, 1993; Cardinal et al., 2001).

Digenic epistatic interactions between all pairs of loci were tested by EPISTACY (Holland, 1998). Interactions with $P < 0.00026$ were considered significant. This threshold was based on an estimate of the number of independent linkage groups in maize with each chromosome arm representing one independent linkage group (Holland et al., 1997). Interaction terms were added to a model by PROC REG (SAS Institute, Inc., 1990). Interaction terms that increased the *R*-square of the model and were significant at $P < 0.05$ were maintained in the model.

RESULTS

In all environments, B73 and De811 differed significantly for ECB tunneling, and there were significant differences among the F_3 lines (Table 1). The F_1 values for ECB tunneling were close to De811 and differed significantly from B73. The highest levels of ECB tunneling in the parents and F_3 lines and the greatest amount of genotypic variation were observed in Ames 1989. The genotypic variation for tunneling was almost 10 times greater for 1989 than 1990 at Ames. The combined precipitation in July and August at Ames was lower in 1989 (11 cm) and higher in 1990 (30 cm) than the 40-yr average (21 cm). Precipitation in July and August at Ankeny in 1989 (22 cm) was similar to the 40-yr average (20 cm). The excessive precipitation at Ames 1990 likely increased larval mortality. The variance for genotype (36) and genotype × environment [16, 95% confidence interval (95% CI) = 9–38] in the mean environment were not significantly different from the F_3 lines of B52 × B73 (69 and 10, respectively), while the error variance herein was lower (85 and 141, respectively; Schön et al., 1993). For both experiments the error variance accounts for a large fraction of the phenotypic variance, illustrating the complications with assessing resistance to tunneling. The broad-sense heritability for tunneling was 0.65 (95% CI = 0.55–0.71), which is comparable to previous studies (0.63–0.78; Sadehdel-Moghaddam et al., 1983; Schön et al., 1993; Cardinal et al., 2001).

Seven QTL for ECB tunneling were detected on chromosomes 1, 3, 4, 5, and 8 in the mean environment (Fig. 1 and Table 2). The QTL were associated with 42% of the phenotypic variation, and all exhibited significant additive effects. Significant dominance effects were evident for one QTL (chromosome 1, *umc13*). Alleles from De811 were associated with decreased tunneling at five QTL. Epistatic effects were not detected.

QTL for ECB tunneling in the individual environments differed from those in the mean environment (data not shown). Only the QTL on chromosome 5 (*umc68-umc51*) was detected in all environments, while the QTL on chromosome 3 (*umc102*) was detected in both 1989 environments and the mean environment. The five QTL for Ames 1989 were also detected in the mean environment. Five and four QTL were observed for Ankeny 1989 and Ames 1990, respectively, but only those on chromosomes 3 (*umc102*) and 5 (*umc68-umc51*) were also observed in the mean environment. The QTL on chromosome 1 (*umc13*) was detected in Ames 1990 at LOD 4.8, but was excluded from the model because it did not increase the AIC by at least 2.0. Detection of that QTL was dependent on a cofactor (*bnl12.06a*), indicating that there may be a QTL in the region between *bnl12.06a* and *bnl7.08*.

Anthesis date and ear height are potentially confounding effects on the assessment of resistance to ECB tunneling (Dicke, 1954; Coors, 1987) because larval survival is affected by availability of pollen, and the length of the stalk may determine the amount of tunneling observed. Significant differences for anthesis and ear height were observed among F_3 lines in individual environments and the mean environment (Table 1). The broad-sense heritabilities for those traits were 0.68 (95% CI = 0.55–0.76) and 0.87 (95% CI = 0.83–0.89), respectively. The genetic correlations (r_g) between anthesis date and ear height and ECB tunneling were −0.36 ($P < 0.001$) and 0.35 ($P < 0.05$), respectively.

Seven QTL for anthesis were detected on chromosomes 1, 3, 5, and 7 (Fig. 1 and Table 3), and 11 QTL for ear height were observed on chromosomes 1, 2, 3, 4, 5, 6, and 9 (Fig. 1 and Table 4) in the mean environment. The anthesis QTL on chromosomes 1 (*umc11*) and 3 (*umc92*) are within 15 cM of QTL for tunneling. On chromosome 1, later anthesis was linked with reduced tunneling, while on chromosome 3, delayed anthesis was linked with increased tunneling. The ear height QTL on chromosomes 1 (*umc11*), 4 (*umc31*), and 5 (*umc68*) are within 10 cM of QTL for ECB tunneling. In those regions, decreased ear height is linked to decreased ECB tunneling.

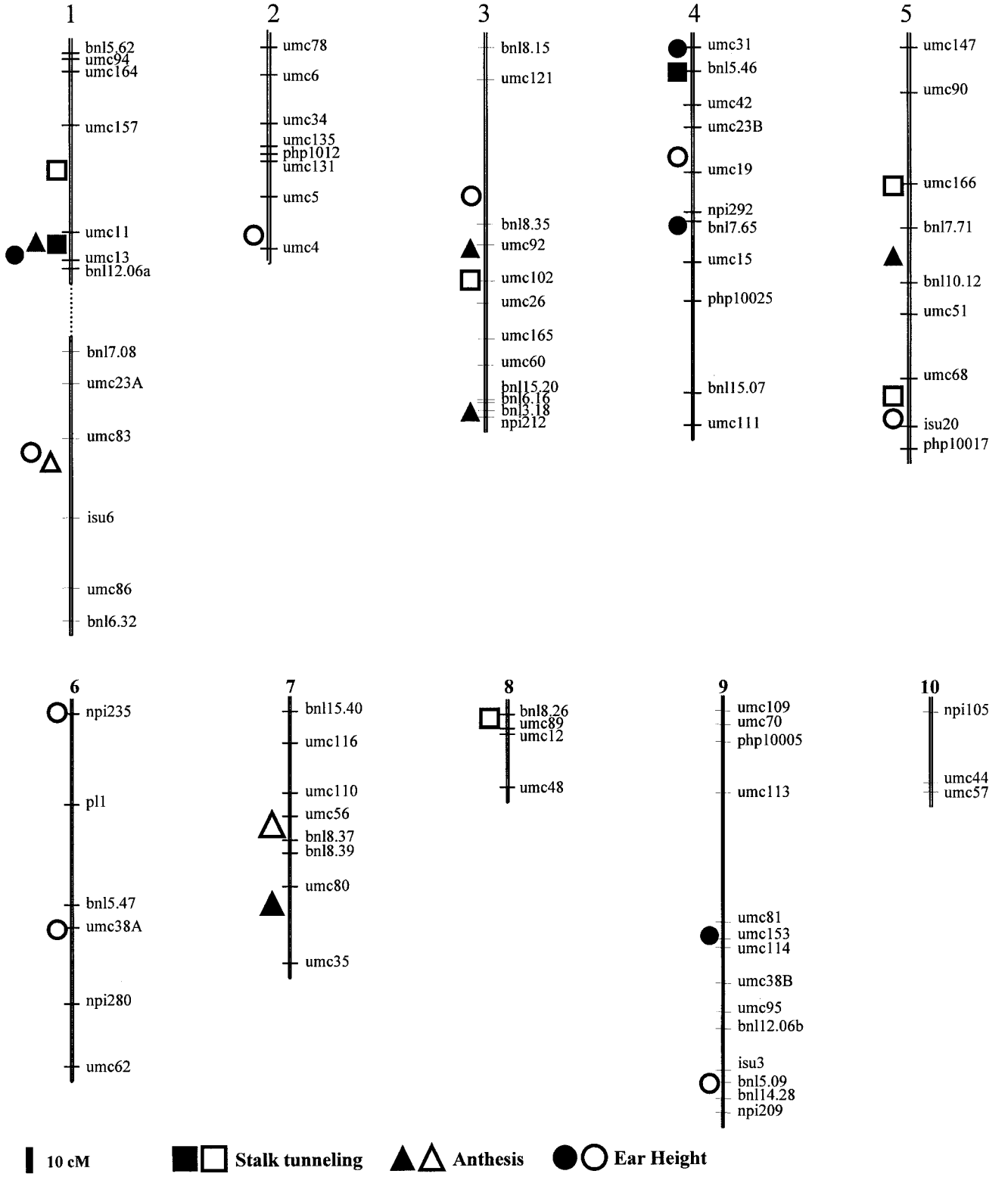

Fig. 1. Genetic map of B73 × De811 F_3 population of maize and location of QTL for stalk tunneling , anthesis, and ear height. Solid shapes (e.g., ■) = De811 allele is associated with an increase in the trait and clear shapes (e.g., □) = B73 allele is associated with an increase in the trait.

DISCUSSION

Four studies of QTL for ECB tunneling have been performed with different resistant parents crossed to the same susceptible parent (B73; Schön et al., 1993; Jampatong, 1999; Cardinal et al., 2001). Comparisons of QTL across populations, while complicated by sampling variation and differences in environments and methodology and limited by the number of common genetic loci, can provide an opportunity to assess genetic heterogeneity of a phenotype (van Ooijen, 1992; Jansen and

Table 2. QTL for ECB stalk tunneling in the F_3 maize population of B73 × De811 in the mean environment.

Chrom.	Genetic locus† (GL)	Distance from GL	Additive		Dominance	
			Effect‡ (cm)	Partial R^2§	Effect (cm)	Partial R^2
		cM				
1	*umc157*	15	−3.3**	5.4	−3.7*	3.1
1	*umc11*	7	3.7**	8.2	−2.0	1.7
3	*umc102*	0	−4.3**	24.7	−0.7	0.3
4	*bnl5.46*	1	2.9**	12.6	0.4	0.1
5	*umc166*	0	−2.7**	9.0	1.1	0.9
5	*umc68*	6	−4.4**	21.3	0.7	0.3
8	*bnl8.26*	0	−2.7**	9.4	−0.2	0.0
Total adjusted R^2¶ = 42%						

* Effect is significant at $P < 0.05$.
** Effect is significant at $P < 0.01$.
† The locus defining the interval that contains the QTL that is proximal to the telomere of the short arm of the chromosome.
‡ Allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait.
§ Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed.
¶ Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model.

Table 3. QTL for anthesis for the F_3 lines of B73 × De811 in the mean environment

Chrom.	Genetic locus† (GL)	Distance from GL	Additive		Dominance	
			Effect‡ (cm)	Partial R^2§	Effect (cm)	Partial R^2
		cM				
1	*umc11*	6	6.3**	10.7	−3.7	2.5
1	*umc83*	7	−6.7**	11.1	−1.4	0.2
3	*umc92*	0	6.1**	10.7	−3.6	2.0
3	*bnl3.18*	0	9.0**	21.8	−4.1*	3.0
5	*bnl7.71*	10	7.7**	14.0	−8.0**	7.3
7	*umc56*	4	−7.2**	8.0	7.4**	6.4
7	*umc80*	9	7.9**	7.7	−10.2**	7.1
Total adjusted R^2¶ = 51%						

* Effect is significant at $P < 0.05$.
** Effect is significant at $P < 0.01$.
† The locus defining the interval that contains the QTL that is proximal to the telomere of the short arm of the chromosome.
‡ Allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait.
§ Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed.
¶ Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model.

Table 4. QTL for ear height for the F_3 lines of B73 × De811 in the mean environment

Chrom.	Genetic locus† (GL)	Distance from GL	Additive		Dominance	
			Effect‡ (cm)	Partial R^2§	Effect (cm)	Partial R^2
		cM				
1	*umc13*	0	6.5**	33.5	−1.0	0.6
1	*umc83*	5	−1.6	0.9	3.2*	4.5
2	*umc5*	9	−3.6**	11.0	0.0	0.0
3	*umc121*	41	−3.5**	11.9	2.9	2.9
4	*umc31*	0	3.2**	8.1	0.6	0.2
4	*umc23b*	8	−5.0**	9.9	1.5	0.8
4	*bnl7.65*	1	5.1**	14.0	3.2*	5.2
5	*umc68*	11	−3.7**	14.0	−2.2	2.2
6	*npi235*	0	−3.9**	14.8	−1.1	0.7
6	*umc38a*	1	−2.5**	7.7	−1.0	0.6
9	*umc81*	4	6.0**	29.3	−0.1	0.0
9	*bnl5.09*	1	−3.4**	12.0	1.7	1.8
Total adjusted R^2¶ = 58%						

* Effect is significant at $P < 0.05$.
** Effect is significant at $P < 0.01$.
† The locus defining the interval that contains the QTL that is proximal to the telomere of the short arm of the chromosome.
‡ Allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait.
§ Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed.
¶ Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model.

Stam, 1994; Zeng, 1994; Visscher et al., 1996). Herein, all comparisons will be made between QTL for ECB tunneling detected in the mean environment and will be based on common marker loci. The F_3 lines of B73 × B52 were evaluated in the same environments in 1989 as the population herein, and this should enhance comparisons of QTL detected in those two populations. Seven QTL were detected for ECB tunneling in the F_3 lines of B73 × B52. Herein, the QTL on chromosome 3 (*umc102*) is in the same region (i.e., within 25 cM), and the alleles from the resistant parent (B52 and De811) were associated with a decrease in tunneling. The QTL on chromosomes 3 (*umc102*) and 5 (*umc68*) are in the same regions as QTL detected in recombinant

inbred lines (RILs) of B73 × B52 (Cardinal et al., 2001), and the QTL on chromosome 5 is also in the same region as one detected in F_3 lines of B73 × Mo47 (Jampatong, 1999). In all populations, alleles from the resistant parent (De811, B52, and Mo47) were associated with decreased tunneling.

On the basis of common genetic loci, four QTL for ECB tunneling herein are in the same regions as QTL for resistance to leaf feeding by the southwestern corn borer (SWCB, *Diatraea grandisella* Dyar) and the sugarcane borer (SCB, *Diatraea saccraralis* Fabricius) in two populations of tropical maize. The QTL on chromosomes 1 (*umc157* and *umc11*), 5 (*umc68*), and 8 (*bnl8.26*) are in the same regions (i.e., within 25 cM) as those for resistance to leaf feeding by SWCB, SCB, or both (Groh et al., 1998). The linkage between resistance to leaf feeding by SWCB and SCB and ECB tunneling was unexpected because resistance to leaf feeding and tunneling by ECB in temperate maize has a low genotypic correlation (0.10–0.33; Russell et al., 1974; Sadeh-del-Moghaddam et al., 1983; Klenke et al., 1986a).

The F_1 progeny of De811 have shown partial dominance for resistance to ECB tunneling (Table 1; Guthrie et al., 1989). Dominance effects were only observed for one QTL in the mean environment (chromosome 1, *umc157*), and the partial *r*-square was small relative to the additive effects. The dominance observed in the F_1 may actually be due to epistasis effects not detected in this experiment.

The biological basis of resistance to stalk feeding by ECB and other insect pests of maize has not been established, but genetic factors for chemical composition and morphological traits associated with resistance have been localized in the genome. The QTL on chromosome 1 (*umc11*) is linked to the same genetic locus as a QTL for concentration of maysin (Byrne et al., 1997), a C-glycosol flavone that inhibits larval growth of the corn earworm (Waiss et al., 1979). Leaf toughness, manifested through cell wall fortification by phenolic acids, has been proposed as a major factor in resistance to ECB feeding (Bergvinson et al., 1994a,b). The QTL for ECB tunneling on chromosome 4 (*bnl5.46*) is in a region associated with leaf toughness in tropical maize populations (Groh et al., 1998).

QTL for tunneling in the mean environment were linked (i.e., within 20 cM) to two and three QTL for anthesis and ear height, respectively. The negative correlation indicates that increased GDD to anthesis is associated with decreased tunneling. This was observed for the QTL on chromosome 3 but not for the QTL on chromosomes 1 (*umc11*), where the De811 allele was associated with increased GDD to anthesis and increased stalk tunneling (Fig. 1). The QTL for stalk tunneling on chromosome 1 (*umc11*) was associated with a smaller percentage of the phenotypic variation (10.7%) than the QTL for tunneling on chromosome 3 (24.7%), and the larger partial *R*-square for the QTL on chromosome 3 could account for the negative correlation between tunneling and anthesis. The correlation between ear height and tunneling may indicate that the length of the stalk was a limiting factor in the amount of observed tunneling. For all linked QTL, the allele associated with increased tunneling was also associated with increased ear height (Fig. 1). Selection for resistance to stalk tunneling could result in inbreds with delayed anthesis and shorter ear heights. In the BS9(CB) population, selection for resistance to leaf feeding and stalk tunneling by ECB resulted in reduced ear height (Novoa, 1987).

Several QTL for resistance to ECB tunneling were detected, only one of which had significant dominance effects. De811 has shown partial dominance for resistance to tunneling, but dominance effects were only detected for one QTL in the mean environment. Delayed anthesis and decreased ear height were associated with decreased tunneling but the correlations were low. So the relationship between these traits in this population is not clear. Assessments of genetic diversity on the basis of DNA polymorphism and pedigrees indicate that De811, B52, and Mo47 were derived from different genetic backgrounds (Hawk, 1985; Lee et al., 1990; Barry et al., 1995; Senior et al., 1998). The detection of different QTL in crosses of B73 to B52, De811, and Mo47 may be due to genetic heterogeneity among the resistant inbreds. Some QTL for ECB tunneling were also detected in studies of resistance to leaf feeding by SWCB and SCB, which suggests that there may be common mechanisms of resistance to these different species and feeding stages. The evidence of genetic heterogeneity among the inbreds, specifically B52 and De811, suggests that breeding could combine these sources of resistance to produce germplasm with higher levels of resistance to ECB tunneling.

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