Theor Appl Genet (2000) 101:907–917

M. Bohn · B. Schulz · R. Kreps · D. Klein
A.E. Melchinger

QTL mapping for resistance against the European corn borer (*Ostrinia nubilalis* H.) in early maturing European dent germplasm

Received: 20 December 1999 / Accepted: 6 June 2000

Abstract The European corn borer (ECB, *Ostrinia nubilalis* Hübner) is a major pest of maize in Central Europe. We mapped and characterized quantitative trait loci (QTLs) involved in resistance of maize against ECB damage, compared them with QTLs for agronomic traits, and evaluated the usefulness of marker-assisted selection (MAS) for improving ECB resistance in early maturing European maize germplasm. A total 226 F_3 families from the cross D06 (resistant) × D408 (susceptible), together with 93 RFLP and two SSR markers were used for the QTL analyses. For each F_3 family we measured the length of tunnels produced by larval stalk mining (TL), stalk damage ratings (SDR), and relative grain yield (RGY) in field experiments, with two replications in two environments in 1 year. The agronomic traits comprised grain yield under insecticide protection (GYP) and manual ECB larval infestation (GYI), the date of anthesis (ANT), and the in vitro digestibility of organic matter (IVDOM) of stover. Estimates of genotypic variance (σ_g^2) were highly significant for all traits. Six QTLs for TL and five QTLs for SDR were detected, explaining about 50.0% of σ_g^2. Most QTLs showed additive gene action for TL and dominance for SDR. No QTL was found for RGY. The number of QTLs detected for the agronomic traits ranged from two for GYI to 12 for ANT, explaining 12.5 to 57.3% of σ_g^2, respectively. Only a single QTL was in common between the two resistance traits, as expected from the moderate trait correlation and the moderate proportions of σ_g^2 explained. Based on these results, MAS for improving ECB resistance can be competitive when cost-effective PCR-based marker systems are applied. However, it remains to be established whether the putative QTL regions for ECB resistance detected in the population D06 × D408 are consistent across other early maturing European maize germplasms.

Key words Insect resistance · *Ostrinia nubilalis* · QTL · Maize

Communicated by H.C. Becker

M. Bohn · D. Klein · A.E. Melchinger (✉)
Institut of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany
e-mail: melchinger@uni-hohenheim.de
Tel.: ++49-711-459 2334, Fax: ++49-711-459 2343

B. Schulz · R. Kreps
KWS Saat AG, Grimsehlstrasse 31, 37555 Einbeck, Germany

Present address:
R. Kreps, Societe Des Mais Europeens,
Centre de Selection de Chartres, Rue du Bois Musquet,
28300 Champhol, France

Introduction

The European corn borer (ECB, *Ostrinia nubilalis* Hübner) is the major pest of maize (*Zea mays* L.) in many maize-growing regions of Central Europe. Damage to the maize plant is mainly caused by feeding of the ECB larvae in the stalk and ear shank. Yield losses are largely attributable to a reduction in kernel number and weight owing mainly to physiological disruption of the plant growth and only to a minor extent to broken stalks, dropped ears, and larval feeding on the grain or lodging (Chiang and Hodson 1950). In a set of eight early maturing commercial maize hybrids, each ECB larval per plant accounted for a 6.1% grain yield loss (Bohn et al. 1999). Total grain yield reduction due to ECB infestation can range from 0.3 t ha^{-1} to more than 3.0 t ha^{-1} (Bohn et al. 1999). In addition, cavities of the ECB larvae increase the occurrence of secondary infections from stalk- and ear-rotting pathogens such as *Fusarium* spp., which reduce yield and deteriorate the quality of grain and forage maize through contamination by mycotoxins (Jarvis et al. 1984).

In contrast to the U.S. Cornbelt, where ECB occurs bivoltine, only one ECB generation is observed in Central Europe (Bohn et al. 1999). Here, natural ECB infestation starts at the pre-tasseling stage of maize and, thus, is similar to the 2nd-ECB generation in the U.S. Corn-

belt. Natural host-plant resistance the against 2nd-ECB generation depends on three mechanisms: non-preference, antibiosis, and tolerance. Non-preference is due to a lack of attractiveness of the host plant as an oviposition site or shelter for the insect. Antibiosis increases the mortality and hampers the growth and feeding of larvae on the host plant. The level of antibiosis to ECB resistance seems to depend on a number of factors such as detergent fiber, cellulose, lignin, and biogenic silica and tissue toughness (Bergvinson et al. 1994; Ostrander and Coors 1997). Tolerance is the ability of a maize plant to withstand feeding of a certain number of insect larvae without economic loss of yield or quality. The development and release of transgenic maize hybrids expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) provided an additional source of resistance to ECB (Armstrong et al. 1995).

In contrast to U.S. Cornbelt maize germplasm, only a few studies on the resistance of early maturing European maize germplasm against ECB are available. Recently, a large number of European elite inbreds was screened for resistance against ECB using artificial infestation, and significant genotypic variation was found for important resistance traits (Melchinger et al. 1998a). In several studies, quantitative trait loci (QTLs) for insect resistance in temperate and tropical maize germplasm against various maize stem-borer species were detected, which explained a large proportion of the genotypic variance (Schön et al. 1993; Bohn et al. 1996, 1997; Khairallah et al. 1998). The authors concluded that marker-assisted selection (MAS) should be at least as effective as conventional phenotypic selection for improving insect resistance.

Based on the results of our previous inbred screening (Melchinger et al. 1998a), we selected two lines, representing extremes with respect to their response to ECB larvae feeding as parents, for a QTL mapping population. The objectives of our study were to: (1) estimate the number, chromosomal position, and genetic effects of the QTLs involved in antibiosis and tolerance against ECB in this mapping population developed from early maturing European dent germplasm, (2) determine associations between ECB resistance and agronomic traits, (3) evaluate the consistency of QTLs for ECB resistance with those mapped in different populations derived from U.S. Cornbelt materials, and (4) evaluate the usefulness of MAS for improving these traits.

Materials and methods

Plant materials

The homozygous early maturing European dent lines D06 and D408 were used as parents. D06 is resistant and D408 susceptible to ECB larval feeding (Melchinger et al. 1998a). During the 1994 summer season, F_2 plants derived from two randomly chosen F_1 individuals from the cross D06 × D408 were selfed to produce 230 $F_{2:3}$ lines. For each $F_{2:3}$ line, 20 F_3 plants were chosen to generate an "Immortalized F_2" population (IF_2) (Gardiner et al. 1993) by randomly crossing ten F_3 plants as females and ten F_3 plants as males in the 1994 winter nursery. The IF_2 families are denoted as F_3 families throughout the remainder of this paper.

Field trials

The F_3 families were evaluated in field experiments at Eckartsweier and Scherzheim in the summer season of 1995. Both sites are located in the Upper Rhine Valley, a major area of grain maize production in Germany with a natural occurrence of ECB. The experiment included 230 F_3 families, both parental lines as duplicate entries, and the F_1 hybrid and a random sample of the F_2 generation as triplicate entries. The experimental design at each site was a split plot with a total of 240 entries comprising main plots. Subplots consisted of one row manually infested with ECB larvae and one row protected by an insecticide. Main plots were arranged in a 10 × 24 α-design with two replications. The experimental unit was a one-row plot with 20 plants, a 4-m length, and a row spacing of 0.75 m. Trials were over-planted and later thinned to the final plant density of 66 667 plants ha^{-1}.

European corn borer treatments

The timing of insecticide treatments and manual infestations was synchronized with light-trap catches of ECB adults. At both environments, the first ECB moths were consistently observed in mid June (15–20) and the last adults were caught in the 2nd half of July (21–26). All plants in the insecticide-protected rows were individually treated three-times from the end of June to the beginning of August with an insecticide granulate (FASTAC SC) at 10–14-day intervals to prevent natural infestation of European corn borer. For manual infestation, ten plants in the center of a row were infested. On average 20 ECB larvae were applied three-times at weekly intervals for a total of about 60 larvae per plant.

Most maize plants were in the mid-whorl stage at the time of first infestation and tasseled, or started silking, at the time of the third infestation. Egg masses for the rearing of ECB larvae were supplied by the entomology laboratory of Dr. P. Aupinel, Institut National de la Recherche Agronomique, Le Magneraud, France. After incubation, neonate larvae were mixed with corncob grits and placed into the whorl and leaf collar of the maize plants using a mechanical dispenser.

Resistance and agronomic traits

The level of antibiosis against ECB was assessed by the tunnel length (TL) in stalks and the damage ratings of stalks. At harvest, stalks of infested plants were split longitudinally to measure tunnel length (in cm) attributable to the feeding of ECB larvae. Measurements were taken only below the primary ear node. All plants of each infested row were assessed individually before harvest by damage ratings on a 1 to 9 scale (1 for intact plants, 2 for breakage within tassels, 3 for breakage directly below the tassel, 4 for breakage within the first node below the tassel, 5 for breakage within the 2nd node below the tassel, 6 for breakage within the third node below the tassel, 7 for breakage within the fourth node below the tassel, 8 for stalk breakage above the ear, and 9 for dropped ears or stalk breakage below the ear) in order to evaluate stalk breakage (Hudson and Chiang 1991). Stalk damage ratings ranged from 1 for intact plants to 9 for stalks broken below the primary ear (Hudon and Chiang 1991). Ten plants from the center of the protected row and the ten plants that were manually infested with ECB larvae were hand-harvested from each subplot and data were separately recorded for each row for grain yield in t ha^{-1} adjusted to 15.5% moisture. To measure the degree of tolerance, the relative grain yield (in %) of each entry was determined by dividing the yield of the infested row by that of the adjacent protected row. Additional agronomic traits recorded from insecticide-protected plots were the date of anthesis (ANT, expressed in days after sowing), and the in vitro digestible organic matter (IVDOM) of stover (in %). IVDOM was determined by near-infrared reflectance spectroscopy according to the procedure described by Degenhardt (1996).

RFLP assays

For subsequent RFLP genotyping, leaf samples were taken from all parental F_2 plants. Genomic DNA was extracted from leaf ma-

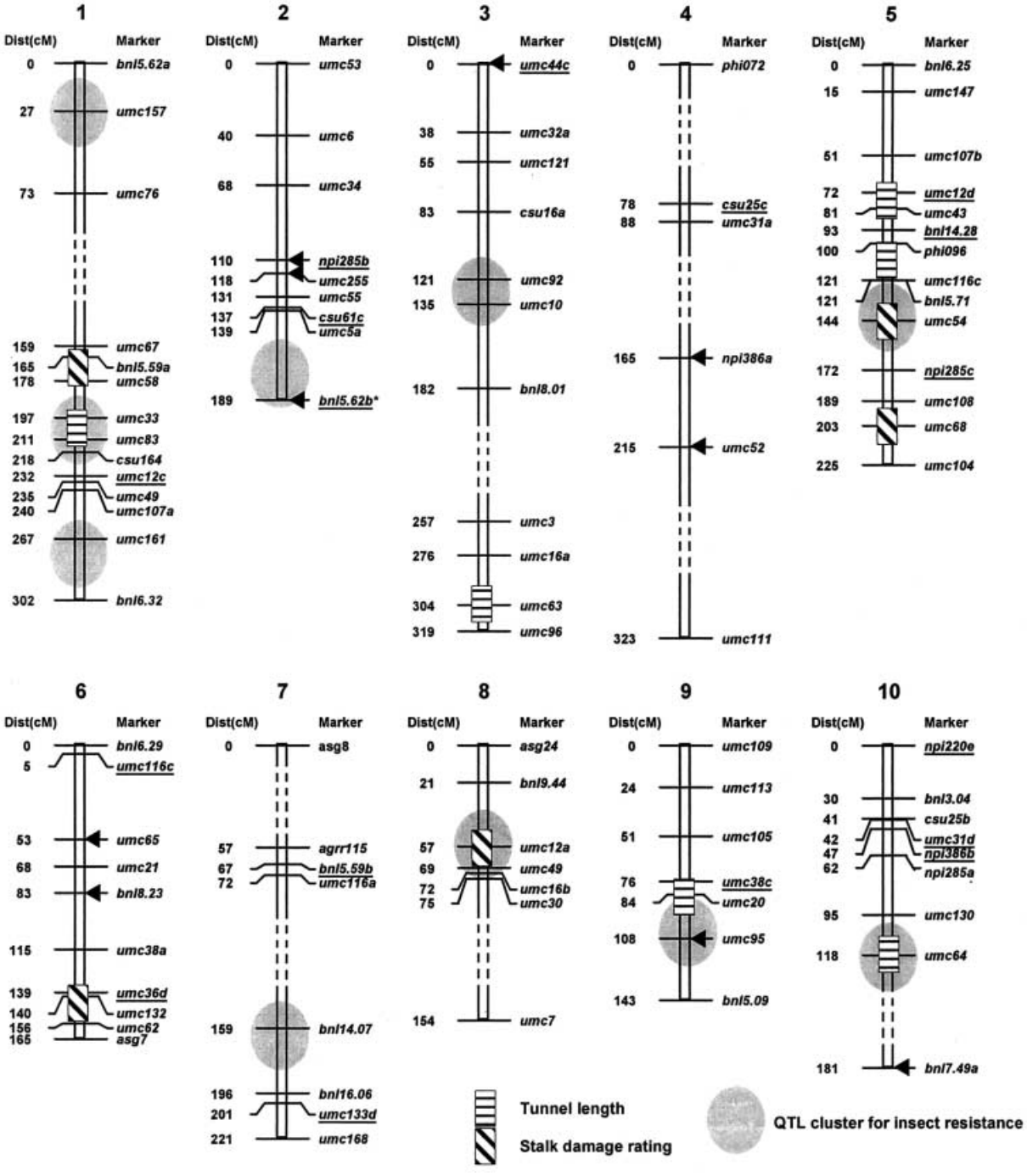

Fig. 1 Linkage map of maize based on 230 F_2 individuals derived from the cross D06 × D408 for 93 RFLP and two SSR marker Loci. *Numbers* to the left of the chromosome indicate distance in cM relative to the first marker. The short arm of each chromosome is shown towards the top of the figure. *Dashed lines* indicate linkage between marker loci with LOD < 2.5. *Underlined* marker loci indicate new loci detected with the respective probe × enzyme combination. Marker loci with a significant distorted segregation are marked by a *black triangle*. Chromosomal regions carrying QTLs for tunnel length and stalk damage ratings are indicated by *boxes.* The *box pattern* is associated with the respective resistance trait. Already published QTL clusters for insect resistance are marked by a *grey ellipse*

terial and digested with the restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII or *Bam*HI. The resulting DNA fragments were separated by agarose-gel electrophoresis and transferred onto uncharged membranes by Southern blotting. Hybridization was carried out by using the chemiluminescence antidigoxigenin-AMPPD protocol of Hoisington et al. (1994). A total of 123 maize DNA probes from the standard probe collection available at the University of Missouri, Columbia, was employed for screening the parents D06 and D408. The resulting 93 polymorphic RFLP probes were applied to the F_2 population.

Simple sequence repeat (SSR) analyses

In addition, two mapped microsatellite (SSR) markers were analyzed (see Fig. 1) The sequences of these primers were obtained

from the maize database and synthesized by Amersham Pharmacia Biotech (Freiburg, Germany). Polymerase chain reaction-amplification and MetaPhor gel-electrophoresis (FMC BioProducts, Rockland, Me.) were performed according to Lübberstedt et al. (1998).

Segregation and linkage analyses

Standard χ^2 tests were employed to test (1) the segregation at each marker for deviations from expected Mendelian segregations, and (2) the observed allele frequency for deviations from the expected allele frequency of 0.5. Because multiple tests were performed (corresponding to the number of marker loci), appropriate type-I error rates were determined by the sequentially rejective Bonferroni procedure described by Holm (1979). Estimates of the level of heterozygosity (%) of parental F_2 plants relative to the heterozygosity in the F_1 (F_1 = 100%) were obtained by dividing the observed number of heterozygous marker loci by the total number of scorable marker loci in the respective plant. Likewise, the percentage of the D06 genome in each F_2 plant was determined by dividing the sum of all D06 marker alleles by twice the number of scorable marker loci in the respective plant.

A linkage map based on 230 F_2 individuals and a total of 93 RFLP and two SSR marker loci was constructed by using the software package MAPMAKER3.0b (Lander et al. 1987) (Fig. 1). Linkage between two markers was declared significant in the two-point analyses when the LOD score ($\log_{10}$ of the likelihood odds ratio) exceeded the threshold of 3.0. After the determination of linkage groups and the correct linear arrangement of marker loci along the chromosomes, recombination frequencies between marker loci were estimated by multi-point analyses and transformed into centiMorgan (cM) by Haldane's (1919) mapping function.

Statistical analyses

Analyses of variance were performed on field data from each subplot within each environment. Adjusted entry means and effective error mean squares were used to compute the combined analyses of variance and covariance across environments. The sums of squares for entries were subdivided into the variation among F_3 families and orthogonal contrasts among (1) both parental lines, (2) the mean of the F_1 and F_2 generation means vs the midparental value ($\bar{P}$) and (3) the overall mean of F_3 families ($\bar{F}_3$) vs $\bar{P}$. A corresponding subdivision was conducted on the entry × environment sums of squares; *t*-tests were used on adjusted entry means across environments for testing the significance of transgressive segregation for the resistance traits as described by Groh et al. (1998a).

Components of variance for the F_3 families were computed considering all effects in the statistical model as random. Estimates of the variance components σ^2_e (error variance), σ^2_{ge} (genotype × environment interaction variance), and σ^2_g (genotypic variance) of F_3 families and their standard errors were calculated as described by Searle (1971, p. 475). Heritabilities ($\hat{h}^2$) for F_3 families were calculated on an entry mean basis (i.e. the average across repetitions and environments) and exact confidence intervals on $\hat{h}^2$ were estimated according to Knapp et al. (1985). Phenotypic ($\hat{r}_p$) and genetic ($\hat{r}_g$) correlation coefficients for F_3 families were calculated among resistance and agronomic traits by applying standard procedures (Mode and Robindon 1959). Estimates of r_g were calculated only when estimates of σ^2_g were significantly ($P< 0.01$) greater than zero for both traits under consideration.

The QTL analyses were performed with a subset of 226 F_3 families for which both complete molecular and phenotypic data were available. The method of composite interval mapping was employed for QTL detection and estimation of their effects as described by Bohn et al. (1997). A LOD threshold of 2.5, corresponding to a comparison-wise type-I error of $P_c< 0.0032$, was chosen for declaring a putative QTL as significant and to ensure a genome-wise type-I error of $P_g<0.30$. Cofactors were selected by stepwise regression. Final selection was for the model that minimized Akaike's information criterion with a penalty =1 (Jansen 1993). The joint analyses of QTLs for agronomic and resistance traits were based on the F_3 family average across the two environments. QTL positions were determined at the local maxima of the LOD-curve plot in the region under consideration. The phenotypic

Table 1 Means of inbred lines D06 and D408, the F_1, F_2 generation, and 226 F_3 families derived from their cross, as well as estimates of variance components and heritabilities among F_3 families for ECB tunnel length (TL), stalk damage ratings (SDR) and relative grain yield (RGY), as well as grain yield under infestation (GYI) and protection (GYP), date of anthesis (ANT), and in vitro digestibility of organic matter (IVDOM) of stover, evaluated at two environments in 1995

Parameters	Entries (no.)	Resistance traits			Agronomic traits			
		TL (%)	SDR (1–9 scale)	RGY (%)	GYI (t ha^{-1})	GYP (t ha^{-1})	ANT (d)	IVDOM (%)
Means[a]								
D06	2	4.1±1.2	1.8±0.2	110.7±18.0	3.5±0.3	3.2±0.3	88.8±7.0	58.4±0.5
D408	2	6.1±1.2	3.3±0.2	58.1±18.0	2.0±0.3	3.4±0.3	87.3±7.0	63.2±0.5
$\bar{P}$[b]	4	5.1±0.6a[c]	2.5±0.1a	84.4±37.7a	2.8±0.2c	3.3±0.2c	88.1±3.5a	60.7±0.4a
$\bar{F}_1$	3	5.0±0.8a	3.0±0.1a	92.4±50.3a	8.3±0.3a	9.0±0.2a	80.6±4.7b	57.1±0.5b
$\bar{F}_2$	3	6.3±0.8a	3.4±0.1a	85.9±50.3a	5.0±0.3b	5.8±0.2b	83.4±4.7ab	58.3±0.5b
$\bar{F}_3$	230	5.5±0.0a	3.0±0.0a	83.3±0.6a	3.8±0.0bc	4.5±0.0bc	86.5±0.1ab	58.6±0.0ab
Variance components (F_3 families)								
$\hat{\sigma}^2_g$		1.0±0.3**	0.28±0.05**	50.0±18.0**	0.57±0.07**	0.53±0.07**	4.2±0.5**	1.7±0.2**
$\hat{\sigma}^2_{ge}$		0.4±0.3	0.03±0.04	64.0±23.0**	0.11±0.03**	0.14±0.03**	0.7±0.1**	0.3±0.1**
$\hat{\sigma}^2_e$		4.9±0.1	0.70±0.03	326.0±11.0	0.40±0.02	0.36±0.01	1.6±0.1	1.6±0.1
Heritability (F_3 families)								
$\hat{h}^2$		0.4	0.59	0.31	0.79	0.77	0.85	0.74
90% C.I. on $\hat{h}^2$		(0.23; 0.54)	(0.47; 0.68)	(0.11; 0.46)	(0.72; 0.83)	(0.70; 0.82)	(0.81; 0.87)	(0.69; 0.81)

** Variance component was significant at the 0.01 probability level

[a] Standard errors are attached

[b] $\bar{P}$ = mean of D06 and D408

[c] Mean values with a different letter are significantly different at the 0.05 probability level

variance ($\hat{\sigma}^2_p$) explained by the i[th] QTL was obtained by the square of the partial correlation coefficient (R^2). The proportion of the genotypic variance explained by all QTLs (Q^2), as well as the presence of QTL × environment interactions was determined as described by Bohn et al. (1996). All computations were performed with software PLABQTL (Utz and Melchinger 1996).

Results

Phenotypic data

Means of the resistant parent D06 and the susceptible parent D408 differed significantly (P <0.05) for stalk damage ratings (SDR) and relative grain yield (RGY) but not for tunnel length (TL) (Table 1). No significant differences among the midparent value and the F_1, F_2, and F_3 generation means, and consequently no significant midparent heterosis, were observed for these traits. The distribution of the phenotypic means of F_3 families for tunnel length, stalk damage ratings, and relative grain yield followed an approximately normal Gaussian distribution (Fig. 2). Transgressive segregation was observed for tunnel length towards susceptibility.

No significant differences between the means of parents D06 and D408 were detected for grain yield under insecticide protection and the date of anthesis. The susceptible parent D408 had a significantly lower grain yield under infestation and a higher IVDOM than the resistant parent D06 (Table 1). The overall mean of F_3 families for grain yield under protection (GYP) and grain yield under infestation (GYI) was significantly larger than the mean performance of the two parents. The phenotypic mean of the F_2 generation was not significantly different from the overall mean of F_3 families for all agronomic traits. All agronomic traits showed high levels of heterosis except for anthesis. For IVDOM, negative heterosis was observed and the overall F_3 mean was not significantly different from the mean performance of the two parental lines.

Genotypic variances among F_3 families were highly significant (P<0.01) for all traits (Table 1). Genotype × environment interaction variances were significant (P<0.01) except for tunnel length and stalk damage ratings. Heritabilities were low to moderate ($0.31 \leq \hat{h}^2 \leq 0.59$) for resistance traits and high ($0.74 \leq \hat{h}^2 \leq 0.89$) for agronomic traits.

Correlations among resistance traits in F_3 lines families were highly significant (P <0.01) but of moderate size (Table 2). Relative grain yield was negatively correlated with tunnel length and stalk damage ratings. Tunnel length and stalk damage ratings showed a positive association. Correlations among agronomic traits and between resistance and agronomic traits were moderate to low. Correlations of the tunnel length and damage ratings of stalks were positive with grain yield under protection but negative with anthesis and IVDOM. Grain yield under infestation was not associated with tunnel length and stalk damage ratings. The relative heterozygosity of F_2 plants (determined from marker data) was significantly (P<0.01) positively correlated with grain yield under protection and grain yield under infestation, but not with any resistance trait. However, the estimated percentage of the D06 genome in the F_2 plants showed significant negative correlations with tunnel length and

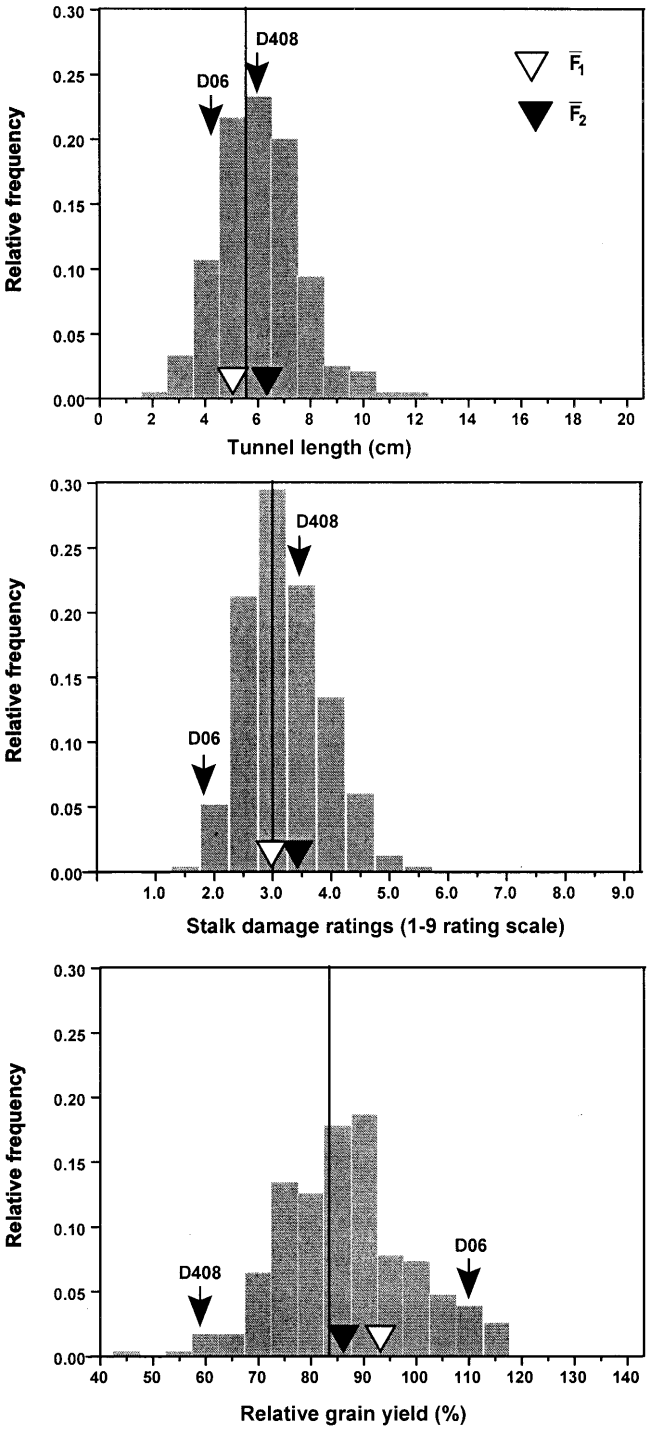

Fig. 2 Histograms for stalk damage ratings, tunnel length, and relative grain yield measured in two environments in 1995, for means of 226 F_3 families derived from the cross D06 × D408. The overall mean is indicated by a *solid line*. *Arrows* indicate the means of parental lines D06 and D408, and *triangles* represent the F_1 and F_2 generation means. Standard errors and means are given in Table 1.

Table 2 Phenotypic ($\hat{r}_p$) and genetic ($\hat{r}_g$, in bold letters) correlation coefficients among resistance and agronomic traits estimated in a population of 226 F_3 lines derived from the cross D06 × D408, and $\hat{r}_p$ between resistance and agronomic traits of F_3 lines and RFLP data of their parental F_2 plants

	Resistance traits			Agronomic traits				Genome composition	
	TL[a]	SDR	RGY	GYI	GYP	ANT	IVDOM	%Het.	%P1
TL		0.51**	–0.24*	–0.08	0.16*	–0.33**	–0.18**	–0.05	–0.15*
SDR	**0.70**$^{++}$		–0.31**	–0.10	0.26**	–0.29**	–0.15*	–0.01	–0.20**
RGY	**–0.43**$^{++}$	**–0.65**$^{++}$		0.56**	–0.16*	0.19**	0.04	–0.06	0.14*
GYI	**0.01**	**–0.08**	**0.73**$^{++}$		0.66*	0.03	–0.22**	0.23**	0.08
GYP	**0.32**$^{++}$	**0.37**$^{++}$	**–0.27**$^{+}$	**0.85**$^{++}$		–0.22**	–0.36**	0.32**	–0.08
ANT	**–0.49**$^{++}$	**–0.37**$^{++}$	**0.27**$^{+}$	**0.09**$^{+}$	**–0.18**$^{+}$		0.27**	–0.16*	0.06
IVDOM	–	–	–	–	–	–	–	–0.12	–0.01

*, ** Phenotypic correlation was significant at the 0.05 and 0.01 probability levels, respectively

$^{+}$, $^{++}$ Genetic correlation exceeded one-time or two-times its standard error, respectively

[a] TL = tunnel length, SDR = stalk damage ratings, RGY = relative grain yield, GYI = grain yield under infestation, GYP = grain yield under protection, ANT = date of anthesis; IVDOM = in vitro digestibility of organic matter of stover, %Het. = level of heterozygosity of parental F_2 individual, %P1 = percentage of genome from the susceptible parent D408 in the parental F_2 individual

Table 3 Parameters associated with QTLs for tunnel length and stalk damage ratings. Parameters were estimated from phenotypic data of 226 F_3 families from the cross D06 × D408 evaluated at two environments in 1995

Bin[b]	Posn.	Marker interval	LOD at QTL position	Genetic effect[a]		Gene action[c]	$\hat{R}^{2}$[d]
				Add.	Dom.		
Tunnel length				cm			
1.07/8	204	*umc33–umc83*	4.19	0.57	NS	A	6.6
3.09	308	*umc63–umc96*	4.26	0.26	3.44	OD	6.3
5.03	78	*umc12d–umc43*	3.15	–0.61	NS	A	5.4
5.05	102	*phi096–umc116c*	3.63	0.60	NS	A	3.5
9.03	84	umc20	3.49	0.52	0.49	D	7.4
10.08	118	umc64	2.81	0.57	NS	A	8.1
Total[e]							54.8
Stalk damage rating				1–9 scale			
1.05	166	*bnl5.59–umc58*	2.84	0.26	–0.34	OD	5.6
5.05	144	*bnl5.71–umc54*	2.89	0.13	NS	A	5.7
5.07	200	*umc108–umc68*	3.31	0.18	–0.29	OD	6.5
6.07	144	*umc132–umc62*	7.13	0.26	0.29	OD	13.5
8.05	58	*umc12a–umc49*	5.52	–0.27	–0.29	D	10.6
Total							51.5

[a] Genectic effects were estimated in a simultaneous fit using multiple regression

[b] Bin locations are designated by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner et al. 1993)

[c] A= additive gene action, ($|d_k/a_k| < 0.2$) or d_k, was not significantly different from zero, PD = partial dominance ($0.2 < |d_k/a_k < 0.8$), D = dominance ($0.8 < |d_k/a_k < 1.2$), OD = overdominance ($|d_k/a_k| > 1.2$)

[d] $\hat{R}^2$= proportion of phenotypic variance explained by the respective QTL

[e] $\hat{Q}^2$= proportion of $\hat{\sigma}^2_g$ explained by the respective QTL

stalk damage ratings (P <0.01) and a positive correlation with relative grain yield.

RFLP marker data

Ten out of the ninety five marker loci showed significant (P <0.001) deviations from Mendelian segregation ratios (see Fig. 1) and two markers deviated significantly (P <0.001) from their expected allele frequency. In most cases, the frequency of genotypes homozygous for the allele from the susceptible parent D408 was reduced. The level of heterozygosity in F_2 plants also displayed a normal distribution and varied from 26.1 to 76.5% with $\bar{x}$ = 49.0% and SD = 8.6%. Large marker intervals that span a distance of more than 50 cM were obtained on chromosomes 1, 3, 4, 7, 8 and 10 one or two marker intervals were detected with not significantly linked (LOD <3.0) flanking markers (Fig. 1). As a consequence, two to three partial linkage groups per chromosome were found. However, the mapping data supported the combining of these partial linkage groups into one linkage group in accordance with published maps (Gardiner et al. 1993). Including all marker intervals with LOD >2.0,

Table 4 Parameters associated with QTLs for grain yield under insecticide protection and infestation with ECB larvae, date of anthesis, and in vitro digestibility of organic matter (IVDOM) of stover. Parameters were estimated from phenotypic data of 226 F_3 families from the cross D06 × D408 evaluated at two locations in 1995

Bin[b]	Posn.	Marker interval	LOD at QTL position	Genetic effect[a]		Gene action[c]	$\hat{R}^{2}$[d]
				Add.	Dom.		
Grain yield under protection				t ha^{-1}			
1.02	34	*umc157–umc76*	5.04	–0.25	0.74	OD	11.8
1.06	162	*umc67–bnl5.59*	2.55	0.14	0.42	OD	5.5
3.01	38	*umc44–umc32*	2.63	0.12	0.28	OD	3.4
9.01	12	*umc109–umc113*	2.57	0.17	0.42	OD	4.2
9.03	74	*umc105–umc38*	3.93	–0.08	0.36	OD	0.8
Total[e]							28.9
Grain yield under infestation				t ha^{-1}			
9.01/2	32	*umc113–umc115*	3.30	0.3	0.3	D	6.5
9.03	78	*umc105–umc38*	3.10	–0.2	0.4	OD	4.2
Total							12.5
Date of anthesis				d			
1.02/3	52	*umc157–umc76*	10.79	1.5	NS	A	20.6
1.07/8	198	*umc33–umc83*	5.11	–0.6	NS	A	7.3
2.04/5	108	*umc34–npi285b*	5.17	0.8	NS	A	3.6
3.04	112	*csu16a–umc92*	6.90	1.0	–0.9	D	12.1
3.08	278	*umc16a–umc63*	9.50	–1.0	NS	A	15.6
4.03	186	*npi386a–umc52*	4.42	0.5	–1.6	OD	4.0
4.11	304	*umc111*	3.41	0.8	NS	A	4.8
5.07	198	*umc108–umc68*	3.37	–0.5	NS	A	4.4
6.01	4	*bnl6.29–umc116c*	3.02	NS	–1.3	OD	3.1
6.06/7	132	*umc38a–umc36d*	6.16	–1.0	1.5	OD	13.4
8.04	32	*bnl9.44–umc12a*	5.40	0.9	NS	A	9.7
10.08	106	*umc130–umc64*	6.36	–0.8	NS	A	9.0
Total				1.5	–2.2		57.3
IVDOM				%			
2.03	46	*umc6–umc34*	14.60	0.93	–0.68	D	18.8
5.02	52	*umc107b–umc12d*	3.35	0.39	NS	A	3.0
5.07	190	*umc108–umc68*	3.83	–0.57	NS	A	9.1
6.07/8	164	*umc62–asg7*	3.90	0.44	NS	A	5.5
8.03	22	*bnl9.44–umc12a*	2.72	0.42	NS	A	4.8
10.08	118	*umc64*	3.06	–0.47	0.36	D	3.6
Total				1.14	–1.04		36.3

[a] Genetic effects were estimated in a final simultaneous fit using multiple regression

[b] Bin locations are designated by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner et al. 1993)

[c] A= additive gene action, ($|d_k/a_k| < 0.2$) or d_k, was not significantly different from zero, PD = partial dominance ($0.2 < |d_k/a_k < 0.8$), D = dominance ($0.8 < |d_k/a_k < 1.2$), OD = overdominance ($|d_k/a_k| > 1.2$)

[d] $\hat{R}^2$= proportion of phenotypic variance explained by the respective QTL

[e] $\hat{Q}^2$= proportion of $\hat{\sigma}^2_g$ explained by the respective QTL

the marker loci span a map distance of 1575 cM with an average interval length of 20.5 cM.

QTL analyses for ECB resistance traits

For tunnel length, six putative QTLs located on chromosomes 1, 3, 5 (two QTLs), 9 and 10 were identified, explaining between 3.5 and 8.1% of $\hat{\sigma}^2_p$ (Table 3). All putative QTLs for tunnel length explained in a simultaneous fit 54.8% of $\hat{\sigma}^2_g$. For stalk damage ratings, five putative QTL were detected on chromosomes 1, 5 (two QTL), 6 and 8, explaining between 5.6 and 12.8% of $\hat{\sigma}^2_p$ and simultaneously 51.5% of $\hat{\sigma}^2_g$. No QTL was detected for relative grain yield. Most putative QTL for tunnel length showed additive gene action, whereas QTL for stalk damage ratings displayed mostly dominance to overdominance. For both resistance traits most of the alleles increasing the resistance against ECB were contributed by the resistant parent D06. However, at one QTL for tunnel length and one QTL for stalk damage ratings the resistance allele originated from the susceptible parent D408 (Table 3). Although moderate genetic correlations between resistance traits were found for tunnel length and stalk damage ratings, only one QTL on chromosome 5 was identified in the same chromosomal bin (5.05).

QTL analyses for agronomic traits

For grain yield under protection, five putative QTLs located on chromosomes 1 (two QTLs), 3 and 9 (two QTLs) were identified explaining up to 11.8% of $\hat{\sigma}^2_p$ (Table 4). Two putative QTLs for grain yield under infestation were detected on chromosome 9, which explained 4.2 and 6.5% of $\hat{\sigma}^2_p$. Twelve QTLs on chromosomes 1, 2, 3, 4, 5, 6, 8, 9 and 10 were found for anthesis. They explained 3.1 to 20.6% of $\hat{\sigma}^2_p$. For IVDOM, six putative QTLs located on chromosomes 2, 5 (two QTLs), 6, 8 and 10 were identified explaining between 3.0 and 18.8% of $\hat{\sigma}^2_p$.

In total, the putative QTLs explained between 12.5% (for grain yield under infestation) and 57.3% (for anthesis) of $\hat{\sigma}^2_g$. Most QTLs involved in the inheritance of grain yield under protection and grain yield under infestation showed dominance to overdominance. For date of anthesis 8 out of 12 QTLs, and for IVDOM four out of six putative QTLs, displayed additive gene action. Common QTLs for tunnel length and anthesis were found on chromosomes 1, 3 and 10; at each common QTL position, alleles with a positive effect on the respective traits were contributed by different parents (Tables 3 and 4).

Discussion

Comparison of QTL for ECB resistance in different populations

Considering the stage of plant development, when the ECB larvae feed on the maize plant, ECB resistance of maize in Central Europe corresponds to the 2nd-generation ECB resistance in U.S. Cornbelt maize. First results on genomic regions affecting ECB resistance of the resistant U.S. inbred B52 were obtained with translocation stocks (Onukogu et al. 1978). B52 and the resistant inbreds DE811 and Mo47 were also most extensively investigated in QTL studies in crosses with the susceptible inbreds B73 and Mo17 (Lee 1993; Schön et al. 1993; Jampatong 1999). In total, 26 QTLs for 2nd-generation ECB resistance were detected. In accordance with the translocation study, the most important QTL were detected on chromosomes 1 and 2.

In this study, we identified six QTLs for tunnel length and five QTLs for stalk damage ratings, which explained approximately one-half of the genotypic variance in F_3 families derived from the cross D06 × D408. Common QTL positions for tunnel length across U.S. Cornbelt and European maize germplasm were detected in chromosomal bins 1.07, 5.05 and 9.03/4, whereas no common QTL was detected for stalk damage ratings. However, QTLs for stalk damage ratings on chromosomes 5 and 6 were also detected in chromosomal bins carrying QTLs for tunnel length. In general, the agreement of QTL results across the different populations and resistance traits was low. Several reasons may explain the observed lack of consistency.

Resistance to 2nd-generation ECB was determined by tunnel length (Lee 1993; Schön et al. 1993; Jampatong 1999) and visual rating on a 1–9 rating scale. Both traits indirectly determine the level of antibiosis but their genetic basis is partly different as indicated by the moderate genetic correlation ($\hat{r}_g = 0.70$) found in our study. Therefore, partly different sets of QTLs are involved in the inheritance of both resistance traits.

Another important reason for the poor consistency of QTL mapping-results across different populations is the low power of QTL detection (Melchinger et al. 1998b). Because ECB resistance traits generally display a low heritability (Table 1), only a moderate to low power of QTL detection can be anticipated with the population sizes ($150 \leq N \leq 250$) employed in most studies. In fact, the estimated values of Q^2 for the resistance traits (Table 3) indicate that about half of the genotypic variance remained unexplained. In addition, with small sample sizes in the mapping population, there is a high risk for a large upward bias in the estimated QTL effects (Melchinger et al. 1998b), which can further reduce the observed consistency across populations. Use of large mapping populations evaluated in a greater number of environments (to warrant a high h^2) could remedy these problems but would be prohibitive considering the high costs for ECB larvae and the considerable labor involved in the evaluation of traits such as tunnel length. An alternative would be the use of indirect traits associated with insect resistance in maize, such as protein concentration in the leaves or leaf toughness (Groh et al. 1998b), which can be measured with high precision and h^2.

QTLs can only be detected if the parental lines contribute different alleles for the trait under study. Therefore, by choosing parents representing extremes for the trait under study, the chances of QTL detection can be increased (Lander and Botstein 1989). The two parents of our mapping population were chosen as extremes from a random set of 115 early maturing European elite maize inbreds evaluated for ECB resistance (Melchinger et al. 1998a). However, the occurrence of transgressive susceptible F_3 families for tunnel length (Fig. 2) suggests that the resistant parent D06 carried some QTL alleles for susceptibility and the susceptible parent D408 carried some resistance alleles. This was confirmed by the result that two QTLs on chromosome 5 for tunnel length were linked in repulsion phase and one out of five QTLs reducing stalk damage originated from the susceptible parent D408 (Table 3). In agreement with our findings, most of the QTL alleles for 2nd-generation ECB resistance in U.S. maize originated from the resistant inbreds B52, DE811 and Mo47, but a few resistance QTL alleles were also found in the susceptible parents (Lee 1993; Schön et al. 1993; Jampatong 1999).

Epistatic effects between QTLs in each of the mapping populations may also account for the observed lack of consistency. In the case of epistasis, the difference between QTL genotype classes depends on other QTLs segregating in the genetic background (Stuber 1995). However, no significant digenic epistatic effects were

detected among the putative resistance QTLs, suggesting a minor importance of epistasis for ECB resistance in the cross D06 × D408.

Several novel ECB resistance QTLs were detected in this study. In simulation experiments it was shown that most QTLs detected in F_2 populations are not false-positives (Beavis 1994). Therefore, these additional QTLs most-likely extend the known pool of loci for improving ECB resistance in maize. Some of these novel ECB resistance QTLs were detected in chromosomal regions with known resistance QTLs against the tropical corn borer species *Diatraea grandiosella* and *Diatraea saccharalis* (chromosomal bins 3.08/9, 6.07/8 and 8.05/6) (Bohn et al. 1997). This is in agreement with previous reports on clustered QTLs involved in resistance of maize against ECB and tropical corn borer species on chromosomes 1, 3, 5, 7, 8, 9 and 10 (see Fig. 1) (Bohn et al. 1997).

No QTL was found for relative grain yield, the direct measure for tolerance. This can be explained by the low $\hat{h}^2$ for this trait caused by its high standard error, which resulted in a low power of QTL detection.

Mode of inheritance

From generation-mean analyses and diallel studies, it was concluded that mainly additive gene action, and to smaller extent also dominance and epistasis, were involved in resistance against 2nd-generation ECB in U.S. germplasm (Jennings et al. 1974). In agreement with these results and previous QTL mapping studies (Lee 1993; Schön et al. 1993), in our cross of two European dent inbreds we confirmed that the majority of QTLs for tunnel length displayed additive gene action. By contrast, QTLs for stalk damage ratings showed dominance or overdominance for both resistance and susceptibility.

Resistance mechanisms

In this study, we indirectly determined the level of expressed antibiosis by tunnel length and stalk damage ratings. Bergvinson et al. (1996) proposed that mechanisms of insect resistance in maize include total cell protein, fiber, and cell-wall phenolic-acid contents as well as peroxidase-mediated production of dehydrodiferulic acid. In a recent study, the association between plant cell-wall composition and 2nd-generation ECB resistance was confirmed but the relationship was found to be highly influenced by the genetic background (Ostrader and Coors 1997). In contrast to Bohn et al. (1996), who identified QTLs for resistance against 1st-generation *D. grandiosella* and *D. saccharalis* in genomic regions on chromosomes 1, 5 and 9 known to carry genes involved in cell-wall biochemistry (i.e. *bk2*, *bm1*, *bm2*), no QTLs for antibiosis mapped to these regions in our study. Therefore, based on the high level of ECB resistance of the parent D06 in combination with its moderate level of IVDOM, it can be hypothesized that resistance mechanisms other than cell-wall fortification are active in the resistant parent D06. This hypothesis is corroborated by the negative correlations found between resistance traits measuring antibiosis and IVDOM (Table 2).

Correlations between resistance and agronomic traits

The negative correlation between the resistance traits and the date of anthesis was reflected in six common QTL positions on chromosomes 1, 3, 5, 6, 8 and 10 (Tables 3 and 4), with an opposite sign of the additive effects. Negative correlations between ECB resistance and the date of anthesis are a common observation in European and U.S. maize germplasm (Russell et al. 1974). Most likely, this is attributable to the better stalk quality of late-maturing genotypes (Hudon and Chiang 1991). If this association is not caused by pleiotropy, but rather by linkage, it will be difficult in conventional breeding to combine the desired alleles for both traits into a single genotype. However, based on graphical genotypes several F_3 families were detected in our study, which showed a high level of ECB resistance associated with early maturity (2–4 days earlier than D408). This demonstrates the potential of molecular markers to identify genotypes with the necessary recombination events between tightly linked QTLs for ECB resistance and maturity.

Marker-assisted selection (MAS) for ECB resistance in maize

Based on theoretical expectations (Lande and Thompson 1990), MAS is most promising for those traits where h^2 is low and Q^2 is high. In our study the ratio $RE = Q^2/h^2$, which is a measure for the relative efficiency of MAS over conventional phenotypic selection (assuming identical selection intensities for both schemes), was 1.37 for tunnel length. This suggests that MAS should be superior over phenotypic selection for this trait, if $\hat{Q}^2$ is estimated without bias. Although *RE* was only 0.87 for stalk damage ratings, MAS may be competitive over conventional selection, which requires costly mass-rearing of insect larvae unless test sites with high natural ECB occurrence are available. Even with a low *RE* value, MAS for insect resistance can be competitive when cost-effective PCR-based marker systems are applied. However, it remains to be established whether the putative QTL regions for ECB resistance detected in the D06 × D408 population are consistent across other early maturing European maize germplasms.

The effectiveness of MAS strongly depends on the accuracy of QTL mapping results. There is a high risk that estimated QTL effects are inflated unless they were estimated either from a very large mapping population or from an independent sample (Melchinger et al. 1998b). Therefore, the prospects of MAS have to be evaluated with caution and additional field trials for ECB resistance should be conducted with the genotypes selected by MAS to confirm the predicted selection response.

Besides the quantitative host-plant ECB resistance, an alternative approach pursued in recent years is the development of transgenic maize hybrids, which carry the *cry-IA* resistance genes isolated from *B. thuringiensis*. With this approach, the mortality of ECB larvae exceeds 99% (Gould 1998) but entails a high risk of being soon overcome by resistant ECB genotypes. One possibility to increase the durability of the Bt resistance genes introduced by genetic engineering would be their marker-assisted backcrossing (MAB) into genotypes with high levels of natural host-plant resistance, because the latter should be more durable owing to its polygenic nature. Thus, by integrating MAB for a single transgene with MAS for resistance QTLs it should be possible to combine the advantages of both approaches.

Acknowledgements This research was supported by grants from the German Ministry of Education and Research (BMBF) and the Gemeinschaft zur Förderung der Privaten Deutschen Pflanzenzüchtung e.V. (GFP). Special thanks to Fa. Südwestdeutsche Saatzucht Dr. R. H. Späth, Rastatt, for providing a field test location at Scherzheim. The authors acknowledge the excellent technical assistance in laboratory work of E. Kokai-Kota. We also thank A. Karg and S. Pluskat for their skilled assistance in conducting the field experiments.

References

Armstrong CL, Parker CB, Pershing JC, Brown SM, Sanders PR, Duncan DR, Stone T, Dean DA, Deboer DL, Hart J et al. (1995) Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringensis*. Crop Sci 35:550–557

Beavis B (1994) The power and deceit of QTL experiments. ASTA Meetings. Chicago, Illinois, December 6–8, 1994

Bergvinson DR, Arnason JT, Pietrzak LN (1994) Localization and quantification of cell-wall phenolics in European corn borer resistant and susceptible maize inbreds. Can J Bot 72:1243–1249

Bergvinson DJ, Arnason JT, Mihm JA, Jewell DC (1996) Phytochemical basis for multiple borer resistance in maize. Insect-resistant maize: recent advances and utilization. Proc Int Symposium. Mexico, D.F. 1995. CIMMYT, pp

Bohn M, Khairallah MM, Gonzales-de-Leon D, Hoisington DA, Utz HF, Deutsch JA, Jewell DC, Mihm JA, Melchinger AE (1996) QTL mapping in tropical maize: I. Genomic regions affecting leaf feeding resistance to sugarcane borer and other traits. Crop Sci 36:1352–1361

Bohn M, Khairallah MM, Jiang C, Gonzales-de-Leon D, Hoisington DA, Utz HF, Deutsch JA, Jewell DC, Mihm JA, Melchinger AE (1997) QTL mapping in tropical maize. II. Comparison of genomic regions for resistance to *Diatraea* spp. Crop Sci

Bohn M, Kreps RC, Klein D, Melchinger AE (1999) Damage and grain yield losses caused by European corn borer (Lepidoptera: Pyralidae) in early maturing European maize hybrids. J Econ Entomol 92:723–731

Chiang HC, Hodson AC (1950) Stalk breakage caused by European corn borer and its efects on harvesting of field corn. J Econ Entomol 43:415–422

Degenhardt H (1996) NIRS-Untersuchungen zur Erfassung futterwertrelevanter Qualitätsparameter von Silomaissorten in einem Gerätenetzwerk. Landbauforschung Völkenrode, Sonderheft 163. PhD thesis, Martin-Luther Universität, Halle-Wittenberg, Germany

Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized F_2-population. Genetics 134:917–930

Groh S, Gonzales-de-Leon D, Khairallah MM, Jiang C, Brgvinson D, Bohn M, Hoisington DA, Melchinger AE (1998a) QTL mapping in tropical maize. III. Genomic regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. Crop Sci 38:1062–1072

Groh S, Khairallah MM, Gonzales-de-Leon D, Willcox M, Jiang C, Hoisington DA, Melchinger AE (1998b) Comparison of QTLs mapped in RILs and their testcross progenies of tropical maize for insect resistance and agronomic traits. Plant Breed 117:193–202

Gould F (1998) Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu Rev Entomol 43: 701–726

Haldane JBS (1919) The combination of linkage values, and the calcualtion of distance between the loci of linked factors. J Genet 8:299–309

Hoisington DA, Khairallah MM, Gonzales-de-Leon D (1994) Laboratory protocols, 2nd edn. CIMMYT Applied Molecular Genetics Laboratory, Mexico, D.F.: CIMMYT

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Statist 6:65–70

Hudon M, Chiang MS (1991) Evaluation of resistance of maize germplasm to the univoltine European corn borer *Ostrinia nubilalis* (Hübner) and relationship with maize maturity in Quebec. Maydica 36:69–74

Jampatong C (1999) Quantitative trait loci for first- and second-generation European corn borer resistance in maize. PhD thesis, University of Missouri, Columbia, Missouri, USA

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205–211

Jarvis JL, Clark RL, Guthrie WD, Berry EC, Russell WA (1984) The relationship between second-generation European corn borers and stalk rot fungi in maize hybrids. Maydica 24: 247–263

Jennings CW, Russell WA, Guthrie WD (1974) Genetics of resistance in maize to first- and second-brood European corn borer. Crop Sci 14:394–398

Khairallah MM, Bohn M, Jiang C, Deutsch JA, Jewell DC, Mihm JA, Melchinger AE, Gonzales-de-Leon D, Hoisington DA (1998) Molecular mapping of QTLs for southwestern corn borer resistance, plant height, and flowering in tropical maize. Plant Breed 117:309–318

Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25: 192–194

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124: 743–756

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lee M (1993) Genetic analysis of resistance to European corn borer and northern corn leaf blight in maize. In: Wilkinson D (ed). Proc 48th Annu Corn and Sorghum Industry Research Conf. American Seed Trade Association, Washington, D.C., pp 213–223

Lübberstedt T, Dussle C, Melchinger AE (1998) Application of microsatellites from maize to teosinte and other relatives of maize. Plant Breed 117:447–450

Melchinger AE, Kreps R, Späth R, Klein D, Schulz B (1998a) Evaluation of early maturing European maize inbreds for resistance to the European corn borer. Euphytica 99:115–125

Melchinger AE, Utz HF, Schön CC (1988b) Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals the low power of QTL detection and large bias in estimates of QTL effects. Genetics 149:383–403

Mode CJ, Robinson HF (1959) Pleiotropism and genetic variance and covariance. Biometrics 15:518–537

Onukogu FA, Guthrie WD, Russell WA, Reed GL, Robbins JC (1978) Location of genes that condition resistance in maize to sheath-collar feeding by second-generation European corn borer. J Econ Entomol 71:1–4

Ostrander BM, Coors JG (1997) Relationships between plant composition and European corn borer resistance in three maize populations. Crop Sci 37:1741–1745

Russell WA, Guthrie WD, Grindeland RL (1974) Breeding for resistance in maize to first and second broods of the European corn borer. Crop Sci 725–727

Schön CC, Lee M, Melchinger AE, Guthrie WD, Woodman W (1993) Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70:648–659

Searle SR (1971) Linear models. John Wiley and Sons, New York

Stuber CW (1995) Mapping and manipulating quantitative traits in maize. Trends Genet 11:477–481

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTLs. J Quant Trait Loci 2: