Published online June 20, 2006

QTL Mapping for Fusarium Ear Rot and Fumonisin Contamination Resistance in Two Maize Populations

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ABSTRACT

Fusarium verticillioides **(Sacc.) Nirenberg (synonym** ***F. moniliforme*** **Sheldon) (teleomorph:** ***Gibberella moniliformis*****) and** ***F. proliferatum*** **(Matsushima) Nirenberg (teleomorph:** ***G. intermedia*****) are fungal pathogens of maize (*Zea mays* L.) that cause ear rot and contaminate grain with fumonisins, mycotoxins that can harm animals and humans. The objective of this study was to identify quantitative trait loci (QTL) for resistance to Fusarium ear rot and fumonisin contamination in two maize populations, comprised of 213 $BC_1F_{1:2}$ families from the first backcross of GE440 to FR1064 (GEFR) and 143 recombinant inbred lines from the cross of NC300 to B104 (NCB). QTL mapping was used to study the genetic relationships between resistances to ear rot and fumonisin contamination and to investigate consistency of QTL across populations. In the GEFR population, seven QTL explained 47% of the phenotypic variation for mean ear rot resistance and nine QTL with one epistatic interaction explained 67% of the variation for mean fumonisin concentration. In the NCB population, five QTL explained 31% of the phenotypic variation for mean ear rot resistance and six QTL and three epistatic interactions explained 81% of the phenotypic variation for mean fumonisin concentration. Eight QTL in the GEFR population and five QTL in the NCB population affected both disease traits. At least three ear rot and two fumonisin contamination resistance QTL mapped to similar positions in the two populations. Two QTL, localized to chromosomes four and five, appeared to be consistent for both traits across both populations.**

Fusarium verticillioides and *F. proliferatum* cause Fusarium ear rot of maize and contaminate the grain with fumonisins, a family of mycotoxins that affects animals and human health (Munkvold and Desjardins, 1997). These toxins are of increasing concern because of mounting evidence of their involvement in a number of human and animal diseases. These diseases include esophageal cancer (Gelderblom et al., 1988, Rheeder et al., 1992) and neural tube birth defects (Hendricks, 1999) in humans, and equine leukoencephalomalacia (Ross et al., 1992) and porcine pulmonary edema (Colvin and Harrison, 1992) in animals. The FDA has published a *Guidance for Industry* that suggests limiting fumonisin concentrations to between 2 and 4 $\mu g\ g^{-1}$ (ppm) for corn flour and other milled corn products used for human consumption (CFSAN, 2001a,2001b).

Quantitative genetic variation exists for resistance to Fusarium ear rot and fumonisin contamination among genotypes (Clements et al., 2004; Robertson et al., 2006) and both disease traits have moderate to high entry mean heritabilities (Robertson et al., 2006; Table 1). Therefore, we expect that phenotypic selection for improved resistance should be effective. However, practical difficulties complicate phenotypic selection for these traits. For example, although many diseases can be evaluated on juvenile plants or before flowering, ear rots and mycotoxin concentrations can only be evaluated on grain produced by mature plants. Also, whereas most polycyclic leaf diseases in maize can be reliably induced either by planting in a disease-prone location or by dropping sorghum grains colonized with fungi into juvenile plant whorls (Carson et al., 2004), two inoculations into ears with calibrated concentrations of liquid spore suspensions are often needed to obtain consistent Fusarium ear rot ratings (Clements et al., 2003a). Similarly, whereas relatively rapid and simple visual ratings can be used to accurately assess resistance to many plant diseases, evaluation of fumonisin contamination resistance requires time consuming and expensive toxin assays on precisely ground and weighed grain samples. Finally, whereas some diseases can be reliably evaluated in greenhouses and off-season nurseries, Fusarium ear rot and fumonisin contamination appear to be strongly affected by environmental factors, restricting their evaluation to target environments that are conducive to disease and mycotoxin accumulation (Shelby et al., 1994). Given the difficulties of phenotypically evaluating Fusarium ear rot and mycotoxin contamination, the availability of PCR-based DNA markers linked to genes with at least moderate effects on resistance could permit more efficient selection strategies based on marker genotyping (Holland, 2004; Robertson et al., 2005).

The effectiveness of marker-assisted selection for quantitative traits depends on the accuracy of QTL position and effect estimates (Holland, 2004). Detecting QTLs and accurately estimating their effects are more difficult for traits of lower heritability (Beavis, 1998). Pérez-Brito

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Published in Crop Sci. 46:1734–1743 (2006).
Crop Breeding & Genetics
doi:10.2135/cropsci2005.12-0450

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Abbreviations: ELISA, enzyme-linked immunosorbant assay; GEFR, (GE440 × FR1064) × FR1064; GEI, genotype × environment interaction; NCB, NC300 × B104; QTL, quantitative trait locus; RIL, Recombinant inbred lines.

Table 1. A summary of parental line means, overall mapping population means and entry mean heritabilities of, and genotypic correlations between, fumonisin content and Fusarium ear rot in the GEFR and NCB populations, based on field evaluations in four and three environments, respectively (Robertson et al., 2006). A small correction to the heritability calculations resulted in slightly higher heritability estimates in the NCB population than were reported in Robertson et al. (2006). The corrected estimates are reported in this table.

	GEFR population		NCB population	
	Fumonisin content	Fusarium ear rot	Fumonisin content	Fusarium ear rot
Parameter estimate	μg g^{-1}	%	μg g^{-1}	%
Mean of more resistant parent	2.2	11	0.8	5
Mean of more susceptible parent	27.1	22	4.0	13
Mean of mapping population	12.2	18	6.8	16
Entry mean h^2	0.75 (0.03)	0.47 (0.06)	0.88 (0.03)	0.86 (0.03)
Genotypic correlation between ear rot and fumonisin content	0.96 (0.07)		0.87 (0.04)	

et al. (2001) demonstrated that resistance to Fusarium ear rot in two tropical maize populations is polygenic with relatively low heritability. They concluded that because many genes, each with small effects, conferred ear rot resistance, and that QTLs for resistance were not consistent across populations, marker-assisted selection for Fusarium ear rot resistance was not likely feasible (Pérez-Brito et al., 2001). Robertson et al. (2006) found moderate to high entry mean heritabilities for fumonisin concentration in two populations of maize, comprised of 213 $BC_1F_{1:2}$ families from the first backcross of GE440 to FR1064 (GEFR) and 143 recombinant inbred lines from the cross NC300 × B104 (NCB) (Table 1). These heritabilities were greater than those reported by Pérez-Brito et al. (2001; $h^2 = 0.26$–0.42); therefore, we expect greater power to detect QTLs for Fusarium ear rot and fumonisin contamination resistance and accurately estimate their effects in the GEFR and NCB populations. Entry-mean heritabilities can be increased by reducing experimental error or by increasing the number of environments in which populations are evaluated (Holland et al., 2003). Entry mean heritabilities may have been higher in the study of Robertson et al. (2006) than in Pérez-Brito et al. (2001) in part because Robertson et al. (2006) used two artificial inoculations per plant and three to four evaluation environments compared to one inoculation per plant and two testing environments used by Pérez-Brito et al. (2001).

In addition to providing the opportunity to conduct marker-assisted selection, QTL mapping provides a powerful method to understand the genetic relationships between correlated traits. Although phenotypic correlations between Fusarium ear rot and fumonisin concentration tend to be low to moderate (Clements et al., 2003b; Clements et al., 2004) and fumonisin can accumulate to high levels in symptomless or only slightly rotted grain (Bacon and Hinton, 1996; Munkvold and Desjardins, 1997; Clements et al., 2004), Robertson et al. (2006) reported high genotypic correlations between Fusarium ear rot and fumonisin concentration (Table 1). These high genotypic correlations suggest that although Fusarium ear rot and fumonisin concentration are not highly phenotypically correlated, genotypes with greater potential to resist ear rot also tend to have greater potential to avoid contamination by fumonisin. Further, it suggests that the genetic components of resistance are largely the same for the two traits (Robertson et al., 2006).

The objectives of this project were to identify QTLs for resistance to Fusarium ear rot and fumonisin contamination in two maize populations, to evaluate the consistency of QTL positions across populations and environments and to evaluate the extent to which QTLs for ear rot resistance had pleiotropic effects on fumonisin contamination.

MATERIALS AND METHODS

Population Development

GE440 and NC300 were identified in preliminary studies as potential sources for resistance to Fusarium ear and kernel rot and low fumonisin contamination (Clements et al., 2001; Robertson et al., 2006). Two segregating populations, derived from the crosses GE440 × FR1064 and NC300 × B104, were created. Resistant inbred GE440 (derived from the open-pollinated variety Hastings Prolific) was crossed and backcrossed once to the susceptible inbred FR1064 (an improved B73 type), and BC_1F_1 plants were self-pollinated to form 213 $BC_1F_{1:2}$ families. This population will be referred to as the GEFR population. A second population was formed by crossing inbred NC300 (derived from the tropical hybrids Pioneer X105A, Pioneer X306B, and H5; Senior et al., 1998) to inbred B104 (derived from the BS13 strain of Iowa Stiff Stalk Synthetic; Hallauer et al., 1997). A random sample of F_2 plants from this cross were self-pollinated to form $F_{2:3}$ families. $F_{2:3}$ families were grown ear-to-row, and a single self-fertilized ear was harvested from each row without selection. This procedure was repeated until 143 F_6-derived recombinant inbred lines (RILs) were developed. F_7 or F_8 generation seeds were used in this study to represent the RILs. This population will be referred to as the NCB population.

GEFR Population Evaluation

In 2002, the GEFR population was grown at two locations, Mt. Olive, NC, and Urbana, IL. The experiment was planted in a randomized complete block design with two replications at each location. Plots consisted of single-rows containing individual $BC_1F_{1:2}$ families. In 2003, the GEFR population was grown at two locations, Clayton and Plymouth, NC, with single-row plots of each $BC_1F_{1:2}$ family replicated twice at each location. The treatment design in 2003 was a replication within sets design, wherein each $BC_1F_{1:2}$ family was randomly assigned to one of three sets. Each set also contained the two parent lines and seven check lines (B73, Mo17, K55, NC300, NC390, NC402, and Va35). The experimental design for each set was an 8×10 α-lattice with two replications at each location. Sets were planted in adjacent plots at each location. At all locations, plots were 3.05 m in length with 0.91 m between plots, and were overplanted and thinned to approximately 20 plants.

NCB Population Evaluation

In 2002, the NCB population was grown at Clayton, NC, with single row plots of each RIL in a randomized complete

block design replicated twice. Each complete block also contained the two parent lines. In the summer of 2003, the population was grown at two locations, Clayton and Plymouth, NC. The NCB population plus the two parent lines and the check inbred B73 were evaluated using a 12 × 12 lattice design with two replications at each location. At both locations, plots were 3.05 m in length with 0.91 m between plots. Plots were overplanted and thinned to approximately 20 plants.

Artificial Inoculation Techniques

To represent the heterogeneity of pathogen species in the natural environment, we inoculated ears with three isolates each of *F. verticillioides* and *F. proliferatum* (Clements et al., 2003a). *Fusarium verticillioides* isolates ISU95082, ISU94445, and ISU94040 and *F. proliferatum* isolates 310, 37–2, and 19 were removed from glycerol storage at −80°C and cultured separately on potato dextrose agar. Inoculum was prepared by washing conidia from the cultures with 0.05% (v/v) Triton X-100 (Fisher Biotech, Fairlawn, NJ) and diluting the resulting conidial suspension of the six isolates to a final concentration to approximately 1×10^6 conidia mL^{-1} in water.

At each inoculation, the primary ear of each plant was inoculated with 10 mL of inoculum. To reduce the chance of escapes, two inoculations, 1 wk apart, were performed on primary ears of all plants in each plot, except for the NCB population in 2002, where only 12 plants were inoculated per plot. Silk channel inoculation (injecting inoculum into the silk channel while the silks are fresh) was performed approximately 10 d after mean silking date for each experiment within each location, and direct ear inoculation (injecting inoculum directly through the husk onto the ear) was performed 7 d later (Clements et al., 2003a).

Phenotypic Data Collection

When all plants had reached physiological maturity, primary ears were hand-harvested and air-dried to approximately 140 g kg^{-1} moisture. After drying, ears were rated by visually identifying the percentage of kernels exhibiting visible Fusarium ear rot symptoms on each inoculated ear. Ear rot ratings were estimated in 5% increments. The grain from all inoculated ears within a plot was then shelled, bulked, and ground to a fine particle size with a Romer mill (Series II Mill, Romer Labs Inc., Union, MO). The fumonisin concentration was obtained from 25 g of ground grain from each plot, which was evaluated in triplicate by enzyme linked immunosorbant assay (ELISA) (Clements et al., 2003a). One family in the GEFR population and four lines in the NCB population were mostly barren in all environments, preventing measurement of ear rot or fumonisin concentration in those lines.

Genotyping and Linkage Map Construction

For the GEFR population, young leaves from eight plants per family and per parent line were sampled for DNA extraction, and for the NCB population, four plants per RIL and parental line were sampled for extraction. In the NCB population, 142 of the 143 lines were genotyped. DNA extractions were performed with DNeasy Plant Mini Kit system (Qiagen, Inc., Valencia, CA). Simple sequence repeat (SSR) primers were selected for screening in both populations with consensus map information from the Maize Genetics and Genomics Database (http://www.maizegdb.org/; verified 11 April 2006) to reference location of marker loci. Parental lines in both populations were screened with SSR primers to identify polymorphisms (Senior et al., 1998). Polymerase chain reaction products were separated by electrophoresis in 4% (w/v) SFR agarose gels (Amresco, Solon, OH) and visualized by staining gels with 0.05% (w/v) ethidium bromide and exposing them to ultraviolet light.

Locus orders and recombination frequencies were estimated using multipoint mapping in MapmakerEXP (Whitehead Institute, Cambridge, MA). Recombination frequencies were then transformed to centimorgans by Haldane's mapping function in MapmakerEXP. Segregation distortion was tested with chi-square tests by Windows QTL Cartographer version 2.5 (Wang et al., 2005).

Statistical Analyses

Details of the statistical analysis to obtain line means for subsequent QTL analysis were given by Robertson et al. (2006). Briefly, fumonisin data on the GEFR population were natural log transformed and fumonisin data on the NCB population were square root transformed to remove heterogeneity of variance. Genotype least square means across environments and across the two common environments were estimated for each trait within each population by a single mixed models analysis in PROC MIXED of SAS version 8.2 (Littell et al., 1996; SAS Institute, 1999). For these analyses, genotypes were considered fixed effects and all other effects (environments, replications, incomplete blocks, sets, and genotype × environment interactions) were considered random. Heritabilities across environments and across the two common environments were estimated for each trait each population on an entry-mean basis from the univariate mixed model analyses (Holland et al., 2003). For these analyses, all effects were considered random.

QTL Detection and Estimation

Composite interval mapping (CIM) was implemented in Windows QTL Cartographer version 2.5 to provide initial models for the multiple interval mapping procedure (MIM). For CIM, the forward regression method was used for cofactor selection and a 10-cM window size was used for the genome scans. The threshold LOD score to declare significance at $\alpha = 0.05$ was estimated empirically with 300 permutations (Churchill and Doerge, 1994). QTL analysis was performed for each trait within the two common environments and across all environments.

QTL peaks from the CIM analysis (with a LOD threshold value of 2.0 and minimum 5-cM minimum between QTLs) were used as the initial models for MIM in Windows QTL Cartographer 2.5. MIM models were created and tested in an iterative, stepwise fashion, searching for new QTLs to add to the current model, and testing their significance after each search cycle. New models were accepted if they decreased the Bayesian Information Criterion (BIC) (Piepho and Gauch, 2001.) The BIC favors models with higher likelihoods, but includes a penalty for each additional parameter added to the model, to help prevent overfitting the models. After no additional QTLs could be added to a model according to the BIC, each pair of QTLs in the model was tested for epistatic interactions. Epistatic interactions were maintained in the model if they decreased the BIC. While deriving the model using multiple interval mapping, we were mindful not to overfit the model such that the proportion of total variation due to QTLs exceeded the heritability, which can occur even when BIC is used as a model selection criterion. If this occurred, the QTLs associated with the smallest amount of variation was dropped from the model and the remaining QTL effects were re-estimated. This process continued until we obtained

a model that explained a proportion of the phenotypic variance that was equal to or less than the heritable proportion of phenotypic variance.

When no additional QTL main effects could be added to the model, the model with minimum BIC was chosen and QTL effects were simultaneously estimated using the "summary" option. The proportion of phenoptyic variation explained by each QTL was estimated from the simultaneous fitting of QTL positions in the final model by Windows QTL Cartographer. Genetic variability explained by QTLs mapped for each trait was calculated as the total phenotypic variation explained by QTLs combined divided by the entry mean heritability of the trait.

QTL main and epistatic effects for fumonisin concentration were estimated by natural log transformed (GEFR population) or square root transformed (NCB population) data. To report QTL effects in original units, we back-transformed the population mean and marginal genotypic effects for each QTL allele class. Additive and epistatic effects were estimated from the back-transformed genotypic mean estimates following Holland (2001). QTLs for maturity were not estimated because Robertson et al. (2006) found that in both the GEFR and NCB populations the genotypic correlation between fumonisin concentration and silk date was not significant and the genotypic correlations between ear rot and silk date were low.

Multivariate QTL Mapping

To investigate the effects of QTLs on ear rot and fumonisin contamination simultaneously, we conducted multivariate multiple regression analysis on the two traits, using marker loci as regressor variables. For each population, the initial regression analysis model contained one marker locus nearest each QTL peak from the final MIM models for both traits. Multivariate analysis was conducted using the MANOVA option of PROC GLM in SAS version 8.2 (SAS Institute, 1999). A single degree of freedom contrast was defined for each marker locus or epistatic interaction. The contrast with the largest Type III *P*-value of Wilks' Lambda statistic (which tests the null hypothesis of no marker effect on the two traits simultaneously) was dropped from the model. The resulting new model was tested in the same way, and this process continued iteratively until all marker loci had significant ($P = 0.05$) effects according to the Wilks' Lambda statistic.

The genetic covariance between the two traits associated with each marker locus in the final model was estimated by the method of moments, using the Type III mean cross products for each marker contrast obtained from the Proc GLM analysis. To obtain unbiased estimates of the covariances, the coefficients of the covariance components were based on the equations of Charcosset and Gallais (1996). These estimates were used to calculate the proportion of total genotypic covariance between the two traits due to each marker locus.

QTL × Environment Interactions

QTLs detected in the final MIM models for each trait and population were tested for QTL × environment interactions. A single marker locus closest to each QTL identified by MIM was tested for environmental interactions by PROC GLM in SAS version 8.2. The model used for all analyses was:

$$Y_{ijk} = \mu + E_i + L_j + G(L)_{jk} + LE_{jk} + \varepsilon_{ijk},$$

where μ = overall mean; E_i = effect of environment i; L_j = effect of marker locus j; $G(L)_{jk}$ = effect of line k within locus j; and LE_{ik} = the interaction of locus j with environment i; and ε_{ijk} = residual variation effect. Loci with significant ($P < 0.05$) locus × environment interaction effects were then analyzed by single factor analysis in SAS PROC GLM within each individual environment to estimate environment-specific QTL effects.

RESULTS

In the GEFR population, 453 SSR markers were screened and 105 polymorphic markers were chosen to genotype the population. In the NCB population, 258 SSR markers were screened and 113 polymorphic markers were chosen to genotype the population. The length of the GEFR map was 1969 cM, and the average distance between markers was 20.7 cM (Supplemental Fig. 1). Segregation distortion was observed at 25% of the markers. The length of the NCB map was 1993 cM, and the average distance between markers was 19.4 cM (Supplemental Fig. 2). Segregation distortion at $P = 0.05$ was observed at 19% of the markers. The segregation distortion seen in both populations was within the typical range seen in maize populations (Lu et al., 2002). Locus orders were consistent with consensus genetic maps of maize (www.maizegdb.org).

Fumonisin contamination was significantly affected by genotype × environment interaction (GEI) in the GEFR ($P < 0.0001$) and in the NCB ($P = 0.0021$) populations. Ear rot was also significantly affected by GEI in both populations ($P < 0.0001$). Despite the significant GEI for both disease traits in both populations, the mean genetic correlations of traits measured in different environments were always at least 0.70 and the main effects of genotypes were highly significant ($P < 0.0001$) (Robertson et al., 2006). Because of these high correlations, means over environments were appropriate to identify QTLs, but we then tested these detected QTLs for interactions with environments. To avoid potential bias because of GEI when comparing QTLs across populations, we also computed genotypic means across the two environments in which both populations were evaluated (Clayton and Plymouth, NC, 2003) and conducted MIM on the basis of these means for cross-population comparisons.

QTL ANALYSES

QTLs were identified in both populations for both ear rot and fumonisin contamination resistance (Tables 2 and 3 and Supplemental Fig. 1 and 2). Over all environments, in the GEFR population, seven QTLs were identified for ear rot resistance, with the beneficial allele (the allele that reduces mean ear rot) always derived from the GE440 parent (Table 2). These seven QTLs explained 47% of the phenotypic variation and 99% of the genotypic variation. In the NCB population, five ear rot QTLs were identified which explain 31% of the phenotypic variation and 36% of the genotypic variation (Table 2). At two of these QTLs the NC300 allele was favorable, and at three of the QTLs the B104 allele was favorable.

The ear rot resistance QTL with the largest effect in the GEFR population was on chromosome one near

Table 2. Chromosome and map positions, nearest flanking marker loci, effects, and variances associated with QTL identified through multiple interval mapping across environments in the two maize populations for Fusarium ear rot resistance.

Chromosome	QTL position	Bin	Left marker	Right marker	Effect†	Percent phenotypic variation explained
					%	
			GEFR population			
1	75.90	1.02	bnlg1953	phi001	6.08‡	18.4
1	221.89	1.10	bnlg1347	bnlg2331	3.37	5.7
2	7.90	2.02	bnlg1017	bnlg1017	3.07	4.4
2	151.90	2.08	bnlg2144	bnlg1662	3.16	5.8
4	52.70	4.03	bnlg2280	umc2280	2.55	3.4
4	182.30	4.09	umc1086	umc1101	3.10	5.0
5	99.60	5.05	umc2111	umc2111	3.06	3.8
			Total variation explained§:			46.5
			NCB population			
2	258.90	2.09	bnlg469	umc1696	−3.00	5.1
3	75.64	3.06	bnlg1063	bnlg1160	4.38	11.2
4	21.38	4.03	umc1294	umc2082	3.47	5.6
5	146.84	5.06	umc1524	phi048	2.86	4.6
6	132.07	6.07	umc2375	bnlg1740	−2.88	4.8
			Total variation explained§:			31.3

† QTL effect estimated as the difference between homozygous FR1064 genotypes and heterozygous GE440/FR1064 genotypes in the GEFR population and as the difference between homozygous NC300 and homozygous B104 genotypes in the NCB population. Effects of ear rot resistance are reported in percent rotten kernels.

‡ Positive effects refer to GE440 (in the GEFR population) or B104 (in the NCB population) as the origin of the beneficial allele, and negative effects refer to FR1064 (in the GEFR population) or NC300 (in the NCB population) as the beneficial allele.

§ Total variation associated with all of the QTL in the model fitted simultaneously.

marker bnlg1953 and explained 18% of the phenotypic variation (Table 2). The ear rot resistance QTL with the largest effect in the NCB population was on chromosome three near marker bnlg1063 and explained 11% of the phenotypic variation. In both populations, each of the remaining ear rot resistance QTLs identified explained less than 10% of the phenotypic variation.

Over all environments, in the GEFR population, nine fumonisin contamination resistance QTLs were identified, as well as one epistatic interaction between QTLs (Table 3). The combined QTL and epistasis model explained 67% of the phenotypic variation and 89% of the genotypic variation. In the NCB population, six fumonisin contamination resistance QTLs and three

Table 3. Chromosome and map positions, nearest flanking marker loci, effects, and variances associated with QTL identified across environments through multiple interval mapping in the two maize populations for fumonisin contamination resistance.

Chromosome	QTL position	Bin	Left marker	Right marker	Back-transformed effect†	Percent phenotypic variation explained
					$\mu g\ g^{-1}$	%
			GEFR population			
1	95.00	1.03	phi001	bnlg1811	5.27‡	10.3
2	177.10	2.08	bnlg1662	bnlg1606	3.97	6.2
3	189.90	3.09	umc1489	umc1594	4.09	6.1
4	53.70	4.03	umc2280	umc2061	2.58	5.5
4	144.80	4.08	bnlg2244	umc1086	3.71	9.2
4	193.30	4.09	umc1086	umc1101	3.33	7.7
5	96.60	5.05	umc2111	umc2111	5.80	13.1
7	24.00	7.02	umc1193	umc2098	3.71	6.6
9	36.90	9.02	dupssr6	dupssr6	1.83	0.1
3.09 × 4.03§					−4.17	1.8
			Total variation explained¶:			66.6
			NCB population			
1	109.20	1.05	bnlg1884	umc1335	3.71	16.5
3	75.60	3.06	bnlg1063	bnlg1063	2.07	8.2
4	8.00	4.02	umc1294	umc2082	3.32	9.3
5	146.00	5.06	bnlg278	umc1941	0.86	1.8
6	140.00	6.08	bnlg1740	umc2059	−4.05	15.7
8	81.30	8.03	umc1034	umc1172	2.66	10.7
1 × 8					18.52	10.7
4 × 8					12.23	1.2
6 × 8					−18.00	7.0
			Total variation explained¶:			81.1

† QTL effect estimated as the difference between homozygous FR1064 genotypes and heterozygous GE440/FR1064 genotypes in the GEFR population and as the difference between homozygous NC300 and homozygous B104 genotypes in the NCB population. Effect estimates are presented as back-transformed data to approximate their original value of $\mu g\ g^{-1}$.

‡ Positive effects refer to GE440 (in the GEFR population) or B104 (in the NCB population) as the origin of the beneficial allele, and negative effects refer to FR1064 (in the GEFR population) or NC300 (in the NCB population) as the beneficial allele.

§ Epistatic interactions are presented using the chromosome and bin location (if necessary to specify QTL) to designate which QTL is involved in the interaction.

¶ Total variation associated with all of the QTL in the model fitted simultaneously.

epistatic interactions between QTLs were identified (Table 3). These QTLs explained 81% of the phenotypic variation and 92% of the genotypic variation.

In the GEFR population, two fumonisin contamination resistance QTLs were identified that each explained over 10% of the phenotypic variation, one on chromosome one near marker phi001 that explained 10% of the variation and one on chromosome five near marker umc2111 that explained 13% of the phenotypic variation (Table 3). In the NCB population, three fumonisin QTLs were identified that each explained more than 10% of the phenotypic variation (Table 3). These major QTLs were located on chromosome 1 near bnlg1884 (associated with 17% of the phenotypic variation), on chromosome 6 near umc2059 (associated with 16% of the phenotypic variation), and on chromosome 8 near umc1034 (associated with 11% of the phenotypic variation).

In the GEFR population, eight QTLs explained a combined 65% of the covariance between ear rot and fumonisin concentration (Table 4, Fig. 1). Two QTLs were unique to ear rot, as they were detected with multiple interval mapping of ear rot alone but were not detected in the multivariate marker analysis (Fig. 1). One nonpleiotropic ear rot QTL was on chromosome 1 near bnlg1347 (associated with 6% of the phenotypic variation), and the other was on chromosome two near bnlg1017 (associated with 4% of the phenotypic variation) (Table 2). One QTL was unique to fumonisin contamination (Fig. 1), and it was located on chromosome nine near dupssr6 but explained only 0.1% of the phenotypic variation (Table 3). However, if the true effect of this QTL were really that small, it could not be reliably estimated with our population size (Beavis, 1998).

In the NCB population, five QTLs explained 31% of the covariance between ear rot and fumonisin contamination (Table 4, Fig. 1). One QTL, located on chromosome 2 near bnlg469, was unique to ear rot and explained 5% of the phenotypic variation (Table 2). One QTL, located on chromosome eight near umc1034, was unique to fumonisin contamination and explained 11% of the phenotypic variation (Table 3).

Table 4. Proportion of total genotypic covariance between, and genotypic variance of, fumonisin concentration and ear rot in the GEFR and NCB populations explained by marker loci nearest to QTL identified across environments in a multivariate analysis.

Marker locus	Bin	Genetic covariance between fumonisin and ear rot	Genetic variance for fumonisin	Genetic variance for ear rot
		% associated with locus		
		GEFR population		
phi001	1.03	0.19	0.10	0.35
bnlg1606	2.08	0.11	0.06	0.16
umc1594	3.09	0.05	0.03	0.06
umc2280	4.03	0.04	0.03	0.03
bnlg2244	4.08	0.01	0.03	−0.01
umc1101	4.09	0.05	0.05	0.04
umc2111	5.05	0.13	0.14	0.10
umc1193	7.02†	0.07	0.08	0.05
	Total:	0.65	0.52	0.78
		NCB population		
bnlg1884	1.05	0.04	0.08	0.01
bnlg1063	3.06	0.14	0.10	0.14
umc2082	4.03	0.06	0.05	0.06
umc1524	5.06	0.06	0.03	0.08
umc2059	6.08	0.01	0.03	0.00
	Total:	0.31	0.29	0.29

† Bin location based on bin position of marker closest to umc1193.

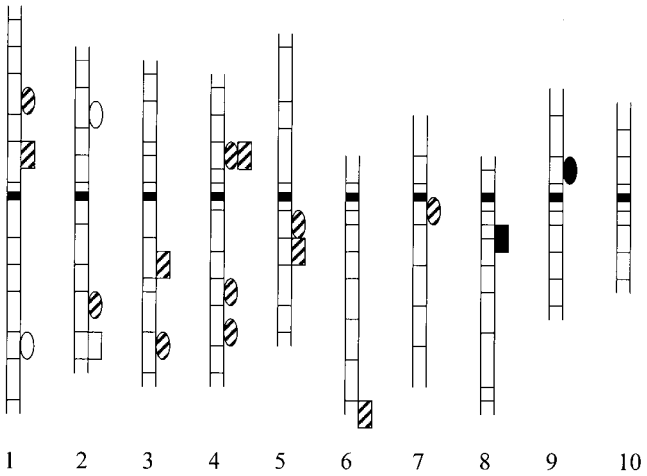

Fig. 1. Schematic depiction of the maize chromosomes, with horizontal lines demarking bins (Davis et al., 1999). Centromeres are shown as thick black lines. Ovals represent QTLs mapped in GEFR population and rectangles represent QTLs mapped in the NCB population on the basis of means across all testing environments. Hash marks indicate QTL for both Fusarium ear rot and fumonisin contamination, black shapes indicate QTL for fumonisin contamination only, and white shapes indicate QTL for Fusarium ear rot only. Genetic maps and QTL locations for individual populations are presented in Supplemental Fig. 1 and 2.

QTLs detected by MIM on the basis of genotypic means across environments were tested for interactions with environments. Among QTLs for both traits detected in the GEFR population, only one locus, one QTL for ear rot, located near phi001 (bin 1.02), exhibited significant ($P < 0.001$) locus × environment interaction. This QTL was significant ($P < 0.0001$) in only two of the four individual environments (Mount Olive, NC, 2002 and Urbana, IL, 2002) when using single marker analysis at phi001. The QTL effects varied in magnitude, but not sign, across environments.

Among the QTLs identified on the basis of genotypic means across environments in the NCB population, only one QTL region exhibited significant locus × environment interaction. The QTL located near the marker pair umc1941-umc1524 (0.8 cM apart in bin 5.06) had significant locus × environment interactions for both ear rot ($P < 0.05$) and fumonisin content ($P < 0.01$). This QTL was significant ($P < 0.05$) for fumonisin content only at Plymouth, NC in 2003, but was almost significant ($0.05 < P < 0.10$) at Clayton, NC in 2002. Its effect on ear rot was significant ($P < 0.05$) at Clayton in 2002 and almost significant at Plymouth ($0.05 < P < 0.10$) in 2003. However, this QTL had no effect on either trait at Clayton in 2003. The QTL effects differed in magnitude, but not sign, across environments.

The GEFR and NCB maps cannot be compared directly because different sets of markers were used in them. Therefore, consistency of QTL positions between the two populations was judged by referencing chromosomal bin position of markers in the maize IBM2 Neighbors con-

Table 5. QTL for Fusarium ear rot resistance identified with multiple interval mapping based on means across two environments (Clayton and Plymouth, NC in 2003) in which both GEFR and NCB populations were evaluated.

Chromosome	QTL position	Bin	Left marker	Right marker	Effect†	Percent phenotypic variation explained
					%	
			GEFR population			
1	67.90	1.02	bnlg1953	phi001	3.74‡	6.7
2	189.43	2.08	bnlg1606	bnlg1520	3.00	4.1
4	35.30	4.03	umc2150	umc2281	3.54	6.9
4	187.33	4.09	umc1086	umc1101	3.64	6.1
5	86.31	5.03	umc1355	umc2111	2.73	3.7
9	136.29	9.05	umc1078	bnlg1270	−2.60	3.1
			Total variation explained§:			30.6
			NCB population			
2	260.90	2.10	bnlg469	umc1696	−2.59	5.0
3	75.64	3.06	bnlg1063	bnlg1160	4.23	10.3
4	15.01	4.03	umc1294	umc2082	4.71	10.7
5	146.84	5.06	umc1524	phi048	1.99	2.6
6	132.07	6.07	umc2375	bnlg1740	−2.53	4.6
3 × 4¶					3.15	4.9
			Total variation explained§:			38.1

† QTL effect estimated as the difference between homozygous FR1064 genotypes and heterozygous GE440/FR1064 genotypes in the GEFR population and as half the difference between homozygous NC300 and homozygous B104 genotypes in the NCB population. Effects of ear rot resistance are reported in percent rotten kernels.
‡ Positive effects refer to GE440 (in the GEFR population) or B104 (in the NCB population) as the origin of the beneficial allele, and negative effects refer to FR1064 (in the GEFR population) or NC300 (in the NCB population) as the beneficial allele.
§ Total variation associated with all of the QTL in the model fitted simultaneously.
¶ Epistatic interaction is presented using the chromosome to designate which QTL is involved in the interaction.

sensus map (or other consensus maps, as required; www.maizegdb.org). Furthermore, the phenotypic evaluations were conducted in different sets of environments. Therefore, we also conducted multiple interval mapping on phenotypic means across the two environments that were common to the two studies (Clayton, 2003 and Plymouth, 2003) to make cross-population comparisons.

Among the QTLs identified by means across all environments, three ear rot QTLs and two fumonisin QTLs were putatively consistent across populations (Tables 2 and 3). For ear rot resistance, three QTLs (bins 2.08/2.09, 4.03, and 5.05/5.06) were putatively consistent QTL. For fumonisin contamination resistance, two QTL regions (bins 4.02/4.03 and 5.05/5.06) were putatively consistent QTL. Two QTL regions were consistent across populations for both traits (bins 4.02/4.03 and 5.05/5.06).

QTL mapping based on phenotypic means across the two common environments (Clayton and Plymouth in 2003) identified QTLs that were consistent across populations in bins 1.04/1.05, 3.06/3.07, and 4.03 (Tables 5

Table 6. QTL for fumonisin contamination resistance identified with multiple interval mapping based on means across two environments (Clayton and Plymouth, NC in 2003) in which both GEFR and NCB populations were evaluated.

Chromo- some	QTL position	Bin	Left marker	Right marker	Back Trans- formed Effect†	Percent phenotypic variation explained
					μg g^{-1}	%
			GEFR population			
1	100.00	1.04	phi001	bnlg1811	0.43‡	6.7
1	191.70	1.09	umc1085	umc2047	0.19	1.0
2	149.60	2.08	mmc0271	bnlg2144	0.44	8.6
3	174.90	3.07	umc1489	umc1594	0.32	3.3
4	53.70	4.03	umc2280	umc2061	0.37	8.7
4	133.90	4.08	dupssr34	bnlg2244	0.26	5.3
4	180.30	4.09	umc1086	umc1101	0.32	6.8
5	94.30	5.05	umc1355	umc2111	0.55	12.2
7	37.20	7.02	umc2098	umc1134	0.37	6.2
8	7.00	8.03	umc1360	umc1778	0.30	3.7
			Total variation explained§:			62.5
			NCB population			
1	110.19	1.05	bnlg1884	umc1335	0.37	7.5
2	14.46	2.02	bnlg1017	umc2403	0.39	6.4
3	75.64	3.06	bnlg1063	bnlg1160	0.42	9.0
6	139.96	6.08	bnlg1740	umc2059	−0.41	8.4
10	71.14	10.04	umc2163	umc2043	0.09	1.0
3 × 10¶						5.2
			Total variation explained§:			37.5

† QTL effect estimated as the difference between homozygous FR1064 genotypes and heterozygous GE440/FR1064 genotypes based on log-transformed data in the GEFR population and as half the difference between homozygous NC300 and homozygous B104 genotypes based on square root-transformed data in the NCB population. Effect estimates are presented as back-transformed data to approximate their original value of μg g^{-1}.
‡ Positive effects refer to GE440 (in the GEFR population) or B104 (in the NCB population) as the origin of the beneficial allele, and negative effects refer to FR1064 (in the GEFR population) or NC300 (in the NCB population) as the beneficial allele.
§ Total variation associated with all of the QTL in the model fitted simultaneously.
¶ Epistatic interaction is presented using the chromosome to designate which QTL is involved in the interaction.

and 6). One QTL identified was potentially consistent across populations for fumonisin contamination resistance (bins 1.04/1.05) (Table 6). The QTL region identified in bins 3.06/3.07 was potentially consistent for fumonisin contamination resistance across populations (and also affected ear rot resistance in the NCB population). This is partly congruent with the results of the analysis of means across all environments, in which QTLs for both ear rot and fumonisin contamination resistance in the NCB population were identified in bin 3.06 (Tables 2 and 3) and a QTL was identified in bin 3.09 for fumonisin contamination resistance in the GEFR population (15 cM from the QTLs identified in the GEFR population in the common environment analysis, Tables 3 and 6).

A QTL region in bin 4.03 was potentially consistent across populations (and also affected fumonisin contamination resistance in the GEFR population, Tables 5 and 6). QTLs were also identified in bins 4.02/4.03 for both traits in both populations in the analysis of means across all environments (Tables 2 and 3).

DISCUSSION

Across environments, the largest QTLs for either trait explained only 18% of the phenotypic variation, with most QTLs for both traits explaining less than 10% of the phenotypic variation (Tables 2 and 3). The genetic architecture of these traits appears complex, with many small effect QTLs controlling resistance to these two traits. The complexity of the genetics governing these traits and the large influence of environments hinder the accurate identification of QTL positions and estimates of their effects. Inaccuracies in QTL mapping will reduce the success of a marker assisted selection program. Furthermore, we caution that our QTL effect estimates are likely overestimated because of the moderate population sizes used. QTL mapping in finite populations leads to upwardly biased estimates of the proportion of variance explained by QTLs (Beavis, 1998; Melchinger et al., 1998); estimating the amount of this bias requires cross-validation (Utz et al., 2000).

By comparing QTLs identified between the two populations in the two common environments, we identified several QTL regions that were consistent across populations. The largest QTL for each trait was consistent with at least one QTL for either trait in the other population, suggesting that some resistance genes are common between the populations. However, the population-consistent QTLs identified explained only small amounts of phenotypic variation, further highlighting both the complexity of the resistance and the likelihood that the different resistant parental lines contain different resistance QTLs that could be combined into one line.

Both disease traits were affected by overall genotype × environment interactions (Robertson et al., 2006); therefore QTLs detected in MIM analyses of line means across environments were further analyzed for QTL × environment interactions. Only one QTL region within each population interacted significantly with environments, indicating that most of the QTLs had stable effects across environments. This is congruent with Robertson et al.'s (2006) finding that the genetic correlations of genotypic values measured in different environments were high for both traits. The two QTL regions that interacted with environments interactions seem to be affected by either a change in magnitude between locations or by QTLs not being significant in all environments. However, there was no case of a QTL that had significant positive effects in one environment and significant negative effects in a different environment. Such QTLs may, in fact, exist, but we likely would never have detected them in the original MIM analysis of genotype means across environments. In summary, the QTLs detected were generally stable across environments with some minor exceptions.

QTLs in the GEFR population evaluated across all environments explained more of the genotypic variability for both traits and more of the genotypic covariance between traits than did QTLs in the NCB population. This is probably largely due to the NCB population being smaller than the GEFR population, and hence provides lower power to detect QTLs. It may also be due in part to the difference in structure of the two populations. The NCB population consists of inbred lines which have undergone more generations of recombination, such that linkage disequilibrium between loci with equal recombination frequencies is lower in the recombinant inbred lines than in the first backcross generation. Therefore, a denser linkage map may be required in the NCB than in the GEFR population to achieve the same power to detect the same number of QTLs.

Our experiment could not determine the relative importance of additive and dominant gene action because the NCB population was composed of nearly homozygous recombinant inbred lines and the GEFR population was composed of first backcross generation-derived families (in which the dominant and additive effects of donor parent alleles are confounded). However, we did detect epistasis for fumonisin contamination resistance. Heritabilities were higher for fumonisin contamination resistance than for ear rot resistance, and the higher precision of fumonisin data may have provided greater power to detect QTLs and QTL × QTL interactions.

Robertson et al. (2006) reported high genetic correlations between ear rot and fumonisin contamination in these two populations, on the basis of this same phenotypic data set. On the basis of that result, they suggested that the genetic mechanisms for resistance to ear rot and fumonisin contamination were largely identical. However, we found fewer QTLs with effects on the two traits than would be expected given the high genetic correlations. Multivariate analysis of markers closest to the detected QTLs demonstrated that we detected only 31% of the total estimated genetic covariance in the NCB population and 65% of the total estimated genetic covariance in the GEFR population. These may be underestimates of the true genotypic covariances associated with markers, since the multivariate analyses were conducted directly at marker loci, where the effects of linked QTLs are estimated with a downward bias due to recombination (Edwards et al., 1987). This contrasts with the univariate analyses conducted with multiple interval

mapping, which permitted the estimation of QTL effects at their most likely positions, which may be in intervals between markers. Nevertheless, the relatively low proportion of genotypic covariance that was associated with markers near QTLs suggests that not all QTLs were identified. It is possible that a large number of genes with small effects that will be hard to detect (i.e., the polygenic background) may explain much of the remaining genetic covariances. Greater power to detect QTLs and estimate QTL effects could be gained by increasing population size, improving ear rot phenotyping methods, increasing the number of environments for phenotyping, and increasing the number of markers used (Robertson et al., 2005).

Our results are consistent with those of Pérez-Brito et al. (2001), who identified many QTLs, each with small effects on Fusarium ear rot resistance. Pérez-Brito et al. (2001) used two mapping populations for ear rot QTL identification. In their first population, on the basis of bin position, QTL regions were identified that were consistent with QTLs in the GEFR population (bins 1.10, 2.02, 2.08, and 4.09) and two ear rot QTL regions were identified that were consistent with QTLs in the NCB population (bins 2.09 and 6.07). In their second population, they identified, on the basis of bin position, one QTL region that was consistent with a QTL identified in the GEFR population (bin 4.03), and two regions that were consistent with QTLs identified in the NCB population (bins 4.03 and 6.07), and one region that was consistent with a QTL identified across the NCB and both Pérez-Brito populations (bin 6.07). Fine mapping of QTLs in the different populations could determine the true consistency of these QTLs.

Marker assisted breeding could prove very useful when breeding for a trait, such as resistance to Fusarium ear rot or fumonisin contamination, that is very difficult to phenotype and is highly influenced by the environment, provided that the true QTLs can be accurately identified (Robertson et al., 2005). The number of loci conditioning a trait influences the potential efficiency of marker assisted breeding. Bernardo (2001) used computer simulation to determine the usefulness of marker-assisted selections assuming that all of the QTL positions affecting yield were known. He found that it is more advantageous to make selections on the basis of known QTLs if there are only a few loci controlling the trait (e.g., ten loci). With large numbers of loci controlling a trait, estimates of QTL effects become imprecise and selections based on genotypes can become less and less useful, and may even become detrimental as more and more QTLs are discovered. Therefore, in practice, marker-assisted selection is most effective when a few QTLs, each with moderate to large effects on target traits and consistent effects across breeding populations, can be identified (Holland, 2004).

In our study, we found numerous QTLs with small to moderate effects, and limited consistency across populations. Therefore, it is appears that marker-assisted selection cannot be generally recommended for these traits at this time. However, given the costs and effort required to obtain accurate fumonisin concentration data, a program focused on improving agronomically good lines, such as FR1064, with alleles at the most important resistance QTLs from resistant but agronomically poor lines, such as GE440, via marker-assisted backcrossing may be relatively economically efficient. For breeding programs in general, however, phenotypic recurrent selection or pedigree breeding approaches, targeted at improving both ear rot and fumonisin contamination resistance, while also selecting for yield and other agronomic traits may be more efficient. With sufficient resources, such traditional methods can be combined with marker-assisted breeding by screening juvenile plants for larger effect QTLs before pollinations, with the goal of increasing the proportion of favorable QTLs in the population each generation. Because of the high environmental effect on these traits, further studies may need to be performed to confirm the presence, location, and effects of ear rot and fumonisin contamination resistance QTLs and to more precisely map the QTLs to minimize linkage drag.

We hesitate to speculate about resistance mechanisms or candidate genes because each of the QTL regions spans about 20 cM, or about 1% of the maize genetic map. If this relates to 1% of all estimated ~59 000 maize genes (Messing et al., 2004), then we expect that almost 600 genes may reside within each QTL region. Relating QTLs to candidate genes and potential mechanisms will require finer-scale genetic mapping of these regions.

ACKNOWLEDGMENTS

This research was supported by a USDA-IFAFS multidisciplinary training grant to North Carolina State University (award number 2001-52101-11507) and by a grant from the Corn Growers Association of North Carolina. We thank Dr. Major Goodman for the NCB population and Mr. Dale Dowden of Monsanto, Mt. Olive, NC, for providing research plots. Special thanks to Brooke Peterson and Stella Salvo for technical assistance, and to Jennifer Tarter, Terry Flint, Carrie Jacobus, Mike Price, Kim Schwartzburg, and Greg Obrian for field inoculation assistance. Additional thanks to David Rhyne and Tricia Bair for laboratory technical assistance.

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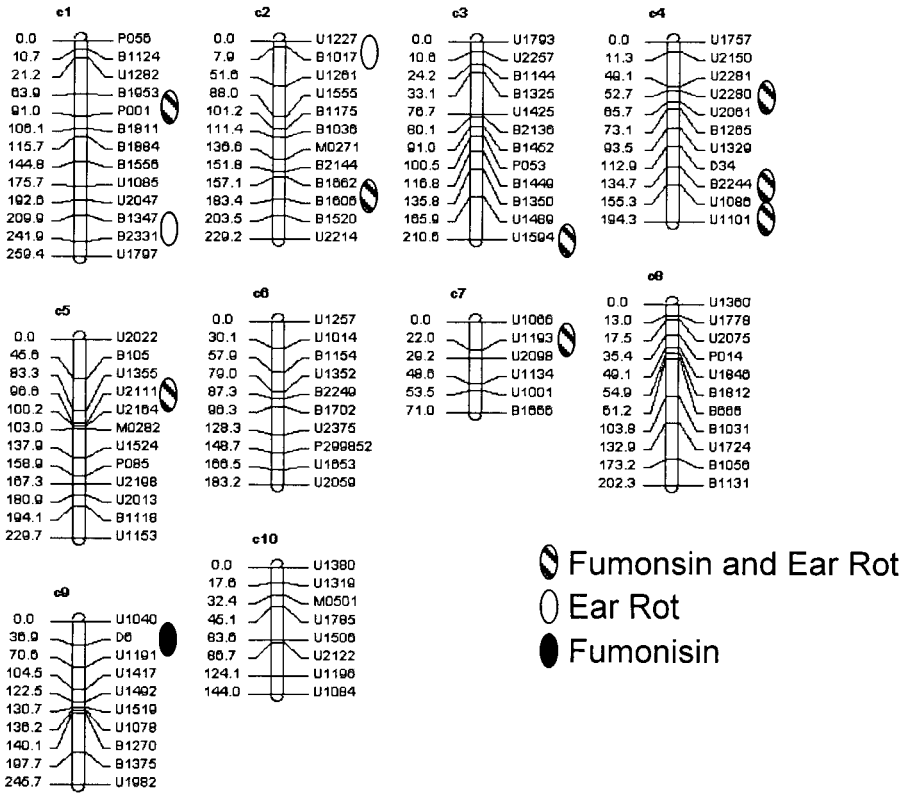
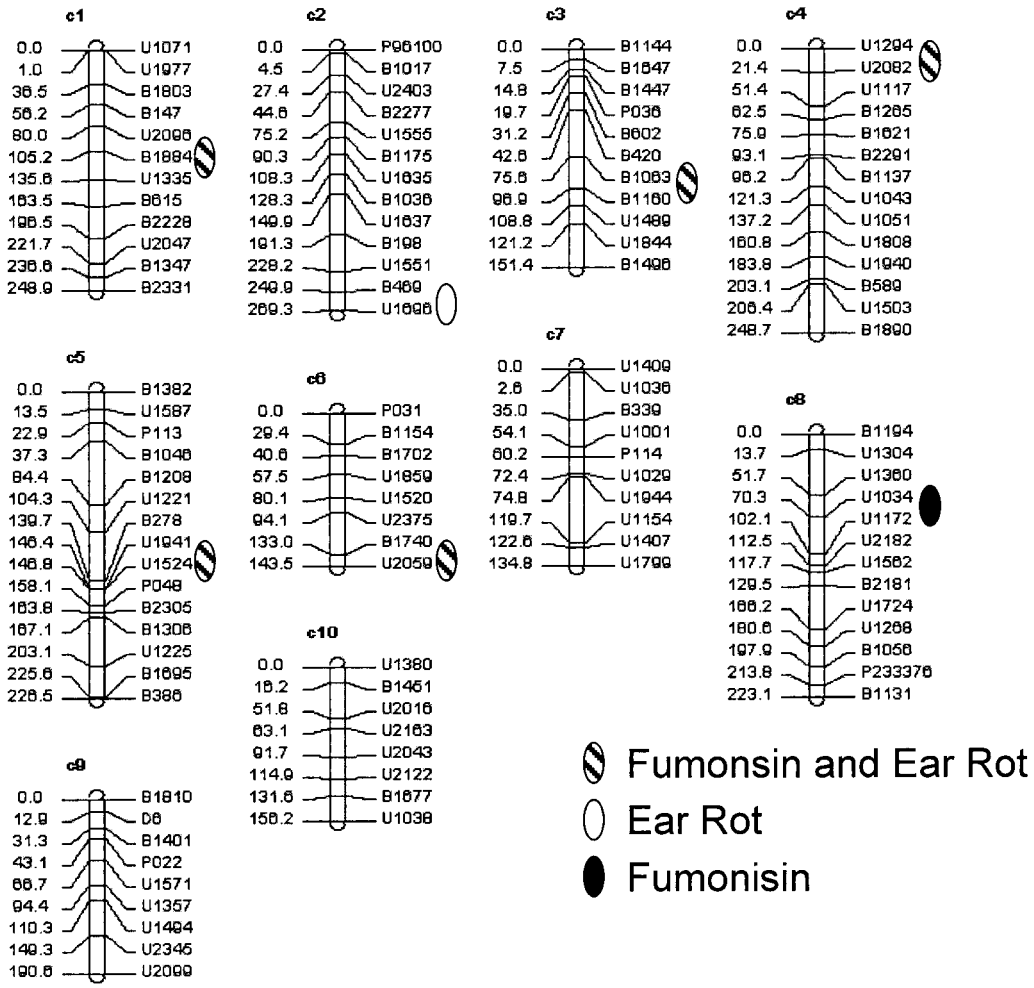


Supplemental Fig. 1. Chromosomal locations of markers significant for mean fumonisin contamination resistance and ear rot resistance across environments in the GEFR population. Ovals are centered on maximum likelihood positions of QTL and extend 10 cM to either side, to reflect typical uncertainty levels in QTL positions (Cardinal et al., 2001). Regions significant for both rot and fumonisin (cross-hatched ovals) were determined by multivariate analysis. QTL locations are based on multiple interval mapping using QTL cartographer version 2.5.



Supplemental Fig. 2. Chromosomal locations of markers significant for mean fumonisin contamination resistance and ear rot resistance across environments in the NCB population. Ovals are centered on maximum likelihood positions of QTL and extend 10 cM to either side, to reflect typical levels in QTL positions (Cardinal et al., 2001). Regions significant for both rot and fumonisin (cross-hatched ovals) were determined by multivariate analysis. QTL locations are based on multiple interval mapping using QTL cartographer version 2.5.