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Comparative QTL mapping of resistance to *Ustilago maydis* across four populations of European flint-maize

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Abstract We mapped and characterized quantitative trait loci (QTLs) for resistance to *Ustilago maydis* and investigated their consistency across different flint-maize populations. Four independent populations, comprising 280 F_3 lines ($A \times B^I$), 120 F_5 lines ($A \times B^{II}$), 131 F_4 lines ($A \times C$) and 133 F_4 lines ($C \times D$), were produced from four European elite flint inbreds (A, B, C, D) and genotyped at 89, 151, 104, and 122 RFLP marker loci, respectively. All F_n lines were evaluated in field trials with two replications in five German environments. Genotypic variances were highly significant for the percentage of *U. maydis* infected plants (UST) in all populations, and heritabilities exceeded 0.69. Between five and ten QTLs were detected in individual populations by composite interval mapping, explaining between 39% and 58% of the phenotypic variance. These 19 different QTLs were distributed over all ten chromosomes without any clustering on certain chromosomes. In most cases, gene action was dominant or overdominant. Fourteen pairs of the detected QTLs for UST displayed significant digenic epistatic interactions, but only two of them did so after arcsin $\sqrt{\text{UST}/100}$ transformation. Significant QTL × environment interactions occurred frequently. Between two to four QTLs were common between pairs of populations. Population $C \times D$ was also grown in Chartres, a location with a high *U. maydis* incidence. Two out of six QTLs identified for Chartres were in common with QTLs detected across five German environments for $C \times D$. Consequently, marker-assisted or phenotypic selection based on results from natural infection seem to be suitable breeding strategies for improving the resistance of maize to *U. maydis*.

Key words Maize · *Ustilago maydis* · QTL mapping · RFLP · Resistance

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Introduction

Common smut of maize (*Zea mays* L.) caused by *Ustilago maydis* (DC.) Corda is a world-wide distributed pest except in Australia (Christensen 1963). Local infection by *U. maydis* may result in the formation of galls on almost all above-ground parts of the maize plant, yet they are usually most prevalent on ears, tassels, stalks, and nodal shoots. Stunting and even death of plants can be the consequence of early infection. Hence, *U. maydis* has the potential to cause severe yield losses in grain and forage maize production. For a long time, *U. maydis* spores contaminating forage maize have been suspected to be toxic or otherwise detrimental to animals, but this is still a matter of controversy. Nevertheless, in some Latin American countries smut galls named "huitlacoche" serve as a delicacy in human nutrition, obviously without being harmful (Kealy and Kosikowsky 1981).

The extent of infection by *U. maydis* strongly depends on the climatic conditions and the plant materials employed (Christensen 1963). For example, sweet-corn cultivars are generally more susceptible to common smut infection than field-corn cultivars (Agrios 1988). For field corn, annual yield losses in the USA range from 1 to 5% but may exceed 10% under epidemic conditions (Shurtleff 1980). In Germany, on average 1 to 3% of the plants in a maize field are infected with *U. maydis*. However, in 1976 more than 50% of the maize plants were infected in several parts of Germany (Zscheischler et al. 1979). Since control of *U. maydis* by fungicides is not practicable, resistance breeding is the most promising strategy

for preventing high yield losses in epidemic years and environments.

A high level of genetic variation for resistance to *U. maydis* has been found in maize germplasm of different origins (Christensen 1963; Kostandi and Geisler 1989). An oligogenic and additive-to-dominant mode of inheritance of resistance to *U. maydis* was indicated using different types of segregating populations (Christensen 1963). Translocation lines were employed to identify chromosomal regions involved in resistance to *U. maydis*. Evidence for single major or race-specific resistance genes, such as the *Rp* loci in the pathosystem *Z. mays-Puccinia sorghi*, is so far lacking.

For maize breeders, response to natural infection by *U. maydis* has been the major criterion for evaluating the resistance of plants to common smut. Because of the low level of disease incidence in many years, this procedure is rather ineffective. Environments with high disease pressure of *U. maydis*, or procedures for artificial inoculation, have been employed for a more reliable discrimination of resistant and susceptible genotypes (Christensen 1963). Besides causing additional costs, both procedures might not reflect the situation of natural infection due to different *U. maydis* strains, infection pathways, or environmental conditions. An alternative strategy could be indirect selection for an increased *U. maydis* resistance based on molecular markers associated with resistance loci identified under conditions of natural infection in adapted germplasm.

In the present study, we have used four European flint populations to characterize QTLs for resistance against *U. maydis* identified after natural infection in Germany. The objectives of our research were to: (1) determine the number, genomic positions, and gene effects of QTLs involved in common smut resistance, (2) investigate the consistency of these QTLs across different populations, (3) assess the importance of digenic epistatic and QTL × environment (QTL × E) interactions, and, for one population, to (4) compare these results with those obtained at a location with a high common smut incidence in France.

Materials and methods

Plant materials

Four early maturing homozygous European flint lines, KW1265, D146, D145 and KW1292, subsequently named A, B, C and D, respectively, were crossed to produce a random set of 280 F_3 lines of cross A × B, an independent sample of 120 F_5 ($F_{4:5}$) lines of cross A × B, 131 F_4 ($F_{3:4}$) lines of cross A × C, and 133 F_4 ($F_{3:4}$) lines of cross C × D. The four populations are designated as populations $A \times B^{I}$, $A \times B^{II}$, A × C, and C × D, respectively. Testcrossed progenies of the same families as used for this investigation were employed in previous studies on forage yield and quality traits (Lübberstedt et al. 1997 a, b; 1998 a). Lines A and D are private inbreds developed by KWS Kleinwanzlebener Saatzucht AG; lines B and C are public inbreds proprietary to the University of Hohenheim, Germany.

RFLP assays and linkage maps

The RFLP assays of all four populations and the segregation and linkage analyses of the RFLP marker loci have been described in detail elsewhere (Schön et al. 1994; Lübberstedt et al. 1998 a). The RFLP data and marker linkage maps given by these authors were also used in the QTL analyses presented here. A total of 89, 151, 104, and 122 RFLP marker loci, well distributed over the maize genome, were used to genotype 275 F_3 lines for $A \times B^{I}$, 113 F_5 lines of $A \times B^{II}$, 131 F_4 lines of A × C, and 133 F_4 lines of C × D. The 275 F_3 lines employed for $A \times B^{I}$ in this study represent a random sample of the 344 F_3 lines used in the study of Schön et al. (1994). Populations $A \times B^{I}$ and $A \times B^{II}$ were derived from independent samples of the cross A × B.

Agronomic trials

Population $A \times B^{I}$ was evaluated at five environments in Germany (1993: Eckartsweier, Hochburg, Krozingen, Zell; 1994: Eckartsweier). The experiment included 300 entries in a 30 × 10 alpha design (Patterson and Williams 1976) with two replications: 280 F_3 lines and both parent lines included ten times each. Field experiments with progenies of the three smaller populations were conducted in adjacent trials at five environments in Germany (1992: Eckartsweier and Krozingen; 1993: Eckartsweier, Hochburg, and Krozingen). In addition, C × D was evaluated at Chartres (France) in 1995. Each experiment included 150 entries: the 131 or 133 F_4 lines in the case of A × C and C × D, respectively, and the 120 F_5 lines in the case of $A \times B^{II}$ plus each parent line included up to 15 times for completion. The experimental design in each environment was a 15 × 10 alpha design with two replications. Plots consisted of single rows, 0.7-m apart and 4-m long. Plots were overplanted and later thinned to a final plant density of 9 plants m^{-2} with a total of 27 plants.

Individual plants were scored for the presence or absence of *U. maydis* galls 6 weeks after mid-silking. Plants with one or more galls were counted as infected. The percentage of plants infected by *U. maydis* (UST) was subjected to subsequent analyses. In Chartres the percentage of ear galls (EAG) and stem galls (STG) was also recorded.

Statistical analyses

The analysis of the untransformed (UST, EAG, STG) or transformed (arcsin $\sqrt{\mathrm{UST}/100}$) phenotypic data and QTL mapping were performed as described in detail by Lübberstedt et al. (1998 b). Briefly, adjusted entry means and effective error mean squares were used to compute the combined analyses of variance across environments for each population. The sums of squares for entries were subdivided into the variation among F_n lines (F_3 lines in $A \times B^{I}$, F_4 lines in A × C and C × D, F_5 lines in $A \times B^{II}$) and the orthogonal contrasts among both parent lines P1 and P2, as well as the mean of both parents ($\bar{P}$) and the mean of F_n lines ($\bar{F}_n$). A corresponding subdivision was conducted on the entry × environment interaction sums of squares. Estimates of variance components σ_g^2 (genotypic variance), σ_{ge}^2 (genotype × environment interaction variance), and σ^2 (error variance) of F_n lines and their standard errors were calculated as described by Searle (1971, p 415). For testing significant differences of σ_g^2 across populations, *F*-tests were employed according to the approximation given by Satterthwaite (1946). Heritabilities (h^2) on an entry mean basis were estimated as described by Hallauer and Miranda (1981, p 90):

$$\hat{h}^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_{ge}^2/e + \hat{\sigma}^2/re},$$

where r = number of replications and e = number of environments. Exact 90% confidence intervals of $\hat{h}^2$ were calculated according to

Knapp et al. (1985). The significance of transgressive segregation was tested in each population by a *t*-test (Groh et al. 1998).

We employed the method of composite interval mapping (CIM) (Zeng 1994) for the mapping of QTLs and an estimation of their effects, as described by Bohn et al. (1996). A LOD threshold of 2.5 was chosen for declaring a putative QTL significant. For each population and environment, and also for the joint analyses across environments, co-factors were selected by stepwise regression (Draper and Smith 1981). Final selection was for the model that minimized Akaike's information criterion with a penalty = 3.0 (Jansen 1993). For each population, QTL positions were determined at the local maxima of the LOD ($\log_{10}$ odds ratio)-plot curve in the regions under consideration. The proportion of the phenotypic variance ($\hat{\sigma}^2_p$) explained by a single QTL was obtained by the square of the partial correlation coefficient (Melchinger et al. 1998). Putative QTLs were examined for the presence of QTL × E and digenic epistatic interactions (Lübberstedt et al. 1997 a). Each QTL was represented by a 20 cM interval with the local LOD maximum as the center. For a comparison of QTLs across two populations, the positions of 20-cM intervals of one population were adjusted relative to the nearest flanking RFLP markers in common with the other population, adopting the same procedure as described in detail by Lübberstedt et al. (1998 a). In cases lacking common RFLP markers among two populations for some genomic regions, the positions of 20-cM intervals were compared relative to closely linked RFLP markers polymorphic in only one of both populations. QTLs with overlapping 20-cM intervals were considered as being common, irrespective of the sign of additive or dominance effects. All computations were performed with the software package PLABQTL (Utz and Melchinger 1996).

Results

Agronomic-trait analysis

Means of populations ($\bar{F}_n$) for UST ranged in individual environments from 0.84% ($A \times B^{II}$: Krozingen 1992) to 18.77% ($A \times C$: Krozingen 1993). The population mean $\bar{F}_n$ of $C \times D$ at Chartres was 14.77% for UST, 13.82% for EAG, and 2.22% for STG. Overall means of populations across the five environments in common for $A \times B^{II}$, $A \times C$, and $C \times D$ differed significantly ($P < 0.05$) and were greatest for $A \times C$ (Table 1). Population means $\bar{F}_n$ across the three environments in common were significantly ($P < 0.05$) different between $A \times B^{I}$ and $A \times B^{II}$ or $A \times C$, but not between $A \times B^{I}$ and $C \times D$. Means of individual F_n lines significantly ($P < 0.05$) transgressed the means of both parent lines in all four populations. The population mean $\bar{F}_n$ was significantly ($P < 0.05$) greater than the midparent value ($\bar{P}$) for UST in $C \times D$, but was not different in the other populations. Means of parent lines differed significantly ($P < 0.05$) in populations $A \times B^{I}$ and $C \times D$, but not in $A \times B^{II}$ and $A \times C$.

Estimates of genotypic variances (σ^2_g) among F_n lines and genotype × environment interaction variances (σ^2_{ge})

Table 1 Estimates of means, variance components, and heritabilities for the percentage of plants infected by *U. maydis* in parent lines (P1 and P2) and F_n lines (from the cross P1 × P2) from maize populations $A \times B^{I}$ (280 F_3 lines), $A \times B^{II}$ (120 F_5 lines), $A \times C$ (131 F_4 lines), and $C \times D$ (133 F_4 lines) evaluated in five environments in Germany

Parameters	$A \times B^{I}$	$A \times B^{II}$	$A \times C$	$C \times D$
Means[a]				
P1	10.41 ± 0.72	5.58 ± 0.54	7.04 ± 1.09	17.15 ± 1.06
P2	2.54 ± 0.72	1.14 ± 0.54	12.92 ± 1.09	1.62 ± 1.06
$\bar{P}$[b]	6.47 ± 0.51	3.36 ± 0.30	9.98 ± 0.77	9.39 ± 0.75
Overall mean of F_n lines	0.00–38.85	0.00–19.50	0.70–31.27	0.00–32.04
$\bar{F}_n$	6.47 ± 0.32	3.65 ± 0.29	10.76 ± 0.62	6.08 ± 0.48
EWE92[c]	–	4.29 ± 0.34	10.32 ± 0.72	6.17 ± 0.55
KRO92	–	0.84 ± 0.13	2.53 ± 0.29	1.15 ± 0.17
EWE93	6.94 ± 0.37	1.01 ± 0.22	11.47 ± 0.70	8.26 ± 0.76
HOC93	5.12 ± 0.31	4.88 ± 0.44	10.69 ± 0.70	5.47 ± 0.50
KRO93	11.24 ± 0.54	7.22 ± 0.57	18.77 ± 1.05	9.37 ± 0.87
ZEL93	5.56 ± 0.37	–	–	–
EWE94	8.83 ± 0.40	–	–	–
Variance components				
$\hat{\sigma}^2_g$	23.82 ± 2.46**	6.91 ± 1.28**	42.69 ± 6.30**	25.59 ± 3.82**
$\hat{\sigma}^2_{ge}$	6.23 ± 1.36**	4.97 ± 1.11**	16.80 ± 2.96**	10.96 ± 1.97**
$\hat{\sigma}^2$	39.60 ± 1.62	19.52 ± 1.10	49.72 ± 2.86	33.98 ± 1.96
Heritability (F_n lines)				
$\hat{h}^2$	0.82	0.70	0.84	0.82
90% C.I.[d]	(0.78; 0.85)	(0.60; 0.77)	(0.78; 0.87)	(0.76; 0.86)

**, * Significant at the 0.01 and 0.05 probability level, respectively

[a] Standard errors are attached

[b] $\bar{P}$ = mean of P1 and P2; $\bar{F}_n$ = means of F_3 ($A \times B^{I}$), F_4 ($A \times C$, $C \times D$), or F_5 lines ($A \times B^{II}$), respectively

[c] EWE92: Eckartsweier 1992; KRO92: Krozingen 1992; EWE93: Eckartsweier 1993; HOC: Hochburg 1993; KRO: Krozingen 1993; Zel93: Zell 1993; EWE94: Eckartsweier 1994

[d] Confidence intervals (C.I.) of $\hat{h}^2$ were calculated by the method of Knapp et al. (1985)

Table 2 Parameters associated with putative QTLs significantly affecting the percentage of plants infected by *U. maydis* in F_n lines of maize populations $A \times B^I$, $A \times B^{II}$, $A \times C$, and $C \times D$, across five environments

Bin[a]	$A \times B^I$				$A \times B^{II}$				$A \times C$				$C \times D$			
	a[b]	d[b]	R^{2c}	Q × E	a	d	R^2	Q × E	a	d	R^2	Q × E	a	d	R^2	Q × E
	——%——				——%——				——%——				——%——			
1.02	− 2.0**	1.4	4.6													
1.04/05					− 1.3**	2.1	16.0	**	2.3**	− 0.6	8.6	**	− 3.2**	2.2	18.0	**
1.09									3.1**	− 3.0	13.1	**				
2.03/04	− 2.4**	− 2.8*	8.5										1.9**	1.4	13.6	
2.06	1.6**	2.8*	4.4	**	0.0	5.8**	14.1	*								
2.08									1.8**	− 3.0	8.4	*				
3.05	2.1**	− 0.7	10.0	**												
3.06	− 1.8**	− 1.7	5.5										− 1.6**	0.4	10.2	
3.08					− 1.0**	− 1.2	13.2		− 2.3**	5.0	11.5					
4.03/04	1.5**	0.8	5.8										− 2.7**	− 1.3	22.5	**
4.09													1.6**	2.3	11.3	
5.01/02	− 2.5**	− 3.2**	15.4		− 0.4	− 4.2**	10.2		− 3.1**	2.5	12.4		− 2.9**	− 0.8	13.7	
5.03									3.5**	0.3	22.5	**	2.2**	2.7	9.5	
6.07		–			− 0.3	3.5*	9.9									
7.04													− 1.6**	0.9	11.3	
8.06													− 0.9*	− 2.9	9.0	
9.01	2.5**	0.4	10.9													
9.05	− 1.3**	0.3	4.3													
10.04									− 1.7**	− 7.1**	11.9	*	1.4**	− 3.5	16.1	
Total	17.7	14.1	46.0		3.0	16.8	38.6		17.8	21.5	50.4		20.0	18.4	57.5	

*, ** Significant at the 0.05 and 0.01 probability level, respectively

[a] Dissection of chromosomes into Bin regions and QTL positions refer to the marker linkage map published in the Maize Genetics Newsletter 70 (1996)

[b] a = additive effect and d = dominance effect of QTL

[c] Percent phenotypic variance explained by QTLs

Table 3 Parameters associated with putative QTLs significantly affecting the percentage of plants infected by *U. maydis* in F_4 lines of maize population C × D across five environments in Germany (UST-D) and for a stress environment with a high incidence of *U. maydis* at Chartres in France. In the latter case, besides UST (UST-F) the percentage of plants with visible ear galls (EAG) or stem galls (STG) was determined

Chromosome[a]	EAG				STG				UST-F				UST-D			
	a[b]	d[b]	R^{2c}	Pos.[d]	a	d	R^2	Pos.	a	d	R^2	Pos.	a	d	R^2	Pos.
	%				%				%				%			
1					− 1.0**	0.5	9.3	0								
1													− 3.2**	2.2	18.0	38
1	− 6.3**	3.9	10.4	200					− 5.9**	− 1.0	9.8	206				
2	4.8**	− 6.0	17.1	86	1.0**	− 0.6	8.9	78	4.9**	− 4.5	17.8	86	1.9**	1.4	13.6	82
3													− 1.6**	0.4	10.2	82
4	− 3.1**	6.1	11.6	40					− 2.4*	12.0*	12.3	34	− 2.7**	− 1.3	22.5	38
4													1.6**	2.3	11.3	192
4	3.7**	0.0	10.6	238	1.2**	2.6	11.5	250	3.8**	4.4	10.7	248				
5													− 2.9**	− 0.8	13.7	50
5													2.2**	2.7	9.5	66
7													− 1.6**	0.9	11.3	76
7	− 6.6**	− 0.1	21.0	108					− 6.1**	− 1.2	20.1	108				
8													− 0.9*	− 2.9	9.0	74
9	4.4**	0.1	12.6	128					3.7**	1.7	9.7	128				
10	3.7**	− 4.4	9.4	80									1.4**	− 3.5	16.1	72
Total	32.6	16.2	52.1		3.2	3.7	22.3		26.8	24.8	47.2		20.0	18.4	57.5	

*,** Significant at the 0.05 and 0.01 probability level, respectively
[a] Maize chromosome
[b] a = additive gene effect and d = dominance effect of QTL
[c] Percent phenotypic variance explained by QTLs
[d] Map position according to Lübberstedt et al. (1998 a)

were highly significant ($P < 0.01$) in each population (Table 1). In all four populations, $\hat{\sigma}^2_{ge}$ was smaller than $\hat{\sigma}^2_g$. Genotypic variances differed significantly ($P < 0.05$) between all four populations for the five or three environments in common. Heritability for UST exceeded 0.70 for all populations and was largest for A × C ($\hat{h}^2 = 0.84$).

QTL analyses

A detailed presentation of results from the QTL analyses is given exclusively for means across environments, because: (1) those QTLs are most important for breeding purposes, (2) a test of QTL × E interactions is included, and (3) results are easier to survey. A complete list of the number and designation of the selected co-factors used for each population can be obtained upon request from the corresponding author.

In the joint analyses across five environments, a total of nine, five, seven, and ten QTLs were detected in A × B^{I}, A × B^{II}, A × C, and C × D, respectively, distributed over all ten chromosomes (Table 2). A simultaneous fit of all putative QTLs explained between 38.6% (A × B^{II}) and 57.5% (C × D) of $\hat{\sigma}^2_p$. Individual QTLs explained between 4.3% and 22.5% of $\hat{\sigma}^2_p$ (A × C, chromosome 5; C × D, chromosome 4). All QTLs displayed significant additive gene effects except for three QTLs identified in A × B^{II}, which had only significant dominance effects (chromosomes 2, 5, 6) (Table 2). The sum of absolute additive effects was 17.7%, 3.0%, 17.8%, and 20.0% in A × B^{I}, A × B^{II}, A × C, and C × D, respectively. In seven cases, significant dominance effects, both increasing or decreasing UST, were detected (Table 2). In each population, UST-increasing alleles were contributed by both parents. Only one QTL on chromosome 5 was consistently detected across all four populations (Table 2).

Significant ($P < 0.05$) digenic epistatic interactions of the type dominance × dominance were detected between the QTLs located on chromosomes 2 and 6 in A × B^{II} ($\widehat{dd} = 11.7\%$). Significant ($P < 0.05$) digenic epistatic interactions of the type additive × dominance were detected in A × B^{I} between the QTLs located on chromosomes 2 (Bin region 2.03) and 9 (Bin region 9.01) ($\widehat{ad} = -2.7\%$), and in A × B^{II} between the QTLs on chromosomes 5 and 3 ($\widehat{ad} = -1.8\%$). For C × D, four pairs of QTLs displayed significant epistatic interactions of the type additive × additive, four of the type type additive × dominance, and three of the type dominance × dominance. None of the digenic epistatic interactions were detected for the transformed UST data (arcsin $\sqrt{UST/100}$) in populations C × D and A × B^{II}.

In total, 11 QTLs displayed significant ($P < 0.05$) QTL × E interactions (Table 2). For C × D, two out of six QTLs identified in Chartres were also detected across five German environments (Table 3). All QTLs detected for EAG or STG, except one for each trait, were in common with the QTLs for UST in Chartres (Table 3).

Phenotypic correlations between UST and rust ratings (Lübberstedt et al. 1998 b) for the means of F_n lines were not significant ($P < 0.05$) across the three environments in common (Eckartsweier 1993, Hochburg 1993, and Krozingen 1993) for populations A × B^{II}, A × C, and C × D, or across all five environments for population A × B^{I} (Table 4). Genotypic correlations ranged between −0.06 and 0.13. The number of QTLs in common for UST and rust ratings varied between zero and three for individual populations (Table 4).

Discussion

As a consequence of the dramatic increase of maize production in Germany during the past three decades, one of the urgent questions was whether this would be accompanied by an increased incidence of maize diseases. While this holds true for the European corn borer (Langenbruch and Scewczky 1995), it does not apply to most other diseases, including *U. maydis*. However, individual years of high pest incidence, such as 1976 for *U. maydis* (Zscheischler et al. 1979), raise the farmers demand for resistant varieties. *U. maydis* incidence between 1992 and 1994 exceeded 5% in our field trials at different German locations and was, therefore, slightly increased compared to the averages given in former reports (Zscheischler et al. 1979). This was partly due to the increased susceptibility of lines A, B and C, exceeding the median for UST in a study of

Table 4 Phenotypic and genotypic correlations for resistance to common smut and common rust in maize populations A × B^{I}, A × B^{II}, A × C and C × D as well as the number of QTLs in common for both traits

Item	A × B^{I}	A × B^{II}	A × C	C × D
Phenotypic correlation	−0.36	0.05	0.08	−0.04
Genotypic correlation	0.13	0.08	0.00	−0.06
Number of QTLs detected for common smut	9	5	7	10
Number of QTLs detected for common rust	9	4	7	13
QTLs in common (with the same mode of gene action and sign of additive effects)	0	1 (0)	2 (1)	3 (1)

137 European maize inbred lines recorded at nine environments in 1993 and 1994 (unpublished results).

In populations $A \times B^{I}$ and $C \times D$, the means of susceptible lines A and C were significantly higher than those of lines B and D, respectively. In contrast, no significant differences were observed between the parent lines of populations $A \times B^{II}$ and $A \times C$. Nevertheless, in all four populations, intermediate-to-high heritabilities ($\hat{h}^2 > 0.69$) (Table 1) were obtained indicating their usefulness for this QTL mapping study. Distributions of UST values were skewed to the right in all four populations but transformation of UST values into arcsin $\sqrt{UST/100}$ values had little effect on QTL detection. Therefore, the original data were directly used for QTL analyses considering also that the *F*-tests employed in the regression approach implemented in PLABQTL are rather robust against deviations from the usual assumptions required with the maximum-likelihood approach (Utz and Melchinger 1996).

Although the number of genes involved in resistance to *U. maydis* is unknown, the trait was previously considered to be oligogenically inherited (Christensen 1963). Early studies using crosses of resistant inbred lines and susceptible translocation stocks identified at least three regions in each cross conferring resistance to *U. maydis* (Burnham and Cartledge 1939; Saboe and Hayes 1941). Schön (1993) identified nine QTLs for resistance to *U. maydis* in testcrosses of 345 F_3 lines of the cross $A \times B$. In our study, a total of 19 different QTLs distributed over all ten chromosomes, and between five and ten QTLs for individual populations, were detected (Table 2). Hence, the inheritance of resistance to *U. maydis* in maize seems to be comparatively complex, as holds true also for resistance to gray leaf spot (Bubeck et al. 1993) and partial resistance to common leaf rust (Lübberstedt et al. 1998 b).

Different from the race-specific *Rp* loci mediating resistance to *P. sorghi* (Hooker 1985), no reports exist about major resistance genes involved in resistance to *U. maydis*. In agreement with these findings the largest QTLs identified in our study explained only 22.5% of $\hat{\sigma}^2_p$ (Table 2). This estimate might even be inflated due to the small population sizes of $A \times C$ and $C \times D$ (Utz and Melchinger 1994). It is rather low compared to the 59% explained by a major QTLs identified for resistance to maize streak virus (Welz et al. 1998) using 196 $F_{2:3}$ lines and the same statistical procedures and software package as employed in our study. All except three QTLs in $A \times B^{II}$ displayed significant additive effects (Table 2). Moreover, seven out of the 31 QTLs detected over all four populations displayed significant dominance effects (Table 2). By comparison, only three QTLs mapped for resistance to *P. sorghi* in the same four populations showed significant dominance effects (Lübberstedt et al. 1998 b). More than 50% of the 31 QTLs displayed dominant or overdominant gene action ($|d/a| > 0.8$). Dominant or overdominant gene action caused increased resistance for about one-half of these loci and increased susceptibility for the other half, while earlier studies indicated intermediate to dominant inheritance of resistance to *U. maydis* (Christensen 1963).

Little is known about the relationship between a prevalent additive or dominant mode of inheritance and the nature of minor resistance genes. This is in contrast to major fungal resistance genes (Pryor 1987), where a prevailing dominant or recessive gene action seems to be the consequence of a biotrophic or necrotrophic life cycle of the respective pathogen. Nevertheless, resistance to *U. maydis* seems to include morphological (e.g., presence of ligules, tightness of the husk) and physiological (concentration of a smut inhibitor) factors (Christensen 1963). In addition, specific mechanisms might be involved for different parts of the plant because some QTLs identified in Chartres 1993 affected *U. maydis* incidence exclusively at stems or ears (Table 3). This is in agreement with former observations that certain inbreds are preferably infected at definite sites of the plant (Christensen 1963).

In the present study, QTLs for resistance against *U. maydis* displayed moderate-to-poor consistency across populations. Similar findings were reported for complex forage maize traits, resistance against *P. sorghi* (Lübberstedt et al. 1998 a, b), and resistance against gray leaf spot in maize (Bubeck et al. 1993). Possible reasons for inconsistencies of QTLs across populations are: (1) different sets of polymorphic and detectable QTLs in each of the three crosses, (2) a low power of QTL detection, (3) QTL × E interactions, and (4) epistasis. Among the three populations $A \times B^{II}$, $A \times C$, and $C \times D$, of about equal size and evaluated at the same five environments, between two ($A \times B^{II}$ and $C \times D$) and four ($A \times C$ and $C \times D$) QTLs were in common (Table 2). Owing to the pedigree relationship of lines A and D with a co-ancestry coefficient (Malecot 1948) $f = 0.13$, on one side, and lines B and C ($f = 0.06$), on the other side, a reversed sign of additive effects was expected to prevail for common QTLs between populations $C \times D$ and $A \times B^{II}$. However, most of the "common" QTLs differed either in their gene effects (significant additive or dominant gene action) or in the sign of their significant additive effects. In conclusion, at least some of the "common" QTLs for resistance against *U. maydis* might be linked rather than being identical QTLs.

In small populations of about 100 individuals or families, the power of QTL detection even for traits with high heritabilities is rather low, and estimates of the proportion of $\hat{\sigma}^2_p$ explained by the detected QTL might be severely inflated (Utz and Melchinger 1994). According to these latter authors, the power for detecting a QTL explaining 6% of $\hat{\sigma}^2_p$ is 31% for a trait with $h^2 = 0.7$ and a population size of 100 F_2 individuals. Hence, the probability of the simultaneous detection of

the same QTL in two independent samples of 100 F_2 individuals is only about 10%. Since most QTLs detected in our study explained less than 15% of $\hat{\sigma}_p^2$, the consistency of QTLs across populations might be severely impaired by the low power of QTL detection in small populations. This may be an explanation for the inconsistencies between populations $A \times B^I$ and $A \times B^{II}$. Furthermore, the statistical power of the test for dominance effects was rather low for the $F_{4:5}$ lines of population $A \times B^{II}$ compared to $A \times B^I$, where $F_{2:3}$ lines were evaluated. Only 12.5% of the parental F_4 plants of $F_{4:5}$ lines are expected to be heterozygous for a given marker locus and, hence, to be informative with regard to the estimation of dominance effects. Estimates of dominance effects, and consequently the degree of dominance, might be biased due to the low number of heterozygotes per locus. This might be an explanation for the high proportion of overdominant gene action for the QTLs detected in $A \times B^{II}$ compared to $A \times B^I$.

Genotype × environment and QTL × E interactions are frequently associated with quantitative resistances (Bubeck et al. 1993; Dingerdissen et al. 1996; Geiger and Heun 1989; Lübberstedt et al. 1998 b). This also holds true for resistance to *U. maydis*, where 11 QTLs displayed QTL × E interactions across the four populations investigated (Table 2). In contrast to most other QTL studies (Melchinger 1997), some QTL × E interactions were related to different signs of dominance ($A \times B^I$, $A \times B^{II}$: chromosome 2) or additive ($A \times C$: chromosome 1) effects in individual environments. In contrast, only a few QTLs showed QTL × E interactions in a study of forage traits using testcross progenies of the same populations evaluated in five environments (Lübberstedt et al. 1998 a). One explanation for an increased level of QTL × E interactions for resistance against *U. maydis* compared to forage traits might be race-specific expression of QTLs and, therefore, characteristic QTL patterns in each environment depending on the prevalent smut races. Alternatively, different climatic and growing conditions for maize populations at individual environments might affect the expression of QTLs involved in developmental, morphological, and chemical characters affecting resistance against *U. maydis*.

In our study, 11 cases of significant digenic epistatic interactions were identified for $C \times D$ and three different ad and dd epistatic interactions were detected in $A \times B^I$ and $A \times B^{II}$. The relatively large estimate for the absolute dd effect (11.7%) obtained in $A \times B^{II}$ might be upwardly biased due to the small number of double heterozygotes in a population of $F_{4:5}$ lines of this sample size. Furthermore, most digenic interactions could be attributed to extreme values of UST, since for transformed arcsin $\sqrt{\text{UST}/100}$ values of $C \times D$ and $A \times B^{II}$ no digenic epistatic interactions were detected. Dissection of partial resistances by QTL mapping revealed digenic epistatic interactions between QTLs involved in resistance against common rust in maize (Lübberstedt et al. 1998 b) and rice blast (Wang et al. 1994), but not for other disease resistances (Young 1996).

Sweet corn was found to be generally more susceptible to common smut (Christensen 1963; Agrios 1988) and common rust (Gingera et al. 1994) than field corn. One explanation for these observations might be common factors responsible for an increased susceptibility to both fungi in maize. In our study, partial resistances to common rust and common smut were not correlated in all four populations (Table 4). In addition, only a few QTLs were in common, mostly with an opposite sign for additive effects or a different type of gene action (dominant vs additive). In conclusion, partial resistances to common rust and common smut seem to be independently inherited, at least in our materials.

Resistance to *U. maydis* can be improved by: (1) phenotypic selection based on an evaluation of the germplasm in target environments with natural infection, (2) phenotypic selection at special locations prone to high *U. maydis* infection pressure, or (3) marker-assisted selection. A fourth alternative is genetic engineering. However, the success of the only transgenic strategy proposed so far, using virally encoded antifungal toxins expressed in maize plants (Kinal et al. 1995), remains to be demonstrated.

Phenotypic selection under natural infection conditions looks promising, with an average *U. maydis* incidence in the four populations of between 3.7 and 10.8% (Table 1). In each of the four populations, F_n lines with less than 1% infected plants were found. Under the same natural infection conditions, F_n lines with a *U. maydis* incidence exceeding 30% were observed in each of the three crosses. In cases of demand, such lines could be a starting point for the development of European maize hybrids for the production of "huitlachoche".

In hybrid breeding the relationship between line *per se* and testcross performance is of fundamental importance. Despite a prevailing dominant or overdominant mode of gene action, six out of nine QTLs affecting resistance to *U. maydis* detected in $A \times B^I$ were in common with six out of nine QTLs identified across two populations of testcross (TC) progenies derived from 345 $F_{2:3}$ lines of population $A \times B^I$ (Schön 1993). All six common QTLs displayed the same sign of additive gene effects for line *per se* performance and gene substitution (α-) effects for testcross performance. Two of the three QTLs not identified in the TC populations showed an overdominant mode of inheritance (chromosome 2: Bin region 2.03; chromosome 5). Hence, at least for our materials, line *per se* performance seems to be a reliable predictor of TC performance and is generally not covered by the dominant gene action of alleles from the dent tester.

In order to allow selection for resistance against *U. maydis* in locations and years of low smut incidence, breeders employ *U. maydis*-prone environments like

Chartres with 14.8% infected plants in C × D compared to 9.3% in Zell, the German location with the highest *U. maydis* incidence in C × D (Table 1). An important question is whether the results obtained in such an environment coincide with those found under "natural" conditions in locations of the target region. For F_n lines of C × D, the phenotypic correlation between Chartres and the mean across the five German environments ($\hat{r}_p = 0.61$) was lower than the phenotypic correlations between individual German environments and the mean across the remaining four German environments ($0.62 < \hat{r}_p < 0.77$), except for Krozingen 1992 ($\hat{r}_p = 0.40$) with an extremely low level of smut incidence. Accordingly, only two out of six QTLs identified for Chartres (UST-F) and ten QTLs identified across the five German environments (UST-D), respectively, were in common. In addition, all six QTLs detected for Chartres explained only 12.5% of the UST-D R^2. This poor agreement between Chartres and the German environments might be caused by deviating growing conditions for maize in both mega-environments or by different races of *U. maydis* altering the relative susceptibility of F_n lines to common smut. In consequence, phenotypic or marker-assisted selection based on information obtained from *U. maydis*-prone locations might be of limited value for improving smut resistance in the target environments.

The detection of associations between QTLs and markers is the first step in different breeding strategies based on marker-assisted selection (MAS). It determines the proportion p of $\hat{\sigma}^2_g$ explained by the putative QTLs and, hence, is a key factor for the relative efficiency (RE) of MAS compared to conventional selection. The relative efficiency of a single cycle of MAS in comparison with conventional selection can be estimated as $RE = (p/h^2)^{1/2}$ (Lande and Thompson 1990), given the same selection intensity for both selection schemes. For all four populations RE values were below 0.84 due to the high heritabilities of UST and the moderate p values. Hence, in the approach outlined above, conventional selection for resistance to *U. maydis* seems to be superior to MAS. However, markers might be helpful if direct selection is impossible because of a low level of *U. maydis* infections, as in 1997, resulting in low heritabilities. For materials grown in the greenhouse or winter nursery, MAS allows the completion of two selection cycles per year with phenotypic selection in every second cycle. Further advantages of marker-based approaches are the identification of QTLs with dominant alleles conferring resistance to *U. maydis* and the possibility to select in both sexes prior to flowering, making selection more efficient even in the case of a high level of *U. maydis* incidence. Nevertheless, owing to the poor consistency of QTLs across populations, QTL mapping must be performed in each population separately as a pre-requisite for marker-assisted selection. In conclusion, MAS for resistance to *U. maydis* might be economically reasonable only in connection with marker-based programs with primary emphasis on other traits like grain yield or forage quality.

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