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The *Rp3* disease resistance gene of maize: mapping and characterization of introgressed alleles

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Abstract The *Rp3* locus of maize conditions race-specific resistance to a fungal rust pathogen, *Puccinia sorghi*. Both morphological and DNA markers were employed to characterize alleles of *Rp3* and to accurately position *Rp3* on the maize genetic map. DNA marker polymorphisms distinctive to each *Rp3* allele were identified, allowing the identification of specific *Rp3* alleles in cases where rust races that differentiate particular alleles are not available. In a population of 427 progeny, *Rp3* and *Rg1* were found to be completely linked, while *Lg3* was approximately 3 cM proximal on the long arm of chromosome 3. In this same population, 12 RFLP markers were mapped relative to *Rp3*; the closest markers were UMC102 (about 1 cM distal to *Rp1*) and NPI114 (1–2 cM proximal). These and additional DNA probes were used to characterize the nature and extent of flanking DNA that was carried along when six different *Rp3* alleles were backcrossed into a single background. Depending upon the allele investigated, a minimum of 2–10 cM of polymorphic DNA flanking the *Rp3* locus was retained through the introgression process. In addition, many of the probes that map near *Rp3* were found to detect an additional fragment in the *Rp3* region, indicating that portions of this chromosomal segment have been tendemly duplicated. The materials and results generated will permit marker-assisted entry of *Rp3* into different maize backgrounds and lay the foundation for the eventual map-based cloning of *Rp3*.

Key words Restriction fragment length polymorphism · Near-isogenic lines · *Puccinia sorghi* · Introgression · Gene duplication

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Introduction

Fungal rusts of the genus *Puccinia* are among the most devastating pathogens in agriculture. Control of these diseases is accomplished primarily by breeding for resistant varieties. Single, commonly dominant, resistance genes that provide high levels of race-specific resistance have been found in several crop species (Hooker and Saxena 1971). When characterized, these resistance loci have been observed to act in a gene-for-gene manner where a resistant (or incompatible) interaction occurs only when a dominant avirulence gene in the pathogen is matched with a resistance allele in the host (Flor 1971). As with other gene-for-gene resistance loci, the protection that these genes provide is generally short-lived due to the appearance of new pathogen races that are virulent on the previously resistant host. The exact mode of action of gene-for-gene resistance loci has not been determined, although the genetics of these systems (Bennetzen and Hulbert 1993) and the molecular cloning of the first resistance gene of this type (Martin et al. 1993) suggest that they are the recognition molecules in a signal transduction process.

The *Rp* loci, maize genes that specify resistance to the common rust disease caused by *Puccinia sorghi*, have been particularly well-characterized. Hooker and coworkers mapped several *Rp* genes and introduced 14 alleles of *Rp1*, 6 alleles of *Rp3*, 2 alleles of *Rp4*, and *Rp5* into the same inbred background (Hagan and Hooker 1965; Hooker and Russell 1962; Lee et al. 1963; Wilkinson and Hooker 1968). Subsequent studies indicated that *Rp1* is not actually a single gene but contains a series of linked *Rp* genes with different specificities (Bennetzen et al. 1991; Hulbert and Bennetzen 1991; Saxena and Hooker 1968). This cluster of genes exhibits allele-specific instability associated with unequal crossing-over between tandemly repeated DNAs in the *Rp1* region (Bennetzen et al. 1988; Hong et al. 1993; Hulbert and Bennetzen 1991; Pryor 1987; Sudupak et al. 1993). Detailed instability and fine structure analyses of *Rp3* have not yet been performed.

In this study, we have identified linked DNA markers that differentiate between our collection of *Rp3* alleles and have determined the map position of *Rp3* relative to morphological and DNA markers. In addition, we report the use of this information to determine the efficiency of a ten-generation backcross introgression process in breaking the linkage between the resistance gene and flanking sequences. These results also demonstrated a high level of tandem duplication of low-copy-number DNAs in the *Rp3* region.

Materials and methods

Plant material

Six near-isogenic lines (NILs) differing in the *Rp3* allele (*Rp3*-A through *Rp3*-F) were generated by Hooker and coworkers by backcrossing ten or more generations into the fully rust-susceptible line R168. We have utilized and described these lines previously (Hulbert et al. 1991). A *P. sorghi*-susceptible stock with the genotype *Lg3 Rg1 rp3/lg3 rg1 rp3* (no. 313C) was obtained from the Maize Genetics Stock Center (University of Illinois, Urbana, Ill. USA).

Mapping cross and analysis

The *Lg3 Rg1 rp3/lg3 rg1 rp3* stock from the Maize Genetics Stock Center was crossed to an *Rp3*-D/*Rp3*-D line in the R168 background that was homozygous for *lg3 rg1*. Liguleless (*Lg3/lg3*) and ragged (*Rg1/rg1*) progeny of this cross were selected and test-crossed to a homozygous *lg3 rg1 rp3* inbred. A total of 427 test-cross individuals were scored for *Rp3*-D [with rust race KS1 (Hulbert et al. 1991)], *Rg1*, and *Lg3*. A random sample of 76 individuals from this population was also typed with restriction fragment length polymorphism (RFLP) probes.

DNA sources and preparation

Plasmids containing maize RFPL probes were generously supplied by D. Grant (Pioneer Hi-Bred International, Johnston, Iowa, USA) and D. Hoisington (University of Missouri, Columbia, Mo., USA). Probe fragments were excised with the appropriate restriction enzyme and purified by agarose gel electrophoresis. Maize DNAs were extracted from 15-day-old seedlings approximately as described by Saghai-Maroof et al. (1984).

RFLP analysis

Maize DNAs were digested, electrophoretically resolved, nylon filter blotted, and hybridized with oligo-labelled probes as previously described (Hulbert et al. 1990). Polymorphisms for DNA probes that mapped near *Rp3* in the NILs were investigated with separate digestions employing restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, and *Sac*I. These four enzymes, plus *Bam*H1, *Dra*I, *Kpn*I, *Sal*I and *Xho*I, were used in the mapping of *Rp3*-D relative to 12 different RFLP probes.

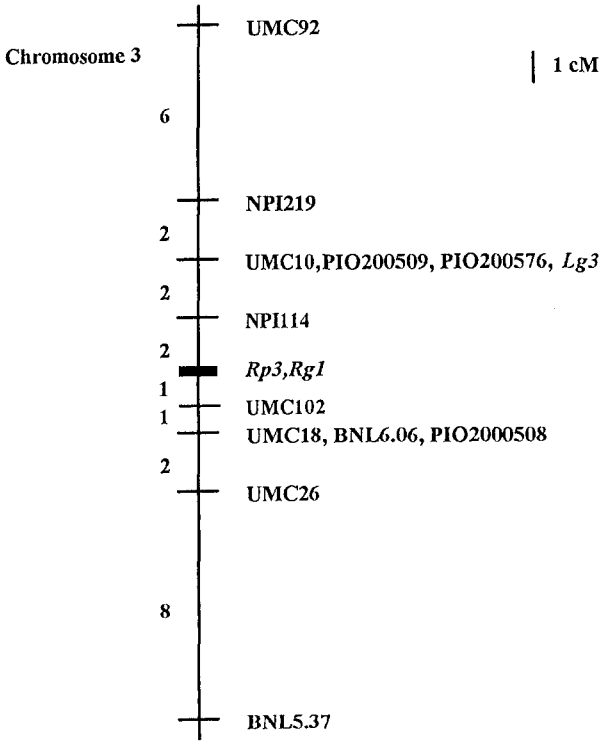
Results

Linkage of *Rp3* to morphological and DNA markers

Complete linkage was observed between *Rp3*-D and *Rg1* in an analysis of 427 test-cross progeny segregating for these traits, while this same population yielded 13 recombinants between *Rp3*-D and *Lg3*. Since *Lg3* has been positioned about 2 cM proximal to *Rg1* on the long arm of chromosome 3 (Coe and Neuffer 1993), these data are in good agreement with our predicted position of *Rg1* and *Rp3*-D as about 3 cM distal to *Lg3*.

Various laboratories have mapped numerous DNA markers to this approximate region of the maize genome (Burr et al. 1988; Coe et al. 1987; Grant et al. 1989; Helentjaris et al. 1986), and we acquired several of these probes. Of the 32 markers acquired and tested, 12 were found to be polymorphic at sites linked to *Rp3* in the two parents used in the mapping cross described above. These probes were employed in analysis of 76 randomly chosen test-cross progeny, and the results are displayed in Fig. 1. The size of the progeny population analyzed was not sufficient to identify recombinants between all of the polymorphic markers used, and map distances should be considered approximations. However, the order of the markers or cosegregating marker groups was unambiguous because each of the progeny represented single meiotic products and no double crossovers were observed between any closely linked markers. These data placed NPI114 and UMC102 as the 2 most tightly linked probes; NPI114 was positioned about 2 cM (two recombination events) proximal and UMC102 mapped about 1 cM (one recombination event) distal.

Fig. 1 Genetic map of the region around *Rp3*, using morphological and DNA markers. Results are derived from the analysis of 76 randomly chosen progeny of a cross of *Lg3 Rg1 rp3/lg3 rg1 Rp3*-D individuals to a *lg3 rg1 rp3* tester. Map distances, shown at *left*, are rounded off to the nearest cM



Identification of a misclassified *Rp3* allele by mapping analysis

In parallel to our mapping of morphological and DNA markers relative to *Rp3*-D, we attempted to map *Rp3*-C relative to other DNA and morphological markers not employed in the *Rp3*-D study. Initial results indicated that the rust resistance conditioned by our *Rp3*-C stocks actually segregated as two independent and dominant alleles; F_2 progeny from the cross *Rp3*-C × H95 (susceptible) segregated 75 resistant to 5 susceptible to rust isolate IN3 (χ^2 15:1 = 0.0). F_3 lines with two different specificities were subsequently derived from this cross. One of the lines had a phenotype similar to that of other *Rp3* lines [resistant to KS1, IN1, and IN3, with an intermediate reaction to IN2 (Hulbert et al. 1991)]. The other derived lines were susceptible to KS1 and IN1 while giving necrotic resistance reactions to IN2 and IN3; these are characteristics of lines carrying *Rp1*-A or *Rp1*-F (Hulbert et al. 1991). Hence, our previous characterizations of the race-specificity profile of resistance conditioned by *Rp3*-C (Bennetzen et al. 1991; Hulbert et al. 1991) is in error. This contamination probably occurred before we acquired this line, because all of our "*Rp3*-C" stocks behave as if they contain both *Rp3*-C and an allele of *Rp1* (presumably A or F). More importantly, our discovery of *Rp3*-C stock contamination indicates an additional value for such mapping studies; genotype confirmation.

Analysis of the *Rp3* near-isogenic lines (NILs)

The maize RFLP probes that were employed came from a variety of different mapping programs (Burr et al. 1988; Coe et al. 1987; Grant et al. 1989; Helentjaris et al. 1986) that have not yet completely integrated their maps. Hence, the mapping data we generated for *Rp3* provided a conceptual base from which we could choose additional DNA probes for analysis of the *Rp3* allelic series introgressed into the R168 background. Even if particular RFLPs had not been mapped relative to *Rp3*, we could determine their approximate position by comparison of the regional map in Fig. 1 to the maps generated by these other mapping programs.

A total of 24 RFLP probes were chosen for analysis of the six *Rp3* NILs and the *rp3* allele in the recurrent R168 background. Genomic DNA from each NIL was separately digested with *Eco*RI, *Eco*RV, *Hin*dIII, and *Sac*I and analyzed by gel blot hybridization. Many probes detected no differences between the various NILs, but others yielded different hybridizational patterns with one or more NILs (Fig. 2). As we had previously observed (but to a much greater degree) in the area of the *Rp1* locus (Hulbert and Bennetzen 1991; Hong et al. 1993), many of the probes detected differences in band number as well as in band size (e.g., Fig. 2C lane 5). These data are summarized in Table 1. No single probe differentiated between every *Rp3* allele, but the combined use of UMC102 and PIO200802 could identify

Table 1 RFLP variability in *Rp3* isolines

Probe	Distance from *Rp3* (cM)	*Rp3* allele						
		rp3	*Rp3*-A	*Rp3*-B	*Rp3*-C	*Rp3*-D	*Rp3*-E	*Rp3*-F
PIO200042	22	1	2	1	1	1	1	1
PIO200006	14	1	1	1	1	1	1	1
NPI249	13	1	1	1	2[a]	2[a]	2[a]	2[a]
BNL8.35	10	1	1	1	1	1	1	1
UMC154	9	1	1	1	1	1	1	1
UMC50	9	1	1	1	1	1	1	1
UMC92	7	1	1	1	1	2[a]	1	3[a]
NPI219		1	1	1	1	2	1	3
PIO200576	7	1	1	1	1	2[a]	1	1
PIO200509	5	1	1	1	2[a]	3	1	1
PIO200558	4	1	1	1	1	2	3	4
NPI247	4	1	1	1	1	1	1	1
UMC42	4	1	1	1	1	2	3	4
PIO200511	3	1	2[a]	2[a]	2[a]	3[a]	2[a]	2[a]
UMC97	2	1	1	1	1	1	1	1
UMC161	2	1	1	1	1	2	2	2
UMC10	2	1	1	1	1	2	1	3
NPI114	2	1	1	1	1	2	2	2
Rp3								
PIO200802	0	1	1	1	2	2	3	4
UMC102	2	1	2	3	4	5	5	5
UMC18	3	1	2	1	3	3	3	3
PIO200508	4	1	2	1	3[a]	1	1	1
BNL6.06	7	1	2	1	1	1	1	1
UMC26	11	1	1	2[a]	1	3[a]	2[a]	3[a]
BNL5.37	23	1	1	1	1	1	1	1
UMC165	27	1	1	1	1	1	1	1
BNL8.01	28	1	2[a]	1	2[a]	1	1	2[a]
UMC175	52	1	1	1	1	1	1	1

[a] Band that was polymorphic among the NILs was not the one that was mapped in the test-cross

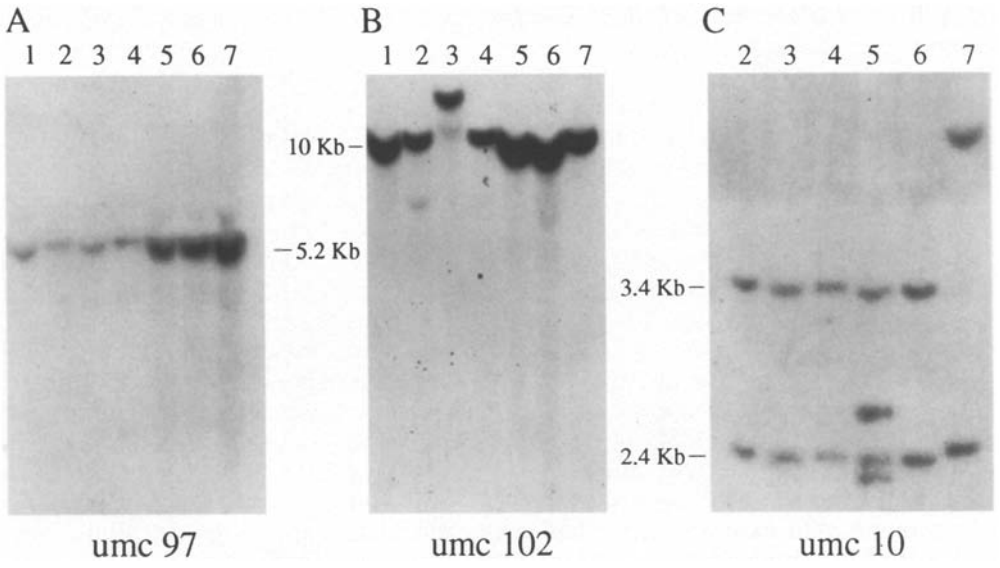


Fig. 2A–C Gel blot hybridization patterns with RFLP probes from the *Rp3* region. **A** DNAs digested with *Eco*RV and hybridized with UMC97. **B** DNAs digested with *Sac*I and hybridized with UMC102. **C** DNAs digested with *Sac*I and hybridized with UMC10. *Lanes 1–7* contain total genomic from the R168 NILs *rp3*, *Rp3*-A, *Rp3*-B, *Rp3*-C, *Rp3*-D, *Rp3*-E, and *Rp3*-F, respectively. Sizes (in kb) are relative to phage lambda DNA digested with *Hin*dIII

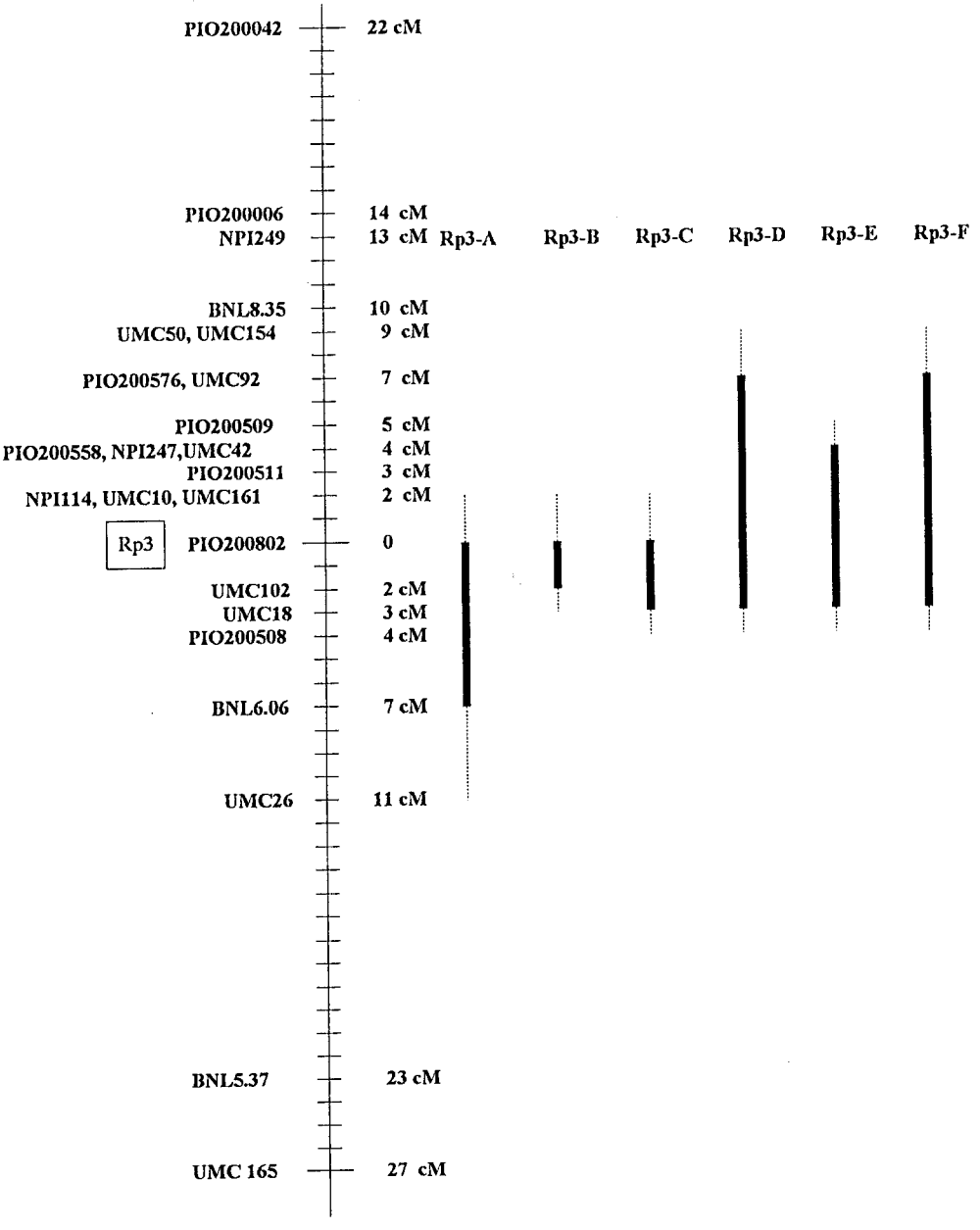


Fig. 3 Characterization of flanking DNA introgressed into the R168 background along with a set of *Rp3* alleles. At the *left* are listed all of the probes used to detect polymorphisms between the *Rp3* NILs, in those cases where the hybridized band was either the only band observed or the one mapped in the study summarized in Fig. 1. Approximate relative positions of each of these probes was derived from a synthesis of the various published maps (Burr et al. 1988; Coe et al. 1987; Grant et al. 1989; Helentjaris et al. 1986), our own mapping study (Fig. 1), and unpublished mapping data (D. Grant, personal communication). Since it is a composite map derived from several different mapping studies, there is a reasonable possibility that some markers are incorrectly positioned. The *vertical bars* to the *right* indicate regions of polymorphism retained in each introgressed NIL (*dark bar*) and the possible extent of additional polymorphism (*dotted bar*) that we could not score due to a lack of markers in the area. This figure is derived from the data summary displayed in Table 1

each *Rp3* allele. Because all *Rp3* alleles have very similar race-resistance specificities with the *P. sorghi* collections that currently exist (Bennetzen et al. 1991; Hulbert et al. 1991), these results indicate that *Rp3* alleles should be more easily identified in these stocks by DNA probes than by the standard screening with a series of rust biotypes.

In several cases a weak band or one that was not the one mapped in the analysis shown in Fig. 1 was found to be polymorphic between the NILs. These are indicated by light shading in Table 1. Since these NILs had been generated by ten generations or more of backcrossing to the same (R168) background, virtually all sites unliked to the selected *Rp3* region should contain R168 alleles. Hence, we expect that these minor variations actually are linked to *Rp3*, but not at the sites where their more intense bands have been mapped. This is exactly the result one would expect if there had been a tandem duplication or duplications of the region containing *Rp3*.

One band, that detected by PIO200042 in *Rp3*-A, was different from our R168 standard, despite being over 20 cM from *Rp3* and having no other differences in the intervening region. This band is the only one detected by this probe, so it cannot be explained as a "distantly tandem" locus. An occasional weakly linked (or unlinked) polymorphism is predicted by a backcross integration program of ten generations, but this retained polymorphism could also be an indication of a selected function in this chromosomal region. However, one possible explanation would be that this actually is R168 information at this site. It is likely that some polymorphism continues to exist within the R168 "inbred", and a rare R168 RFLP allele at this locus could account for this difference between the *Rp3*-A NIL and our R168 controls.

As expected, polymorphisms in the NILs were most commonly detected with DNA markers that had been previously mapped near the position we identified for the *Rp3* gene (Table 1). Different NILs exhibited different polymorphic positions and patterns, ranging from the *Rp3*-B line, with very little polymorphism, to the *Rp3*-D and *Rp3*-F lines, with long polymorphic stretches (Table 1). Befitting a presumably stochastic recombinational process that would remove donor flanking DNA during the recurrent backcross process, most NILs (except *Rp3*-D and *Rp3*-F) were observed to be unique in their extent of polymorphism, and the polymorphisms retained were assymetric relative to *Rp3* (Table 1).

Making the simplifying (although not necessarily correct) assumption that the last confirmed polymorphism in either direction from *Rp3* approximately delimits the extent of retained donor DNA, a map could be drawn of the position and amount of this retained DNA for each *Rp3* allele (Fig. 3). These crude approximations suggest that *Rp3*-B may have as little as 2 cM of retained donor DNA flanking *Rp3*, most of it proximal to the resistance gene. At the other end of the spectrum, *Rp3*-D and *Rp3*-F each have at least 10 cM of DNA surrounding *Rp3* that came in with the linked *Rp3* allele.

Discussion

Map-based opportunities for *Rp3* utilization

The placement of *Rp3* on the maize genetic map, relative to DNA markers, provides a number of opportunities for further utilization of this gene. At an applied level, the efficiency of introduction of *Rp3* into an elite background can be improved, because the gene may now be followed in a recurrent backcross program without the need to perform the laborious (and often undependable) phenotypic screen for rust resistance. Moreover, the loss of unlinked and linked/flanking DNAs ("linkage drag") can also be monitored to greatly enhance the rate at which backcrosses lead to a NIL state (Tanksley et al. 1989). This will be particularly important when genes that negatively affect line performance are tightly linked to a particular *Rp3* allele. The "pyramiding" of different *Rp* genes could be easily accomplished, because these multiple dominant loci could be introduced into a line by following DNA markers. Lines with various *Rp* gene combinations could also be used for studies of synergistic, competitive, or epistatic interactions between these genes (Simcox and Bennetzen 1993).

Many gene-for-gene resistance loci (including *Rp1*) are complex in nature, often consisting of multiple tandemly repeated genes with different recognition specificities (Bennetzen and Hulbert 1993; Hulbert and Bennetzen 1991; Pryor 1987; Saxena and Hooker 1968). At least some, perhaps all, of these gene-for-gene resistance loci are unstable due to unequal recombination events that can reciprocally delete and amplify resistance gene number (Bennetzen et al. 1988; Bennetzen and Hulbert 1993; Hulbert and Bennetzen 1991; Sudupak et al. 1993). Fine structure genetic studies of these loci require easily utilized flanking markers, and *Rp3* can now be subjected to this analysis. Moreover, this fine structure mapping and the identification of flanking markers could provide access to *Rp3* via map-based cloning (Martin et al. 1993). One of the advantages of maize as a study organism is the availability of several thousand mapped DNA probes. Hence, one has a reasonable chance of receiving through the mail a probe that maps within a centiMorgan of the targetted gene. In our study, PIO200802 seems to fit this criterion for *Rp3* (and for *Rg1*).

The use of DNA markers for placement of a gene that confers disease resistance also allows comparison to the position of genes in other plant species that may be mapped with the same DNA probes (Bennetzen and Freeling 1993). In our case, we are currently mapping genes conferring resistance to *Puccinia purpurea* in sorghum (*Sorghum bicolor*) using maize DNA probes. If the sorghum resistance maps to a site comparable

to that at which *Rp1*, *Rp3*, *Rp4*, *Rp5*, or *Rp6* map in maize, then we will suspect that the sorghum gene is probably allelic to the maize rust resistance gene. However, if a gene for sorghum rust resistance maps to a novel site, then we will suspect that it provides resistance by a process not identified in maize. Such a neomorphic function (Bennetzen and Freeling 1993) would be particularly interesting to study, and potentially of exceptional value to transfer to species that have not evolved this resistance mechanism.

Introgression of *Rp3* into the R168 background

Each of the NILs utilized in this study was derived from the introduction of a different *Rp3* allele into the R168 background by ten generations or more of backcrossing. Our estimates of the extent of *Rp3*-flanking DNA retained during the introgression process is limited by the level of polymorphism available between the original recurrent and non-recurrent parents. Thus, some flanking markers may not have differed between the two parents, just as probes like UMC97 (for instance) were not detected to be polymorphic between any of the lines used in this study. However, the employment of many probes in this small region should have partially compensated for this problem. Without additional information on the probe hybridization of the various non-recurrent parents, the values we calculated for retained flanking DNA must be minimum estimates.

On average, ten generations of recurrent crosses to R168 should remove over 99% of the unlinked sequences from the non-recurrent parent. About 18 cM (on average) of *Rp3*-linked sequences should remain through this crossing program (Hanson 1959; Muehlbauer et al. 1988; Stam and Zevin 1981), assuming that chromosome 3 of maize is approximately 180 cM in length. The minimum values of 2–10 cM of flanking DNA that were determined for the various *Rp3* alleles generally agree with these predictions. Hence, recombinational suppression was not detected in the *Rp3* region in any of the heterozygotes generated in the introduction program, nor does there appear to have been any selected bias against *Rp3*-flanking loci during the backcross program.

Investigation of the placement of the retained flanking DNA during these separate *Rp3* introduction programs suggests non-random sites for some recombination events. For instance, *Rp3*-A, *Rp3*-B and *Rp3*-C all seem to have acquired R168 sequences with a boundary between NPI114/UMC10/UMC161 and *Rp3*. Similarly, *Rp3*-D, *Rp3*-E and *Rp3*-F all seem to have undergone a recombination event in a roughly 1 cM interval between UMC18 and PIO200508. Additional probes from these small areas may differentiate recombination sites between these introduction programs, but it is also possible that these boundaries indicate preferred recombination sites in these populations.

Allelic variation at *Rp3*

Hooker and coworkers originally identified multiple alleles of *Rp3* by the unique response of the cultivars in which they were found to an array of different races of *P. sorghi*. Although Hooker's extensive rust collection has not survived, we have assembled a set of ten unique *P. sorghi* races that can be used for *Rp* gene differentiation (Hulbert et al. 1991 and unpublished results). Unfortunately, none of our rust races differentiates very well between any of the *Rp3* "alleles". The one exception to this general result was our observation that *Rp3*-C gave a much broader, and phenotypically distinguishable, range of rust resistance then did other *Rp3* genes (Bennetzen et al. 1991; Hulbert et al. 1991). The results presented here indicate that this observation was mistaken, since our *Rp3*-C stocks were contaminated with another *Rp* gene (probably either *Rp1*-A or *Rp1*-F). Hence, we are now largely unable to distinguish between any *Rp3* alleles with our *P. sorghi* collection.

The DNA markers utilized in this study do allow us to identify each of our *Rp3* NILs with as few as two DNA probes. This provides us with some confirmation of the independence of each of these *Rp3* alleles and confirms the general success of the backcross introgression program. However, this does not prove that any of these *Rp3* alleles actually show functional differences, only that they came from different maize sources. Hence, confirmation that these *Rp3* genes actually represent different alleles will require either identification of new rust races that allow a functional determination or cloning/structural analysis of *Rp3* from each of these NILs.

Duplications in the *Rp3* region

Our analyses of the *Rp1* region have indicated extensive tandem duplication of *Rp* genes (Hulbert and Bennetzen 1991; Sudupak et al. 1993) and other low-copy-number DNAs (Hong et al. 1993). These tandem duplications are included within a region of 5 cM or less, and can include as many as 15–20 copies of the repeated sequence (Hong et al. 1993). Although we have no definitive physical sizes for the region containing these repeats, we know that some repeats occur within as little as 90 kb of each other and that the whole series of tandem repeats covers over 800 kb (Frederick, SanMiguel, Hong, Edwards, Hulbert and Bennetzen, unpublished observations). A very tightly linked, relatively simple series of tandemly duplicated loci homologous to the *Pto* resistance gene of tomato has been observed by Martin et al. (1993). Similarly, Ronald and coworkers found a series of tandemly repeated sequences near the *Xa21* disease resistance gene of rice (Ronald et al. 1992). These molecular results concur with the genetics of gene-for-gene disease resistance loci, which often behave as tightly linked clusters of

related genes with different specificities (Bennetzen and Hulbert 1993). We have not observed anything similar to this high level of probe complexity for DNAs flanking the *Rp3* region. Hence, if *Rp3* is duplicated, then these duplications do not extend many centiMorgans into flanking regions.

Probes from the *Rp3* region often do detect more than one locus, and these generally map to more than one site. This observation, made by all laboratories that have mapped DNA markers in maize (Burr et al. 1988; Coe et al. 1987; Grant et al. 1989; Helentjari et al. 1986), is at least partly due to the fact that maize is actually a "degenerate tetraploid" (Helentjaris et al. 1988; Rhoades 1951; Wendel et al. 1986). However, some of the *Rp3*-linked probes detect loci that map to more than one site near *Rp3*. These sites appear to be separated by several centiMorgans and do not fall into a simple pattern suggestive of a tandem duplication of a large stretch of this region. Similar low-copy-number, widely separated tandem duplications in plants have been detected by others (Gill et al. 1991; S. Hake, personal communication; R. Shoemacher, personal communication), and we have also seen this phenonmenon in other regions of the maize and sorghum genomes (unpublished observation). The origin of these duplications is not clear, but they may be due to multiple centiMorgan tandem duplications of DNA that have been largely obscured by subsequent sequence divergence or local rearrangements. An alternative explanation would be that these sequences are actually very low-copy-number transposable elements that have duplicated themselves preferentially at linked sites due to the preferences transposons often exhibit for transposition to sites linked to the donor site (Dooner and Belachew 1989; Greenblatt and Brink 1962; Nowick and Peterson 1981).

Regardless of the origin of these "distantly tandem" duplications, they can greatly complicate a mapping program. Different laboratories will often map different bands detected by a single probe. When these bands map to two totally unlinked sites in the different investigators' hands, they then conclude that they are mapping different loci. However, when bands map to approximately the same site in each study, then it is assumed that the same loci are being positioned. If this assumption is incorrect, and it may often be in maize, then subsequent efforts to integrate maps from two different studies will generate unreal compromises. In our experiments, polymorphisms between our NILs were observed at what (incorrectly) appeared to be sites only distantly linked to *Rp3*, thereby (incorrectly) suggesting very little removal of the non-recurrent parent's flanking DNA during the introgression process.

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References

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, collinearity and complementarity. Trends Genet 9:259–261

Bennetzen JL, Hulbert SH (1993) Organization, instability and evolution of plant disease resistance genes. Plant Mol Biol 20:575–578

Bennetzen JL,, Hulbert SH, Lyons PC (1991) Genetic fine structure analysis of a maize disease-resistance gene. In: Patil SS, Ouchi S, Mills D, Vance C (eds) Molecular strategies of pathogens and host plants. Springer, Berlin Heidelberg New York pp 177–186

Bennetzen JL, Qin M-M, Ingels S, Ellingboe AH (1988) Allele-specific and *Mutator*-associated instability at the *Rp1* disease-resistance locus of maize. Nature 322:369–370

Burr B, Burr FA, Thompson KA, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Coe EH Jr, Neuffer MG (1993) Gene loci and linkage map of corn (maize) (*Zea mays* L.) (2n = 20). In: O'Brien SJ (ed) Genetic maps, book 6, plants. Cold Spring Harbor Laboratory Press, Plainview, N.Y., pp 157–189

Coe EH Jr, Neuffer MG, Hoisington DA (1987) The genetics of corn. In: Sprague GF, Dudley J (eds) Corn and corn improvement. Am Soc Agron, Madison, Wis., pp 81–258

Dooner HK, Belachew A (1989) Transposition pattern of the maize element *Ac* from the *bz-m2* (*Ac*) allele. Genetics 122:447–457

Flor HH (1971) Current status of the gene-for-gene concept. Adv Genet 8:29–54

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Grant D, Nelson W, Blair D, Katt ML, Martich J, Smith HSC, Bowen SL, Tenborg RA, Fincher RR, Meier R, Beavis WD, Smith OS (1989) Identification and localization of loci affecting yield and other agronomic traits in maize. In: Helentjaris T, Burr B (eds) Development and application of molecular markers to problems in plant genetics. Cold Spring Harbor Laboratory Press, Plainview, N.Y., pp 135–138

Greenblatt IM, Brink RA (1962) Twin mutations in medium variegated pericarp maize. Genetics 47:489–501

Hagan WL, Hooker AL (1965) Genetics of the reaction to *Puccinia sorghi* in eleven corn inbred lines from Central and South America. Phytopathology 55:193–197

Hanson WD (1959) Early generation analysis of lengths of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing. Genetics 44:833–837

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Helentjaris T, Weber DL, Wright S (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353–363

Hong KS, Richter TE, Bennetzen JL, Hulbert SH (1993) Complex duplications in maize lines. Mol Gen Genet 239:115–121

Hooker AL, Russell WA (1962) Inheritance of resistance to *Puccinia sorghi* in six corn inbred lines. Phytopathology 52:122–128

Hooker Al, Saxena KMS (1971) Genetics of disease resistance in plants. Annu Rev Genet 5:407–424

Hulbert SH, Bennetzen JL (1991) Recombination at the *Rp1* locus of maize. Mol Gen Genet 226:377–382

Hulbert SH, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci USA 87:4251–4255

Hulbert SH, Lyons PC, Bennetzen JL (1991) Reactions of maize lines carrying *Rp* resistance genes to isolates of the common rust pathogen, *Puccinia sorghi*. Plant Dis 75:1130–1133

Lee BH, Hooker AL, Russell WA, Dickson JG, Flangas AL (1963) Genetic relationships of alleles on chromosome 10 for resistance to *Puccinia sorghi* in 11 corn lines. Crop Sci 3:24–26

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Muehlbauer GJ, Specht JE, Thomas-Compton MA, Staswick PE, Bernard RL (1988) Near-isogenic lines – a potential resource in the integration of conventional and molecular marker linkage maps. Crop Sci 28:729–735

Nowick EM, Peterson PA (1981) Transposition of the enhancer controlling element system in maize. Mol Gen Genet 228:201–208

Pryor A (1987) The origin and structure of fungal disease resistance genes in plants. Trends Genet 3:157–161

Rhoades MM (1951) Duplicate genes in maize. Am Nat 85: 105–110

Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet 236:113–120

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Saxena KMS, Hooker AL (1968) On the structure of a gene for disease resistance in maize. Proc Natl Acad Sci USA 68:1300–1305

Saxena KMS, Hooker AL (1974) A study on the structure of gene *Rp3* for rust resistance in *Zea mays*. Can J Genet Cytol 16:857–860

Simcox KS, Bennetzen JL (1993) The use of molecular markers to study *Setosphaeria turcica* resistance in maize. Phytopathology 83:1326–1330

Stam P, Zeven AC (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30:227–238

Sudupak MA, Bennetzen JL, Hulbert SH (1993) Unequal exchange and meiotic instability of disease-resistance genes in the *Rp1* region of maize. Genetics 133:119–125

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/technology 7:257–264

Wendel JF, Stuber CW, Edwards MD, Goodman MM (1986) Duplicated chromosome segments in *Zea mays* L.: further evidence from hexokinase isozymes. Theor Appl Genet 72:178–185

Wilkinson DR, Hooker AL (1968) Genetics of reaction to *Puccinia sorghi* in ten corn inbred lines from Africa and Europe. Phytopathology 58:605–608