

Unequal Exchange and Meiotic Instability of Disease-Resistance Genes in the *Rp1* Region of Maize

Mehmet A. Sudupak,* Jeffrey L. Bennetzen[†] and Scot H. Hulbert*¹

*Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502, and [†]Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Manuscript received July 14, 1992

Accepted for publication September 23, 1992

ABSTRACT

The *Rp1* region of maize was originally characterized as a complex locus which conditions resistance to the fungus *Puccinia sorghi*, the causal organism in the common rust disease. Some alleles of *Rp1* are meiotically unstable, but the mechanism of instability is not known. We have studied the role of recombination in meiotic instability in maize lines homozygous for either *Rp1*-J or *Rp1*-G. Test cross progenies derived from a line that was homozygous for *Rp1*-J, but heterozygous at flanking markers, were screened for susceptible individuals. Five susceptible individuals were derived from 9772 progeny. All five had nonparental combinations of flanking markers; three had one combination of recombinant flanking markers while the other two had the opposite pair. In an identical study with *Rp1*-G, 20 susceptible seedlings were detected out of 5874 test cross progeny. Nineteen of these were associated with flanking marker exchange, 11 and 8 of each recombinant marker combination. Our results indicate that unequal exchange is the primary mechanism of meiotic instability of *Rp1*-J and *Rp1*-G.

GENES controlling resistance to plant pathogens have been identified in numerous plant species. Most of these genes are dominant and confer resistance to specific races of a pathogen (HOOKER and SAXENA 1971; CRUTE 1985; KEEN 1990). Race-specific resistance genes interact with corresponding genes for avirulence in bacterial and fungal pathogens in a gene-for-gene manner (FLOR 1955) (reviewed by KEEN 1990). Genes that condition resistance to biotrophic fungi are often tightly clustered in the genome (FLOR 1971; SHEPHERD and MAYO 1972; SAXENA and HOOKER 1968; HULBERT and MICHELMORE 1985; WISE and ELLINGBOE 1985). The *Rp* genes of maize, which condition resistance to the common rust fungus, *Puccinia sorghi*, provide a classic example of this clustering. Most of the 25 *Rp* genes identified by HOOKER and co-workers mapped to two genomic areas (SAXENA and HOOKER 1968; HOOKER and RUSSELL 1962; LEE *et al.* 1963; HAGAN and HOOKER 1965). Each gene could be distinguished by the spectrum of rust isolates to which they conferred resistance. Six of the genes mapped to a locus on chromosome 3 (*Rp3*-A–*Rp3*-F) and 16 genes mapped to an area on the short arm of chromosome 10. The lack of recombination between 14 of these genes in small test cross families suggested that they might be allelic and they were given the *Rp1* designation (*Rp1*-A–*Rp1*-N). Two other genes were designated *Rp5* and *Rp6* as they mapped roughly 1 and 2 map units from *Rp1*-C

and 3 map units from each other. More extensive analysis of *Rp1* has indicated that susceptible individuals, and individuals with the combined resistance of both parents, can be generated from test crosses of certain *Rp1* heterozygotes (SAXENA and HOOKER 1968). A similar analysis with flanking restriction fragment length polymorphism (RFLP) markers verified that such changes in resistance were generated by recombination and suggested that the *Rp1* locus consisted of more than a single cistron (HULBERT and BENNETZEN 1991). Most of the *Rp1* genes mapped within 0.3 map unit of each other, but *Rp1*-G maps 1–3 cM distally, near *Rp5*.

Another interesting aspect of *Rp1* is that some alleles are meiotically unstable. This instability has been observed as a high frequency of susceptibles in test crosses of certain *Rp1* homozygotes (PRYOR 1987; BENNETZEN *et al.* 1988). The mechanism of this instability is unknown. In a previous study (HULBERT and BENNETZEN 1991), susceptible derivatives from an *Rp1*-A homozygote were analyzed for flanking marker exchange. All of the derivatives had recombinant flanking markers but only one nonparental combination of flanking markers was observed. Both nonparental combinations of flanking markers would have been expected if the instability was due to unequal exchange. It is possible, however, that the *Rp1* homozygote used was not a true homozygote since it was made by crossing two unrelated maize lines which were both thought to carry *Rp1*-A. In the present study, we have constructed lines homozygous for two

¹ To whom reprint requests should be sent.

Rp1 area genes, *Rp1-J* and *Rp1-G*, which were heterozygous for flanking markers. Test crosses of these lines were used to analyze the role of recombination in *Rp1* instability. We report here the characterization of the mechanism of meiotic instability of *Rp1-J* and *Rp1-G*.

MATERIALS AND METHODS

Genetic stocks: All of the *Rp1* alleles were originally identified and backcrossed into the R168 genetic background by A. L. HOOKER and co-workers at the University of Illinois, Champaign-Urbana. Many of the *Rp1* genes were also transferred to the B14 inbred background. The maize lines carrying *Rp1-J*, *Rp1-G* and *Rp1-I* used in this study were in the R168 genetic background. The *Rp1-D* gene employed was in the B14 inbred background. The inbreds OH43 and H95 carry no known *Rp* genes and were used as male parents in test crosses with the *Rp1* homozygotes. Maize line 1291, carrying the *oy* seedling morphological marker, was obtained from the Maize Genetics Stock Center, University of Illinois, Champaign-Urbana, and also carries no known *Rp* genes.

Flanking RFLP markers: Several RFLPs have been mapped to the short arm of chromosome 10, where the *Rp1* locus resides (WEBER and HELENTJARIS 1989; BURR *et al.* 1988; BEAVIS and GRANT 1991). Three RFLP loci were genetically positioned with respect to the *Rp1* genes (HULBERT and BENNETZEN 1991) to provide flanking markers for studies of the recombinational behavior of *Rp1*. Two RFLP loci, *NPI285* and *NPI422*, mapped proximally to the *Rp1* area. *NPI422* was the closest proximal RFLP marker, mapping roughly 1 cM from *Rp1* in most crosses. *NPI285* mapped 3–10 cM proximal to *Rp1* depending on the cross. *BNL3.04*, was positioned 1–2 cM distal to *Rp1-G*. Both *Rp1-G* and *BNL3.04* map distally to the other *Rp1* genes (HULBERT and BENNETZEN 1991).

An alternative probe, *KSU3a*, was used to detect the *NPI422* locus. The *KSU3a* clone is a 3.5-kb *EcoRI-HindIII* fragment that was subcloned from a recombinant maize:lambda clone selected by hybridization to the *NPI422* probe. The *KSU3a* and *NPI422* probes often hybridize to the same genomic fragments upon Southern analysis, but *KSU3a* detects additional fragments. These additional bands also cosegregate with the *Rp1* region (data not shown). The additional cosegregating bands detected by this probe makes it a more informative marker in most crosses. The probe used to detect the *BNL3.04* locus was a 1.2-kb *PstI-BamHI* fragment in the 2.2-kb *PstI* insert of the *BNL3.04* clone (HULBERT and BENNETZEN 1991).

The *Rp1* genes were originally identified by screening large numbers of maize inbred lines from diverse sources of germplasm. The *Rp1* genes have been transferred to the maize inbred R168 by backcrossing, creating a series of near isogenic lines. Variation has been maintained among the differential lines at RFLP loci which are closely linked to the *Rp1* locus (HULBERT and BENNETZEN 1991). Hence, these RFLP markers can be used to assay recombination at *Rp1*.

Construction of *Rp1-J* homozygotes with heterozygous flanking markers: To assess the role of recombination in the instability of *Rp1-J* homozygotes, it was necessary to construct F₁ hybrids that were homozygous at *Rp1* but heterozygous at the flanking DNA markers. Two such F₁s were made for *Rp1-J* using three different recombinant maize lines as parents. Line J1 (see Table 1) was used as a parent in both hybrids. The *Rp1-J*-R168 line carries the

RFLP alleles *NPI285-6* and *BNL3.04-2*. Line J1 was derived from a recombination event within the cross (*Rp1-J*-R168/*Rp1-D*-B14)/H95 and has the *BNL3.04-1* allele from the *Rp1-D*-B14 line. Lines J2 and J3 were derived from recombination events between *Rp1* and *NPI285* and carried *Rp1-J* with the *NPI285-3* allele. Line J2 was derived from the above cross, while line J3 was derived from the cross (*Rp1-J*-R168/*Rp1-F*-R168)/1291. Recombinants from these populations were self-pollinated to obtain parental lines which were homozygous at *Rp1-J* and the flanking RFLP markers. Two separate populations were derived by test crossing hybrids of the lines J1 crossed to J2 and J1 crossed to J3 (Table 1).

Construction of *Rp1-G* homozygotes with heterozygous flanking markers: An F₁ hybrid was constructed which was homozygous for *Rp1-G* but heterozygous at flanking markers, essentially the same way the *Rp1-J* F₁s were assembled; from recombinant maize lines which carried *Rp1-G* but had either the distal or proximal marker alleles exchanged with alternate alleles. The *Rp1-G*-R168 line has the *NPI422-10* and *BNL3.04-2* alleles. Line G1 was selected from the cross (*Rp1-G*-R168/*Rp1-I*-R168)/B14. It was derived from a recombination event between *Rp1-G* and *BNL3.04* and had *Rp1-G* with the *BNL3.04-1* allele. Line G2 was derived from the cross (*Rp1-G*-R168 × 1291) × 1291 and carries *Rp1-G* with the *NPI422-9* allele. As with the *Rp1-J* recombinants, the original recombinants in these two populations were self-pollinated to obtain the G1 and G2 lines which were homozygous at *Rp1-G* and the recombinant flanking markers (see Table 2). The hybrid between G1 and G2 was test crossed to generate a family to screen for susceptible derivatives.

The test cross families of both the *Rp1-G* and *Rp1-J* homozygotes were constructed by using resistant F₁s as the female parent so that susceptible individuals would not result from pollen contamination.

Selection and analysis of susceptible test cross individuals: The test cross population from the *Rp1-J* lines were screened for susceptible individuals using the rust isolate KS1 that is avirulent on maize lines carrying *Rp1-J* (HULBERT, LYONS and BENNETZEN 1991). The *Rp1-G* test cross population was screened with the rust isolate 1-4 which is avirulent on maize lines carrying *Rp1-G* (BENNETZEN *et al.* 1988; HULBERT, LYONS and BENNETZEN 1991). Test cross families were screened by planting 100–120 seeds in a 38 × 61 × 8 cm flat and inoculation with the appropriate rust isolate. Inoculated seedlings were incubated for 16 hr in a mist chamber and scored for susceptible individuals 7 days later. Susceptible seedlings from *Rp1-J* test crosses were covered with sporulating pustules while the other seedlings in the flat showed a typical *Rp1-J* response; chlorotic spots with occasional small pustules. Susceptible individuals from the *Rp1-G* test cross were also fully susceptible while resistant individuals had very small necrotic spots typical of the *Rp1-G* response. Susceptible individuals were then transplanted into large pots, and seed was subsequently obtained following self fertilization. The progeny of each seedling were tested to verify susceptibility.

Flanking marker analysis: Total genomic DNA extraction and gel blot analysis was performed as described previously (HULBERT and BENNETZEN 1991). *ScaI* digested genomic DNA of susceptible individuals derived from *Rp1-J* populations and *EcoRI* digested genomic DNA of susceptible individuals derived from *Rp1-G* populations were fractionated in 0.8% agarose gels and transferred to MSI blotting membranes. Membranes were hybridized to the [³²P]dCTP-labeled RFLP probes *NPI285*, *KSU3a* and *BNL3.04* for 24 hr at 65°. Hybridized membranes were washed with 0.1 ×

TABLE 1
Susceptible test cross progeny from *Rp1*-J homozygotes

| Test crosses | Origin ^a | Genotypes at flanking RFLP markers ^b | |
|--|---------------------|---|------------------|
| | | Proximal marker | Distal marker |
| Parent J1 | | <i>NPI285-6</i> | <i>BNL3.04-1</i> |
| Parent J2 and J3 | | <i>NPI285-3</i> | <i>BNL3.04-2</i> |
| Susceptible progeny (J1 × J3) × H95 #1 | CO | <i>NPI285-6</i> | <i>BNL3.04-2</i> |
| #2 | CO | <i>NPI285-6</i> | <i>BNL3.04-2</i> |
| #3 | CO | <i>NPI285-6</i> | <i>BNL3.04-2</i> |
| #4 | CO | <i>NPI285-3</i> | <i>BNL3.04-1</i> |
| (J1 × J2) × H95 #1 | CO | <i>NPI285-3</i> | <i>BNL3.04-1</i> |

^a CO (crossover) indicates that the derivative had nonparental combinations of flanking markers.

^b Only the flanking marker alleles from the F₁ parent are given for the recombinants; the tester parent, H95, is homozygous for *NPI422* and *BNL3.04*.

SSC (1 × SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0) and 0.1% SDS solution at 65° for at least 2 hr. The membranes were then autoradiographed for 24–48 hr at –80°.

RESULTS

Analysis of test cross progeny from *Rp1*-J and *Rp1*-G homozygotes: The screening of test cross progeny of *Rp1*-J homozygotes yielded five susceptible seedlings out of 9772 tested. All five had nonparental combinations of flanking RFLP markers (Table 1). Four of the recombinants (out of 6414) were derived from the J1 × J3 population while the J1 × J2 population yielded only one recombinant out of 3358 seedlings screened. Three of the recombinants had the *NPI285-6* allele of J1 at the proximal RFLP locus and the *BNL3.04-2* allele of J3 at the distal RFLP locus (Table 1). The other two recombinants had the opposite nonparental combination of flanking markers.

The test cross families of *Rp1*-G homozygotes yielded 20 susceptible individuals out of 5874 progeny. Nineteen of these were associated with flanking marker exchange (CO type), whereas one susceptible individual arose on a parentally marked chromosome (NCO type; Table 2). Of the 19 susceptible individuals associated with crossing over, eight had the *NPI422-9* allele of G2 at the proximal RFLP locus together with the *BNL3.04-1* allele of G1 at the distal RFLP locus, whereas 11 carried the *NPI422-10* allele of G1 along with the *BNL3.04-2* allele of G2 (Figure 1). The single NCO type individual carried both proximal and distal flanking markers of the G1 parent.

Susceptibility of the recombinant individuals from the *Rp1*-J and *Rp1*-G populations was verified by progeny testing with the appropriate rust isolate. Progeny from all of the recombinants were found to be completely susceptible, including the individual from the *Rp1*-G population which originated on a parentally marked chromosome.

DISCUSSION

The role of recombination in meiotic instability was examined in two different *Rp1* region genes, *Rp1*-J and *Rp1*-G. Previous mapping experiments have indicated that most *Rp1* genes, including *Rp1*-J, are clustered within about 0.3 cM of each other (SAXENA and HOOKER 1968, HULBERT and BENNETZEN 1991). *Rp1*-G mapped up to 3 cM distally in some crosses. Estimates of meiotic instability have been reported for many of the *Rp1* genes (PRYOR 1987; BENNETZEN *et al.* 1988). As with the present study, previous estimates of instability were conducted by screening test cross populations of *Rp1* homozygotes. It was not possible to test the role of recombination in previous studies because flanking markers were not available. No previous estimate of *Rp1*-J instability is available, but the present frequency (5×10^{-4}) was similar to that reported for a number of different *Rp1* homozygotes. The previous estimates of instability of *Rp1*-G varied from 1.8 to 7×10^{-3} , the highest observed for any *Rp1* area gene (PRYOR 1987; BENNETZEN *et al.* 1988). The frequency of *Rp1*-G instability in the present population (3.4×10^{-3}) lies within this range.

All five susceptible derivatives from *Rp1*-J homozygotes and 19 of the 20 derivatives from *Rp1*-G homozygotes were associated with recombination of closely linked flanking markers. Furthermore, both nonparental combinations of flanking markers were obtained from both types of populations. This indicates that the susceptible derivatives arose by unequal crossing over. Unequal crossing over requires sequence duplications that retain synaptic homology. Studies of recombination between duplicated sequences in maize (DOONER and KERMICLE 1971; ROBBINS *et al.* 1991), *Drosophila* (DAVIS, SHEN and JUDD 1987) and yeast (MALONEY and FOGEL 1987) have indicated that mispairing occurs frequently, possibly as frequently as normal pairing. The high level of instability in some *Rp1* genes is consistent with this observation. The recovery of both possible nonpar-

TABLE 2
Susceptible test cross progeny from *Rp1-G* homozygotes

| Test cross | Origin ^a | Genotypes at flanking RFLP markers ^b | |
|--|---------------------|---|------------------|
| | | Proximal marker | Distal marker |
| Parent G1 | | <i>NPI422-10</i> | <i>BNL3.04-1</i> |
| Parent G2 | | <i>NPI422-9</i> | <i>BNL3.04-2</i> |
| Susceptible progeny (G1 × G2) × H95 #1 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #2 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #3 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #4 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #5 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #6 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #7 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #8 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #9 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #10 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #11 | CO | <i>NPI422-10</i> | <i>BNL3.04-2</i> |
| #12 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #13 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #14 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #15 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #16 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #17 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #18 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #19 | CO | <i>NPI422-9</i> | <i>BNL3.04-1</i> |
| #20 | NCO | <i>NPI422-9</i> | <i>BNL3.04-2</i> |

^a CO (crossover) indicates that the derivative had nonparental combinations of flanking markers while NCO (noncrossover) indicates that it did not.

^b Only the flanking marker alleles from the F₁ parent are given for the susceptible progeny; the tester parent, H95, is homozygous for *NPI422* and *BNL3.04*.

ental combinations of flanking markers in both *Rp1-J*- and *Rp1-G*-derived susceptible progeny is evidence of mispairing in both possible directions (Figure 2).

In a previous analysis of recombination in various *Rp1* heterozygotes, virtually all of the susceptible recombinants exhibited recombinant flanking markers (HULBERT and BENNETZEN 1991). Only one recombinant, from an *Rp1-E/Rp1-F* heterozygote, was reported with parentally marked chromosomes, and this individual died without producing seed which could be progeny tested to verify its susceptibility. It is likely that this individual was not a true susceptible, since the *Rp1-F* phenotype can be difficult to score unambiguously. In one of the heterozygotes analyzed, *Rp1-D/Rp1-F*, both nonparental combinations of flanking markers were obtained, indicating that mispairing and recombination had occurred. The propensity of derivatives from both *Rp1* homozygotes and heterozygotes to exhibit flanking marker exchange indicates that recombination at *Rp1* generally results from interchromosomal events. This varies somewhat from what has been observed at the *A* and *R* loci of maize (LAUGHNAN 1961; ROBBINS *et al.* 1991). Both loci have alleles which carry duplicated sequences capable of mispairing and recombination, and derivatives with altered patterns of pigmentation are often associated with recombinant flanking markers. Both loci, how-

ever, have complex alleles in which derivatives that are not associated with flanking marker exchange (NCO types) make up nearly 50% of the total derivatives (LAUGHNAN 1961; ROBBINS *et al.* 1991). The mechanism by which the NCO derivatives arise is not known. Possibilities include mutation, intrachromosomal recombination and gene conversion following mispairing. Intrachromosomal recombination and gene conversion between tandem repeats have been documented in *Drosophila* (PETERSON and LAUGHNAN 1963; HIPEAU-JACQUOTTE, BRUTLAG and BREGEGERE 1989) and yeast (MALONEY and FOGEL 1987; JACKSON and FINK 1985). NCO-type derivatives from the *R* locus do not appear to have lost a copy of the duplication; this indicates an absence of intrachromosomal events such as unequal sister chromatid exchange or intrachromatid recombination which would result in deletion of a copy of the duplication (ROBBINS *et al.* 1991). It is not clear whether NCO derivatives from complex alleles of the *A* locus have lost a copy of the duplication.

The unequal crossing over model in Figure 2 assumes that the unstable *Rp1* area genes lie on the duplicated sequences which mispair. An alternative model is that *Rp1-J* and *Rp1-G* do not lie on duplicated sequences but are flanked by repetitive sequences which can mispair and recombine. This type of recom-

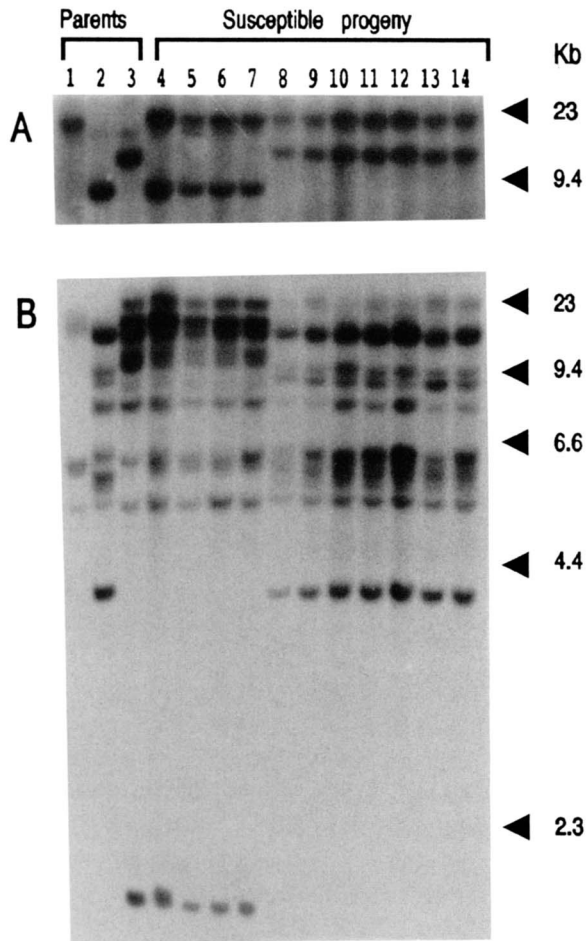


FIGURE 1.—Southern blot analysis to determine flanking marker combinations of susceptible progeny derived from test cross populations of *Rp1-G*. DNA from susceptible *Rp1-G* individuals was digested with *EcoRI* and probed with either the distal RFLP marker, *BNL3.04* (A) or the proximal RFLP marker, *KSU3a* (B). Lanes: 1) tester parent, H95; 2) line *G*₂; 3) line *G*₁; lanes 4–14 are the susceptible progeny numbers (listed in Table 2) 1, 2, 3, 4, 12, 13, 14, 15, 16, 17, 18, 19, respectively.

bination has been documented at a number of human loci (HORSTHEMKE, BEISIEGEL and DUNNING 1987; ARIGA, CARTER and DAVIS 1990; HU, RAY and WORTON 1991; NICHOLLS, FISCHER-GHODSIAN and HIGGS 1987) and at the *white* locus of *Drosophila* (GOLDBERG *et al.* 1983). While mispairing and recombination between dispersed repeated sequences are thought to be important in the creation of the initial duplications of complex loci (GOLDBERG *et al.* 1983; TSUBOTA *et al.* 1989), these are thought to be rare events. The high frequency of unequal crossing over in the *Rp1* area, therefore, argues against this model. Moreover, the high rate of intragenic recombination observed at loci such as *waxy* or *bronze 1* (DOONER 1986; NELSON 1962) indicates that most recombination in the maize genome lies in low copy sequences. The only example of unequal crossing over in maize in which derivatives have been analyzed at the molecular level is the *R*

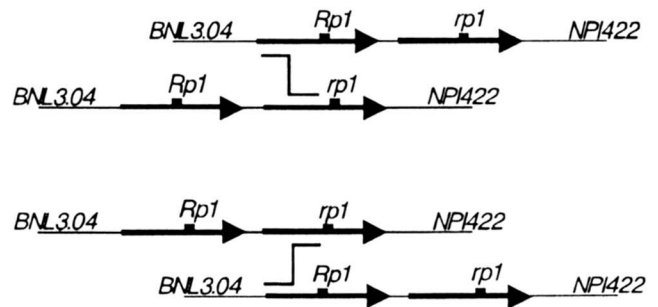


FIGURE 2.—An unequal crossing over model (modified from DOONER and KERMICLE 1971) which explains the instability in the *Rp1* region. The large arrows represent direct repeats carrying the *Rp1* alleles. *NPI422* and *BNL3.04* are the flanking RFLP markers used to assay recombination. Possible mispairing configurations and unequal exchanges are shown. The result of such exchanges will produce susceptible individuals that do not bear detectable *Rp1* alleles and have either of two possible combinations of recombinant flanking markers. The model may be an oversimplification of the structure of the *Rp1* area in at least some maize lines since some lines may carry more than two copies of the duplication.

locus (ROBBINS *et al.* 1991). Molecular analysis indicated that most of the unequal recombination events occurred intragenically. Another indication that *Rp1* instability results from exchange between sequence duplications is that recombination studies have indicated that the *Rp1* area carries more than a single disease resistance locus, some of which (*e.g.*, *Rp5*, *Rp1-G*) are easily separable by recombination. Complex disease resistance loci which carry multiple genes may provide crucial variation to plant species which are coevolving with biotrophic pathogens. Multiple genes can be combined in a single haplotype and new combinations can be generated by recombination. In addition, mispairing and intragenic recombination may add to the arsenal of resistance genes by generating novel genes.

The unusually high level of instability of *Rp1-G* indicates that these duplicated sequences recombine frequently. Our frequency of susceptibles with recombinant flanking markers was 3.2×10^{-3} . The reciprocal class of recombination events, those with two copies of *Rp1-G*, was not detectable in our assay. In addition, since a homozygote was used in the experiment, mispairing is required for the recombination event to be observed. Furthermore, if the duplication that carries the *Rp1-G* genes is involved in the recombination event, not all crossovers will be detectable, depending on the proximity of the crossover to the *Rp1-G* gene carried on the duplication (Figure 2). Since the number of duplicated sequences adjacent to *Rp1-G*, and the frequency in which they freely mispair is not known, it is impossible to estimate the genetic size of the duplications. Collectively, they appear to span over one half a map unit or more of recombination in the current population.

The variable frequencies of instability in different *Rp1-G* homozygote and heterozygote populations is particularly interesting. As stated above, the frequency of susceptible individuals in progeny of *Rp1-G* homozygotes varies from 1.8 to 6.8×10^{-3} . The frequency from heterozygotes in which *Rp1-G* was one of the parents is also very variable (1.8×10^{-2} to 9×10^{-4} ; SAXENA and HOOKER 1968; HULBERT and BENNETZEN 1991), and, in some cases, may be lower than the homozygote frequencies. Only three susceptible recombinants were observed from 3450 test cross progeny of an *Rp1-G/Rp5* heterozygote (M. A. SUDUPAK and S. H. HULBERT, unpublished data). Since *Rp1* recombination and instability requires interchromosomal crossover events, the variable frequency of recombination in heterozygotes might be expected if the duplication(s) which mispair and recombine in *Rp1-G* homozygotes do not pair as well with related sequences in lines such as the *Rp5* line. Similarly, differences in recombination rates from *Rp1* homozygotes may be due to structural differences that have occurred in the different *Rp1-G* stocks. Since unequal crossing over events themselves would alter the copy number of duplications involved, the structural variation that might inhibit further effective pairing could be rapidly generated.

We thank KYUNG HONG for assistance with the RFLP analysis. We are grateful to the Turkish government for a scholarship to M.A.S. This work was supported in part by a grant from the U.S. Department of Agriculture (90-37262-5446). Contribution 93-92-j from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

LITERATURE CITED

- ARIGA, T., P. E. CARTER and A. E. DAVIS III, 1991 Recombinations between *Alu* repeat sequences that result in partial deletions within the *C1* inhibitor gene. *Genomics* **8**: 607-613.
- BEAVIS, W., and D. D. GRANT, 1991 Linkage map based on information from four F_2 populations of maize (*Zea mays* L.). *Theor. Appl. Genet.* **82**: 636-644.
- BENNETZEN, J. L., M. M. QIN, S. INGELS and A. H. ELLINGBOE, 1988 Allele-specific and *Mutator*-associated instability at the *Rp1* disease-resistance locus of maize. *Nature* **332**: 369-370.
- BURR, B., F. A. BURR, K. H. THOMPSON, M. C. ALBERTSON and C. W. STUBERT, 1988 Gene mapping with recombinant inbreds of maize. *Genetics* **118**: 519-526.
- CRUTE, I. R., 1985 The genetic basis of relationships between microbial parasites and their hosts, pp. 80-142 in *Mechanisms of Resistance to Plant Disease*, edited by R. S. S. FRASER. MARTINUS NIJHOFF AND W. JUNK, DORDRECHT.
- DAVIS, P. S., M. W. SHEN and B. H. JUDD, 1987 Asymmetrical pairings of transposons in and proximal to the *white* locus of *Drosophila* account for four classes of regularly occurring exchange products. *Proc. Natl. Acad. Sci. USA* **84**: 174-178.
- DOONER, H. K., 1986 Genetic fine structure of the *bronze* locus in maize. *Genetics* **113**: 1021-1036.
- DOONER, H. K., and J. L. KERMICLE, 1971 Structure of the *R-r* tandem duplication in maize. *Genetics* **67**: 427-436.
- FLOR, H. H., 1955 Host-parasite interaction in flax rust—its genetics and other implications. *Phytopathology* **45**: 680-685.
- FLOR, H. H., 1971 Current status of the gene-for-gene concept. *Adv. Genet.* **8**: 29-54.
- GOLDBERG, M. L., J. SHEEN, W. J. GEHRING and M. M. GREEN, 1983 Unequal crossing-over associated with asymmetrical synapsis between nomadic elements in the *Drosophila melanogaster* genome. *Proc. Natl. Acad. Sci. USA* **80**: 5017-5021.
- HAGAN, W. L., and A. L. HOOKER, 1965 Genetics of the reaction to *Puccinia sorghi* in eleven corn inbred lines from Central and South America. *Phytopathology* **55**: 193-197.
- HIEPAU-JACQUOTTE, R., D. L. BRUTLAG and F. BREGEGERE, 1989 Conversion and reciprocal exchange between tandem repeats in *Drosophila melanogaster*. *Mol. Gen. Genet.* **220**: 140-146.
- HOOKER, A. L., and W. A. RUSSELL, 1962 Inheritance of resistance to *Puccinia sorghi* in six corn inbred lines. *Phytopathology* **52**: 122-128.
- HOOKER, A. L., and K. M. S. SAXENA, 1971 Genetics of disease resistance in plants. *Annu. Rev. Genet.* **5**: 407-424.
- HORSTHEMKE, B., U. BEISIEGEL, A. DUNNING, J. R. HAVINGA, R. WILLIAMSON and S. HUMPHRIES, 1987 Unequal crossing-over between two *alu*-repetitive DNA sequences in the low-density-lipoprotein-receptor gene. *Eur. J. Biochem.* **164**: 77-81.
- HU, X., P. N. RAY and R. G. WORTON, 1991 Mechanism of tandem duplication in the Duchenne muscular dystrophy gene includes both homologous and nonhomologous intrachromosomal recombination. *EMBO J.* **10**: 2471-2477.
- HULBERT, S. H., and J. L. BENNETZEN, 1991 Recombination at the *Rp1* locus of maize. *Mol. Gen. Genet.* **226**: 377-382.
- HULBERT, S. H., P. C. LYONS and J. L. BENNETZEN, 1991 Reactions of maize lines carrying *Rp* resistance genes to isolates of the common rust pathogen, *Puccinia sorghi*. *Plant Dis.* **75**: 1130-1133.
- HULBERT, S. H., and R. W. MICHELMORE, 1985 Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa*) *Theor. Appl. Genet.* **70**: 520-528.
- JACKSON, J. A., and G. R. FINK, 1985 Meiotic recombination between duplicated genetic elements in *Saccharomyces cerevisiae*. *Genetics* **109**: 303-332.
- KEEN, N. T., 1990 Gene-for-gene complementarity in plant-pathogen interactions. *Annu. Rev. Genet.* **24**: 447-463.
- LAUGHNAN, R. J., 1961 The nature of mutations in terms of gene and chromosome changes, pp. 3-29, in *Mutations and Plant Breeding*, Publ. No. 891. National Academy Science, National Research Council, Washington, D.C.
- LEE, B. H., A. L. HOOKER, W. A. RUSSELL, J. G. DICKSON and A. L. FLANGAS, 1963 Genetic relationships of alleles on chromosome 10 for resistance to *Puccinia sorghi* in 11 corn lines. *Crop Sci.* **3**: 24-26.
- MALONEY, D. H., and S. FOGEL, 1987 Gene conversion, unequal crossing-over and mispairing at a nontandem duplication during meiosis of *Saccharomyces cerevisiae*. *Curr. Genet.* **12**: 1-7.
- NELSON, O. E., 1962 The *waxy* locus in maize. I. Intralocus recombination frequency estimates by pollen and by conventional analyses. *Genetics* **47**: 737-742.
- NICHOLLS, R. D., N. FISCHHEL-GHODSIAN and D. R. HIGGS, 1987 Recombination at the human α -globin gene cluster: sequence features and topological constraints. *Cell* **49**: 369-378.
- PETERSON, H. M., and J. R. LAUGHNAN, 1963 Intrachromosomal exchange at the *Bar* locus in *Drosophila*. *Proc. Natl. Acad. Sci.* **50**: 126-133.
- PRYOR, A., 1987 The origin and structure of fungal disease resistance in plants. *Trends Genet.* **3**: 157-161.
- ROBBINS, T. P., E. L. WALKER, J. L. KERMICLE, M. ALLEMAN and S. L. DELLEPORTA, 1991 Meiotic instability of the *R-r* com-

- plex arising from displaced intragenic exchange and intrachromosomal rearrangement. *Genetics* **129**: 271–283.
- SAXENA, K. M. S., and A. L. HOOKER, 1968 On the structure of a gene for disease resistance in maize. *Proc. Natl. Acad. Sci. USA* **68**: 1300–1305.
- SHEPHERD, K. W., and G. M. E. MAYO, 1972 Genes conferring specific plant disease resistance. *Science* **175**: 375–380.
- TSUBOTA, S. I., D. ROSENBERG, H. SZOSTAK, D. RUBIN and P. SCHELDL, 1989 The cloning of the *Bar* region and the B breakpoint in *Drosophila melanogaster*: evidence for a transposon-induced rearrangement. *Genetics* **122**: 881–890.
- WEBER, D., and T. HELENTJARIS, 1989 Mapping RFLP loci in maize using B-A translocations. *Genetics* **121**: 583–590.
- WISE, R. P., and A. H. ELLINGBOE, 1985 Fine structure and instability of the *M1-a* locus in barley. *Genetics* **111**: 113–130.

Communicating editor: B. BURR