# FINE STRUCTURE AND INSTABILITY OF THE *ML-A* LOCUS IN BARLEY

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ABSTRACT

There are many naturally occurring variants at the *Ml-a* locus in barley that confer resistance to the powdery mildew fungus *Erysiphe graminis* f. sp. *hordei*. Since the *Ml-a* locus is bracketed by *Hor-1* and *Hor-2*, genes that encode storage proteins in the endosperm, the *Ml-a* locus is amenable to fine structure analysis. Rare susceptible recombinants, as judged by exchange of flanking markers, were recovered in $F_3$ families from the *Ml-a10* × *Ml-a1*, *Ml-a1* × *Ml-a15* and *Ml-a6* × *Ml-a13* crosses. Some susceptible recombinants were recovered from the *Ml-a6* × *Ml-a13* cross that did not fit the expected $F_3$ family segregation ratios. The *Ml-a6/Ml-a13* recombinants often reverted to resistance in subsequent generations. No recombinants were recovered in the reciprocal cross, *Ml-a13* × *Ml-a6*. The possibility of a transposable element and a possible linear order of six "alleles" at the *Ml-a* locus is discussed.

ALTHOUGH genes conferring specific disease resistance are often used in basic and applied breeding experiments, there have been few analyses of their structure. One interesting feature of variants conferring resistance to an obligate parasite is that they are tightly linked (FLOR 1956; SAXENA and HOOKER 1968; MOSEMAN 1971; SHEPHERD and MAYO 1972). This opens the possibility that there might be only one gene that has many alleles or clusters of closely linked cistrons. These systems have characteristic gene-for-gene specificity as first described by Flor. FLOR's (1955) analysis established that, for each gene for resistance (*R*) in the host, there is a complementary and specific gene for avirulence (*P*) in the pathogen. A host with a particular *R* gene cannot express resistance unless the complementary *P* gene is present in the pathogen.

Gene-for-gene specificity is useful in the study of the fine structure of these loci. When applied to groups of codominant genes, the modified *cis-trans* test described by SHEPHERD and MAYO (1972) is particularly useful in differentiating between functional alleles of one gene or closely linked cistrons. In this test, the *trans* and *cis* arrangements of closely linked genes for specific resistance will exhibit the same phenotype. Each allele conditions its' respective

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resistance. If, on the other hand, the genes are allelic, their specificities may change in the *cis* arrangement in comparison to the *trans* arrangement. Shepherd and Mayos' investigations of the *L* and *M* loci in flax (*Linum usitatissimum* L.) controlling specific interactions with flax rust [*Melampsora lini* (Ehrenb.) Lev.] demonstrated important functional differences among genes of the *M* group as distinct from genes of the *L* group. The interpretation was that the *M* group is a series of separate but closely linked genes, whereas variants in the *L* group are functionally allelic (ELLINGBOE 1976; MAYO and SHEPHERD 1980).

Many variants have been identified in barley (*Hordeum vulgare* L.) that confer resistance to powdery mildew (*Erysiphe graminis* DC. Merat f. sp. *hordei* em. Marchal). These are distributed among seven groups: *Ml-at*, *Ml-a*, *Ml-k*, *Ml-nn* and *Ml-p* on chromosome *5*; and *Ml-g* and *ml-o* on chromosome *4* (JORGENSEN and JENSEN 1976; JENSEN 1980). There is a large cluster of allelic or closely linked variants within the *Ml-a* region, 14 of which have been differentiated by their reactions to many strains of *E. graminis* f. sp. *hordei* (for review see GIESE 1981). JORGENSEN and MOSEMAN (1972) detected rare recombination between *Ml-a1* and *Ml-a3*, and GIESE *et al.* (1981) recovered a possible recombinant between *Ml-a12* and *Ml-a13*.

We have extended the analysis of the *Ml-a* locus using the concept of the modified *cis-trans* test. The *Ml-a* locus was chosen for several reasons. One of these is the large number of naturally occurring variants in the region, six of which have been made near isogenic to the cultivar Manchuria (MOSEMAN 1972). Manchuria has no known *Ml-a* resistance. The six isogenic lines are all resistant to a single race (CR3) of *E. graminis* f. sp. *hordei*; thus, large numbers of progeny may be screened with one strain of the pathogen. The *Ml-a* locus is also bracketed by *Hor-1* and *Hor-2*, genes that encode endosperm storage proteins, and, therefore, can be used as flanking markers (JENSEN *et al.* 1980). The hordein proteins can be assayed by polyacrylamide gel electrophoresis. The objectives of the research reported here were examination of the fine structure of the *Ml-a* locus and exploration of the nature of the specificity conferred by the naturally occurring variants in this region.

## MATERIALS AND METHODS

Table 1 lists the six near-isogenic barley lines and cultivar Manchuria followed by their reaction 7 days after inoculation with *E. graminis* f. sp. *hordei* race CR3. Infection types 0, 1 or 2 signify no disease, flecks with small pustules or small pustules with a hypersensitive reaction, respectively. Infection types 0, 1 and 2 are considered resistant reactions. Infection type 3 represents a significant reduction in mildew development. Infection type 4 signifies a fully susceptible reaction. All barley lines were obtained from J. G. MOSEMAN, Small Grains Collection, United States Department of Agriculture, Beltsville, Maryland..

Culture CR3 of *E. graminis* f. sp. *hordei* is avirulent on each of the six host *Ml-a* lines. Avirulence is defined as the inability to produce disease on a resistant host. CR3, therefore, is presumed to have the corresponding avirulence (*P*) gene for each of the six *Ml-a* specificities. Culture CR3 was propagated as described previously (MASRI and ELLINGBOE 1966). Purity of the mildew culture was monitored by weekly inoculation of sets of differential host lines and evaluation of infection types.

*Experimental design*: The selection scheme for recovering susceptible recombinants in the *Ml-a*

TABLE 1

*Six near-isogenic barley lines and cultivar Manchuria and their reaction to culture CR3 of* E. graminis *f. sp.* hordei

| Isogenic lines | C.I. no.[a] | Gene conditioning reaction to CR3 | Infection type with CR3[b] |
|---|---|---|---|
| Algerian/4* (F14) Man. (R)[c] | 16137 | *Ml-a1*[d] | 0 |
| Franger/4* (F15) Man. (R) | 16151 | *Ml-a6* | 0 |
| Durani/4* (F13) Man. (R) | 16149 | *Ml-a10* | 2 |
| Multan/4* (F15) Man. (R) | 16147 | *Ml-a7(Mu)* | 1–2 |
| Long Glumes/4* (F15) Man. (R) | 16153 | *Ml-a15*[e] | 1–2 |
| Rupee/4* (F13) Man. (R) | 16155 | *Ml-a13* | 0 |
| Manchuria | 2330 | *ml* | 4 |

[a] C.I. = Cereal Introduction number, United States Department of Agriculture.

[b] Infection type: 0 = no observable mildew development; 1 = chlorotic flecking; 2 = necrotic reaction; 3 = significant reduction in mildew development; 4 = abundant mildew development.

[c] Cultivar Algerian crossed to Manchuria followed by three additional backcrosses and then selfing the heterozygotes 14 generations, selecting the resistant plants each generation (MOSEMAN 1972).

[d] Same as original *Ml-a* from Algerian (MOSEMAN 1972).

[e] Previously *Ml-a7(LG)* (WISE and ELLINGBOE 1983).

region is presented in Figure 1. Vertical lines represent the chromosomal segment encompassing the *Ml-a* region. Horizontal slash marks represent putative allelic sites conferring specific resistance to race CR3 of *E. graminis* f. sp. *hordei*. Once the crosses were made, natural self-pollination produced large numbers of segregating $F_2$ progeny. Rare recombination may occur in the $F_1$ between different sites conferring specific resistance. During fertilization, recombinant gametes will most likely fuse with wild-type gametes to form four possible types of "recombinant" seed. Types 1 and 2 will possess both unique sites for specificity, whereas types 3 and 4 will possess neither. Since the recombinant chromosome is paired with a parental chromosome carrying a gene for resistance, all $F_2$ seedlings should be resistant to race CR3 of *E. graminis* f. sp. *hordei*. However, when these $F_2$ plants are progeny tested, segregation will yield offspring homozygous for the recombinant chromosome. Since CR3 possesses avirulence specificities for both resistances used in the parental cross, recombinants with a resistant phenotype will be indistinguishable from the parentals. Recombinants with a susceptible phenotype will, however, be readily distinguishable.

Two recombinant types will be produced: those bearing both unique sites (left-most elements in $F_3$ families 1 and 2) and those bearing neither site (left-most elements in $F_3$ families 3 and 4). The latter combination will be susceptible in phenotype. The phenotype of the former combination should be resistant if the two sites are in separate cistrons. If, however, the two sites are in the same cistron, the phenotype could be resistant or susceptible depending on the unknown molecular recognition mechanism. The outcome easiest to interpret would be for the two-site recombinant to be susceptible. In that case, the two variants would be considered allelic since the *cis*-combination does not give the *trans*, resistant phenotype. Should this class prove resistant, it would be impossible to distinguish between the two models; variants in two loci would yield resistance in *cis* but so could variants at two sites within one gene product if multiple sites within that product can be recognized by the pathogen.

*Crossing scheme*: Parental crosses were made among the six host lines with dominant *Ml-a* variants. Individual parental crosses resulted in approximately 18 hybrid seeds per spike. The parental crosses were identified by spike, and all subsequent progeny resulting from a particular parental cross were kept separate throughout the analysis. (To be sure parental plants were homozygous for their respective genes for resistance, approximately 20 progeny seeds from each parental plant used in a cross were tested for segregation of resistance by inoculation with CR3.) The resulting $F_1$ generation was selfed in the greenhouse during the winter to obtain a maximum $F_2$. To prevent contamination by outcrossing, no susceptible barley was grown in the same greenhouse

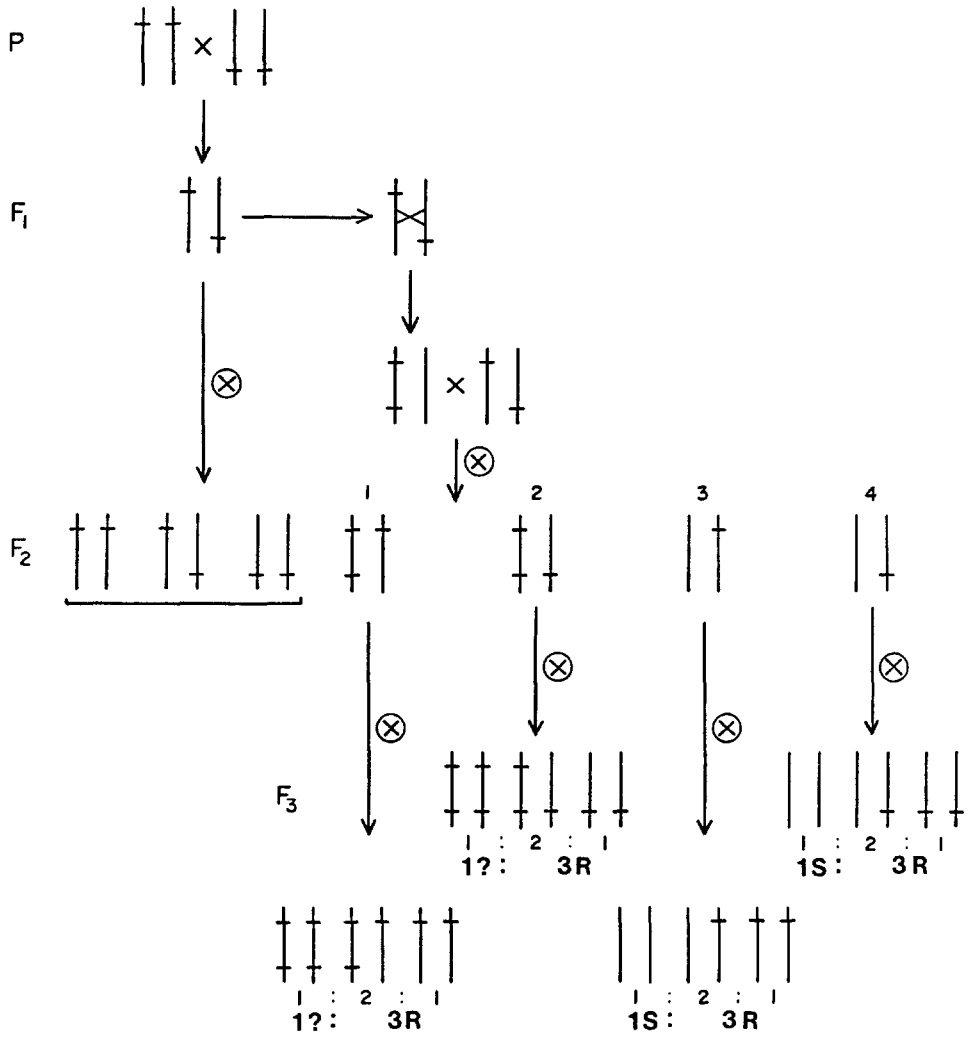


FIGURE 1.—Selection scheme for recovery of susceptible recombinants at the *Ml-a* locus. Vertical lines represent the chromosomal segment encompassing the *Ml-a* region. Horizontal slash marks represent sites conferring specific resistance to race CR3 of *E. graminis* f. sp. *hordei*. P, $F_1$ and $F_2$ plants are all resistant to CR3. All of the $F_2$ plants are selfed; however, all nonrecombinant $F_3$ families are nonsegregating for resistance to CR3. For simplicity, only the recombinant $F_3$ families are diagrammed. The expected phenotypic ratio is illustrated below $F_3$ family types 1–4. R = resistant; S = susceptible.

or in the near vicinity. The $F_2$ seeds were space planted in the field, and a single spike was harvested from each $F_2$ plant to represent the $F_3$ families. This procedure was repeated twice (from P to $F_3$) in successive years.

*Screening procedure*: $F_3$ families were screened for susceptible segregants by planting intact spikes consisting of 25–40 seeds in flats and inoculating the seedlings with culture CR3 of *E. graminis* f. sp. *hordei* when they were approximately 3 inches high. Forty-seven families as well as a susceptible control (Manchuria) were planted per flat. Flats of inoculated seedlings were placed in a Sherer-Gillett model CEL 512-37 or model CEL 25-7 controlled environment chamber with 660 footcan-

dles (ft-c) of light (600 ft-c from white VHO fluorescent tubes and 60 ft-c from 25-watt incandescent bulbs) with a 15-hr photoperiod. The temperature was kept at 16–18°, and the relative humidity was 65 ± 5%. These conditions are optimal for development of the pathogen. Six to 7 days after inoculation the flats were screened for susceptible segregants. Segregating $F_3$ families were repotted and grown to maturity. Five $F_4$ seedlings from the mature recombinant plants were retested with CR3. Five seeds from each putative recombinant were assayed electrophoretically for their hordein-banding patterns by running endosperm protein extracts on 12% sodium dodecyl sulfate (SDS)-polyacrylamide slab gels (DOLL and ANDERSEN 1981). This served two purposes: (1) If classical recombination occurred within the *Ml-a* region, it should be accompanied by exchange of outside markers. (2) The hordein patterns served as a "fingerprint" of each parental line to further rule out any contamination.

*Recombination estimates*: Recombination frequencies were estimated by the maximum likelihood formula:

$$p = \frac{n_2 + 2n_3}{n_1 + n_2 + n_3}$$

where $p$ = recombination frequency, $n_1$ = $F_3$ families not segregating for susceptible types, $n_2$ = $F_3$ families segregating three resistant to one susceptible and $n_3$ = $F_3$ families not segregating for resistant types. In the event that no recombinant plants were obtained, the upper 95% confidence limits were estimated by the method of GIESE *et al.* (1981). The limits will only be correct if the genes involved segregate in a 3:1 or 1:2:1 ratio when tested with the appropriate mildew strains. The formula used to obtain these limits is as follows:

$$P \leq 2/3(1 - (1 - 1.5(1 - \exp(1/N \ln 0.05)))^{0.5})$$

where $p$ = upper limit of recombination and $N$ = the number of nonrecombinant families observed.

Homogeneity of results in recombination experiments was tested using a Chi-square analysis of the appropriate contingency table.

*Studies on the action of recombinants*: Progeny seeds from the recombinant plants were grown and used to make intercrosses among the different recombinants in most pairwise combinations. In the event that only one recombinant was recovered, it was crossed to Manchuria. Manchuria represented the type of recombinant with neither resistance site. Selected recombinants from the *Ml-a6* × *Ml-a13* hybrid were also crossed to each of the parental resistant lines and to Manchuria. Approximately ten hybrid seeds per cross were planted in the field and allowed to self-pollinate. The progeny were planted in flats and tested with CR3 as described previously.

*Preparation of hordein for electrophoresis*: The method of DOLL and ANDERSEN (1981) was used with minor modification.

## RESULTS

$F_3$ families for each cross were derived from approximately 60 $F_1$ plants. Five to 15 $F_1$ plants were produced from each set of parents. The $F_3$ family data from each parental cross type were homogeneous, and the results are pooled in Table 2.

Families segregating three resistant to one susceptible 7 days after inoculation with race CR3 of *E. graminis* f. sp. *hordei* were observed in the *Ml-a10* × *Ml-a1*, *Ml-a1* × *Ml-a15* and *Ml-a6* × *Ml-a13* crosses. Susceptible segregants were accompanied by flanking marker exchange in these crosses. The flanking marker constitution for susceptible progeny of the *Ml-a10/Ml-a1* recombinant was *Hor-1*(a10) and *Hor-2*(a1). In progeny of the *Ml-a1/Ml-a15* recombinant the flanking marker constitution was *Hor-1*(a15) and *Hor-2*(a1). In progeny of 19 of the *Ml-a6/Ml-a13* recombinants the flanking marker constitution was *Hor-1*(a13) and *Hor-2*(a6) (Figure 2). The other two *Ml-a6/Ml-a13* recombi-

TABLE 2

*Reciprocal parental crosses and the segregation pattern of $F_3$ families 7 days after inoculation with race CR3 of* E. graminis *f. sp.* hordei

| | Segregation | | | | Segregation | | | | % recombination[b] | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parental cross[a] | All R | 3R:1S | All S | Parental cross | All R | 3R:1S | All S | Total | Estimate | 95% upper confidence limit of recombination |
| *Ml-a1* × *Ml-a6* | 3599 | 2[c] | | *Ml-a6* × *Ml-a1* | 2366 | | | 5967 | | 0.025 |
| *Ml-a1* × *Ml-a7(Mu)* | 2168 | | | *Ml-a7(Mu)* × *Ml-a1* | 3371 | 2[c] | | 5541 | | 0.027 |
| *Ml-a1* × *Ml-a10* | 4033 | | | *Ml-a10* × *Ml-a1* | 3676 | 1 | | 7710 | 0.013 | |
| *Ml-a1* × *Ml-a13* | 810 | | | *Ml-a13* × *Ml-a1* | 1263 | | | 2073 | | 0.072 |
| *Ml-a1* × *Ml-a15* | 12461 | 1 | | *Ml-a15* × *Ml-a1* | 2802 | | | 15264 | 0.0065 | |
| *Ml-a6* × *Ml-a10* | 1999 | | | *Ml-a10* × *Ml-a6* | 666 | | | 2665 | | 0.056 |
| *Ml-a6* × *Ml-a7(Mu)* | 2915 | | | *Ml-a7(Mu)* × *Ml-a6* | 948 | | | 3863 | | 0.039 |
| *Ml-a13* × *Ml-a10* | 1175 | | | *Ml-a10* × *Ml-a13* | 1269 | | | 2444 | | 0.061 |
| *Ml-a13* × *Ml-a7(Mu)* | 940 | | | *Ml-a7(Mu)* × *Ml-a13* | 1310 | | | 2250 | | 0.066 |
| *Ml-a13* × *Ml-a15* | 611 | | | *Ml-a15* × *Ml-a13* | 598 | 1 | | 1510 | 0.066 | 0.099 |
| *Ml-a15* × *Ml-a7(Mu)* | 3392 | 1[c] | | *Ml-a7(Mu)* × *Ml-a15* | 951 | | | 4344 | | 0.035 |
| *Ml-a6* × *Ml-a13* | 3205 | 12 | 9 | *Ml-a13* × *Ml-a6* | 4886 | 0 | 0 | 8112 | 0.930[d] | |

[a] Female parent × male parent.
[b] % Recombination estimated by maximum likelihood.
[c] Possible contaminants, only one or two susceptible out of 25 total seedlings in the $F_3$ family, significantly different ($P < 0.05$) from a 3:1 ratio.
[d] Recombination estimate based on total from *Ml-a6* × *Ml-a13* cross only.

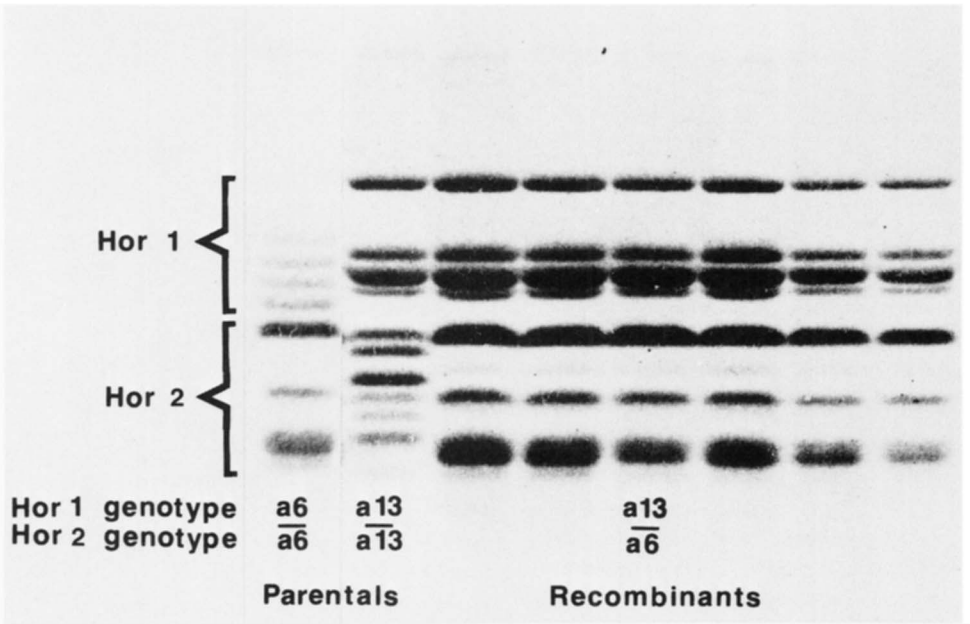


FIGURE 2.—SDS-polyacrylamide gel electrophoresis of hordein polypeptides from progeny of $F_3$ susceptible segregants. The original parental cross was *Ml-a6* × *Ml-a13*. The slower migrating bands are polypeptides encoded by the *Hor-1* locus. The faster migrating bands are polypeptides encoded by the *Hor-2* locus.

nants had the hordein pattern of the *Ml-a13* parent. Three or four progeny from each of the susceptible putative recombinants were retested with CR3. All of these progeny tested from the *Ml-a10/Ml-a1* and *Ml-a6/Ml-a13* recombinants were susceptible. Surprisingly, all the progeny tested from the *Ml-a15/Ml-a1* recombinant displayed type 2 (resistant) reactions. These maintained the exchanged outside marker arrangement between *Hor-1* and *Hor-2*. Progeny from one of the *Ml-a15/Ml-a1* plants giving a type 2 reaction also gave mostly type 2 reactions; however, two seedlings displayed a type 2–3 reaction with severe necrosis.

One 3R:1S segregating family was observed in the *Ml-a15* × *Ml-a13* cross. We were unable to assay the flanking markers in the susceptible progeny of this cross. In crosses with *Ml-a13* and *Ml-a10*, ten families seemed to segregate 3R:1S; however, the seedlings that appeared susceptible had a type 2–3 reaction. These plants were later shown to be resistant.

$F_3$ families that were entirely susceptible were observed in the *Ml-a6* × *Ml-a13* cross. Progeny seeds from these plants had exchange of *Hor-1* and *Hor-2* identical with the 3R:1S families recovered from the same cross (Figure 2). The recovery of these susceptible families was unexpected as judged by the observed frequency of 3R:1S families. If resistance is dominant an entirely susceptible $F_3$ family probably would have been derived from a homozygous $F_2$ plant. Such $F_2$ plants should be the product of two independently derived recombinant gametes. To test this, additional $F_2$ seeds from the same parental

TABLE 3

*Results of powdery mildew tests of $F_2$ segregating progeny from reciprocal crosses between* Ml-a6 *and* Ml-a13

| Cross[a] | Cross no. | Resistant | Susceptible | Flanking marker constitution of susceptible plants |
|---|---|---|---|---|
| *Ml-a6* × *Ml-a13* | 159 | 498 | 0 | |
| | 168 | 302 | 18 | 16 *Hor-1*(a13)-*Hor-2*(a6), 2 *Hor-1* and *2*(a13) |
| | 193 | 167 | 4 | All *Hor-1*(a13)-*Hor-2*(a6) |
| | 331 | 350 | 0 | |
| Total | | 1317 | 22 | |
| *Ml-a13* × *Ml-a6* | 102 | 510 | 0 | |
| | 122 | 307 | 0 | |
| | 357 | 532 | 0 | |
| Total | | 1349 | 0 | |

[a] Female parent × male parent.

crosses were screened (Table 3). The reader is reminded that these $F_2$ seeds originated from a number of $F_1$ seeds and when susceptibles are found they should not be segregating in a 1R:1S or 3R:1S ratio. Twenty-two susceptible plants from a total of 1339 were observed from crosses in which *Ml-a6* was the female parent. No susceptible plants were observed from the reciprocal crosses. The hordein-banding patterns of progeny from the susceptible plants were identical with those observed in the progeny of the $F_3$ susceptible segregants, thus demonstrating genetic recombination and not contamination. It is not known why the data within the *Ml-a6* × *Ml-a13* cross are heterogeneous.

In two cases the hordein-banding pattern in progeny from the *Ml-a6/Ml-a13* $F_3$ susceptible segregants was that of the *Ml-a13* parent. This could be explained by (1) an additional crossover in the region between the first exchange and the *Hor-2* gene, (2) gene conversion or (3) mutation in *Ml-a13* to susceptiblity. This was seen in one of the 3R:1S families and in one of the entirely susceptible families. In the susceptible family it was evident that the *Ml-a13* hordein pattern came from only one of the parents since the *Ml-a6/Ml-a13* recombinant pattern segregated independently of the *Ml-a13* pattern in resulting progeny. The *Ml-a13* hordein pattern was also present in progeny of two of the $F_2$ plants susceptible to CR3. Like the case above, it was clear from progeny of the susceptible $F_2$ plants that the *Ml-a13* hordein pattern came from only one of the parents.

*Further tests on the* Ml-a10/Ml-a1 *recombinant*: Susceptible recombinants could possess both allelic sites for specific resistance or neither site. If the lone *Ml-a10/Ml-a1* recombinant possessed both allelic sites, one might be able to reconstruct the parental specificity by recombination with a line lacking sites for specific resistance. Recombination between the two sites should restore both parental specificities.

Susceptible progeny from the *Ml-a10/Ml-a1* recombinant were crossed with Manchuria. Hybrid plants were allowed to self-pollinate. The progeny were tested with CR3. Nine resistant seedlings were recovered from 3922 plants.

TABLE 4

*Results of inoculation of progeny from entirely susceptible and 3R:1S $F_3$ families with race CR3 of* E. graminis *f. sp.* hordei

| Recombinant family | Resistant | Intermediate | Susceptible |
|---|---|---|---|
| All S | | | |
| 193-1[a] | 5 | | 12 |
| 193-1 | 17 | | 2 |
| 193-1 | 18 | | 1 |
| 193-4 | 7 | 2 | 5 |
| 193-4 | 6 | | 2 |
| 193-4 | 2 | 4 | 7 |
| 193-4 | 9 | | 0 |
| 193-4 | 16 | 2 | 4 |
| 193-4 | 5 | | 13 |
| 193-4 | 20 | | 9 |
| 193-4 | 8 | | 10 |
| 168-4 | 18 | | 0 |
| 168-4 | 10 | 2 | 5 |
| 168-4 | 6 | | 2 |
| 168-4 | 8 | | 2 |
| 168-4 | 5 | | 4 |
| 168-4 | 1 | | 5 |
| 3R:1S | | | |
| 193-2 | 20 | | 0 |
| 193-2 | 16 | 4 | 2 |
| 193-2 | 20 | | 8 |
| 168-1 | 14 | | 0 |
| 168-1 | 14 | | 1 |
| 168-1 | 15 | | 0 |
| 168-3 | 8 | | 1 |
| 168-5 | 10 | | 0 |
| 168-5 | 25 | | 1 |
| 193-3 | 0 | | 20 |

[a] Each line represents a single spike or a portion of a single spike.

These seedlings gave a type 0-1 reaction with no necrosis, the same reaction given by the *Ml-a1* allele.

*Expression of* Ml-a6/Ml-a13 *recombinants*: The occurrence of an excess of completely susceptible families in the *Ml-a6* × *Ml-a13* cross and the lack of such families in the reciprocal cross could be due to a recombination suppressor/enhancer. To test this possibility, it was necessary to examine the recombination of other genes on chromosome 5. This was possible by use of the hordein genes, since all phenotypes are distinguishable by their characteristic banding patterns. There was no significant difference ($P > 0.05$) in the frequency of exchange between *Hor-1* and *Hor-2* in reciprocal *Ml-a6* and *Ml-a13* crosses. There was, however, a significant difference in the frequency of exchange between *Hor-1* and *Hor-2* in the crosses of *Ml-a6* with *Ml-a13* (12.016%) and crosses of *Ml-a6* with *Ml-a1* (7.140%).

*Instability of* Ml-a6/Ml-a13 *recombinants*: When large numbers of progeny

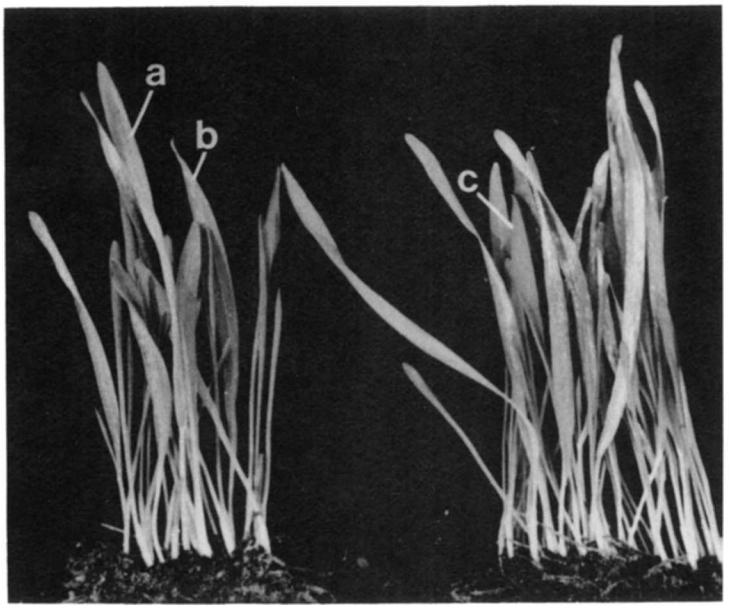


FIGURE 3.—Phenotype of revertant barley seedlings 8–9 days after inoculation with race CR3 of *E. graminis* f. sp. *hordei*. Progeny of a cross between the *Ml-a6/Ml-a13* recombinants 193-1 and 168-4. All seedlings from one group are from a single spike. Reaction type: a, susceptible; b, revertant-intermediate type; c, revertant-resistant type.

TABLE 5

*Results of powdery mildew tests on progeny of crosses between* Ml-a6/Ml-a13 *recombinants and Manchuria and between* Ml-a6/Ml-a13 *recombinants and the susceptible* ml-a6 *isoline*

| Cross[a] | Resistant | Intermediate | Susceptible |
|---|---|---|---|
| 619 Manchuria × 168-4[b] | 42 | 28 | 10 |
| 637 Manchuria × 159-5 | 38 | 46 | 376 |
| 693 168-2 × Manchuria[c] | 25 | 41 | 1014 |
| 708 Manchuria × 168-1 | 2 | 50 | 1341 |
| 711 Manchuria × 159-1-3 | 236 | 281 | 384 |
| 717 Manchuria × 168-7 | 0 | 33 | 1377 |
| 725 168-7 × Manchuria | 0 | 0 | 419 |
| 731 168-2 × Manchuria | 1 | 0 | 444 |
| 732 Manchuria × 168-6 | 171 | 297 | 348 |
| 734 Manchuria × 168-7 | 0 | 3 | 426 |
| 643 *ml-a6* × 159-4 | 139 | 0 | 1841 |
| 699 159-3 × *ml-a6* | 4 | 12 | 1029 |
| 712 *ml-a6* × 159-1-3 | 1 | 0 | 389 |
| 718 193-1 × *ml-a6* | 218 | 357 | 522 |

[a] Female parent × male parent.

[b] 159-1-3, 159-5, 168-4, 168-6, and 193-1 originated from entirely susceptible $F_3$ families.

[c] 159-3, 168-1, 168-2 and 168-7 originated from 3R:1S $F_3$ families.

TABLE 6

*Results of powdery mildew tests on progeny of intercrosses among different* Ml-a6/Ml-a13 *recombinants*

| Cross[a] | Resistant | Intermediate | Susceptible |
|---|---|---|---|
| 479 193-4[b] × 193-2[c] | 0 | 0 | 330 |
| 480 193-1 × 193-2 | 0 | 0 | 852 |
| 481 193-2 × 193-4 | 2 | 0 | 1905 |
| 628 168-5 × 168-4 | 0 | 0 | 570 |
| 640 193-1 × 168-4 | 32 | 7 | 1034 |
| 641 168-1 × 168-6 | 0 | 3 | 1152 |
| 646 159-1-3 × 168-2 | 4 | 2 | 954 |
| 647 168-2 × 159-2 | 7 | 3 | 1394 |
| 650 159-5 × 159-3 | 10 | 2 | 197 |
| 654 193-1 × 168-1 | 0 | 0 | 720 |
| 656 159-1-3 × 159-5 | 1 | 14 | 1533 |
| 657 193-1 × 168-7 | 0 | 3 | 897 |
| 659 168-2 × 159-1-3 | 8 | 0 | 1402 |
| 660 159-5 × 159-1-3 | 0 | 10 | 1070 |
| 663 159-1-2 × 168-6 | 1 | 0 | 2919 |
| 667 159-2 × 193-1 | 9 | 8 | 1904 |
| 668 168-2 × 193-1 | 3 | 12 | 525 |
| 669 159-5 × 159-3 | 0 | 30 | 2430 |
| 677 193-1 × 159-1-2 | 4 | 4 | 1980 |
| 678 159-3 × 159-1-2 | 0 | 7 | 2303 |
| 689 159-3 × 193-1 | 0 | 16 | 1964 |

[a] Female parent × male parent.

[b] 159-1-2, 159-1-3, 159-5, 168-4, 168-6, 193-1 and 193-4 originated from entirely susceptible $F_3$ families.

[c] 159-3, 168-1, 168-2, 168-7 and 193-2 originated from 3R:1S $F_3$ families.

from the *Ml-a6/Ml-a13* susceptible recombinants were inoculated with CR3, some resistant plants were recovered. Resistant plants occurred in progeny from nearly all of the recombinants, regardless of whether they originated from completely susceptible or 3R:1S families. This was unexpected since the progeny from the 3R:1S families originated from a selfed homozygous susceptible plant with the recombinant hordein pattern. Mildew tests of the progeny from $F_3$ susceptible recombinants were repeated three times. Intact heads were planted in flats and inoculated heavily with CR3 on day 0 and day 1. Representative results from one of the tests are presented in Table 4. In some of the cases an intermediate infection was observed. This was not necrotic like a type 2 infection, but the overall development of the mildew was severely limited when compared to the susceptible type (Figure 3). Some plants that appeared immune at 7–8 days developed various degrees of infection by 14 days. By the 10th day, the control plants had died. Progeny from the revertants had no change in their flanking marker constitution. Similar results were obtained in progeny of crosses of *Ml-a6/Ml-a13* recombinants with Manchuria (Table 5). However, resistant progeny among intercrosses of the recombinants were

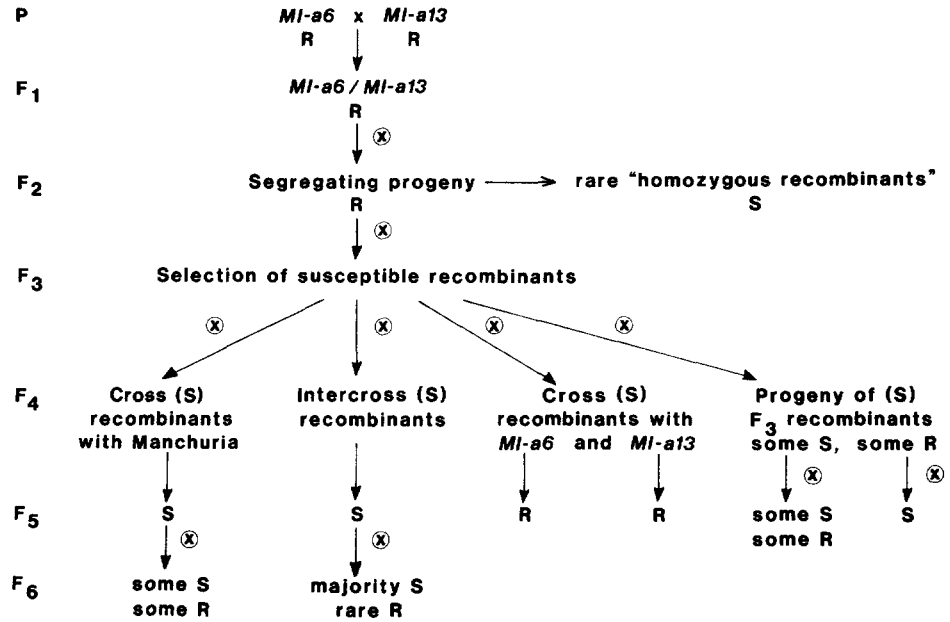


FIGURE 4.—Flow chart of crosses between *Ml-a6* and *Ml-a13* and the results of seedling tests with *E. graminis* f. sp. *hordei* for six generations. R = resistant; S = susceptible; ⊗ = self-pollinated.

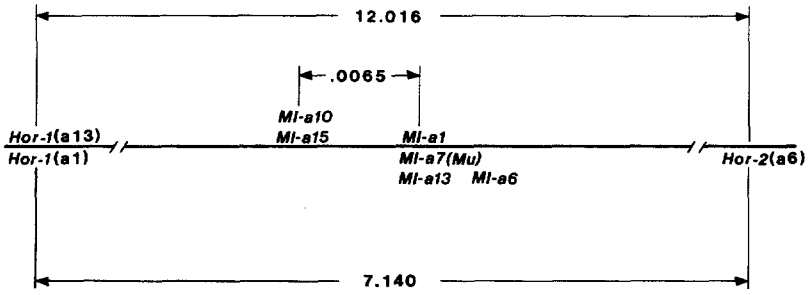


FIGURE 5.—Model of *Ml-a* region on chromosome 5. The centromere is about 66 map units to the left of *Hor-1*(a13). *Ml-a10* and *Ml-a15* and placed to the left of *Ml-a1* based on flanking marker exchange. *Ml-a6* is positioned to the right of *Ml-a13* in accordance with the pattern of flanking marker exchange, although an actual recombination percentage is not shown (see DISCUSSION for details).

rare (Table 6). A diagram of the kinds of results observed in each generation is presented in Figure 4.

## DISCUSSION

The purpose of this study was to determine the structure of genes that confer specific resistance to *E. graminis* f. sp. *hordei* and, particularly, whether variants in the *Ml-a* group are alleles or are closely linked genes. Six naturally occurring variants were used in recombination tests, the aim being completion of the modified *cis-trans* test for codominant genes (SHEPHERD and MAYO 1972).

One susceptible recombinant was observed in the *Ml-a10* × *Ml-a1* cross. This recombinant apparently possessed both allelic sites for specific resistance since resistant seedlings (nine of 3922) were observed in the progeny of crosses between the *Ml-a10/Ml-a1* recombinant and a Manchuria isoline. These are probably the result of recombination restoring *Ml-a1* or *Ml-a10* specificity. The infection type of the resistant seedlings was most similar to *Ml-a1*. If specificity was restored by recombination, then the original susceptible recombinant most likely contained both dominant sites for specific resistance, *Ml-a1* and *Ml-a10*. Since these variants conditioned susceptibility in the *cis* arrangement, but conditioned resistance in *trans*, *Ml-a1* and *Ml-a10* must fall within the same cistron. Secondarily, if intracistronic recombination between the two allelic sites had led to an altered specificity, then the original selection in this cross should have been for both kinds of recombinants, not just for the double recessive (Figure 1). This would alter the maximum likelihood formula by doubling the denominator resulting in a linkage value of 0.0065.

Verifiable recombinants were absent in many crosses. This may reflect the closeness of the alleles at the *Ml-a* locus or more probably it may represent an inability of the different alleles to recombine at all. Studies on the fine structure of the waxy locus in maize resulted in a marked nonadditivity of recombinational frequencies (NELSON 1968). Nelson proposed that this may be due to small differences in gene structure. A similar phenomenon occurred in studies of intragenic recombination at the maize *Adh1* locus (FREELING 1976). Structural differences in the different *Ml-a* "alleles" could explain the lack of recombination.

The percentage of susceptible segregants in the *Ml-a6* × *Ml-a13* cross was 0.93. There was no confirmed recombination between *Ml-a6* and *Ml-a1*, *Ml-a10* or *Ml-a15*, suggesting that these variants are tightly linked (Table 2). If the susceptible segregants in the *Ml-a6* × *Ml-a13* cross were due to recombination between the two recessive alleles, *ml-a6* and *ml-a13*, we would expect to see comparable recombination between *Ml-a13* and *Ml-a1*, *Ml-a10* or *Ml-a15*. This was not observed. The underlying mechanism at present is not understood.

Although the genes used in this analysis behave as units of segregation (MOSEMAN and JORGENSEN 1973), it has recently been suggested that some of the host lines may have more than one gene for resistance. J. H. JORGENSEN (personal communication) has evidence that the lines that possess *Ml-a7(Mu)* and *Ml-a13* contain a second specificity. The second specificity in *Ml-a13* produces type 1-2n (resistant) reactions. If the two variants in the *Ml-a13* line were positioned on either side of *Ml-a1* or *Ml-a6*, which produce type 0 reactions, the original interpretation would remain, since recombination between *Ml-a13* and *Ml-a1* or *Ml-a6* could be detected by the observation of a (three type 0 to one type 1-2n) $F_3$ segregation. No such families were observed, suggesting that this did not occur. Alternatively, if the two variants were positioned on the same side of *Ml-a1* or *Ml-a6*, the selection would proceed as previously described. Crosses with the Multan isoline may be interpreted as the *Ml-a7(Mu)* allele being tightly linked with the other five genes examined.

Alternatively, an additional variant may be masking the effect of recombination between the two primary genes in the cross.

*Reversion among progeny of susceptible segregants*: One segregating family was observed in the *Ml-a1* × *Ml-a15* cross. Type 4 (susceptible) plants had exchanged outside markers. However, type 2 (resistant) infections were observed in progeny of these susceptible plants. In an analogous fashion, progeny of *Ml-a6/Ml-a13* susceptible segregants also had exchanged outside markers and also produced progeny exhibiting resistance. Reversion occurred at a much higher rate than expected if standard recombination or mutation were occurring. Furthermore, susceptible recombinants were only observed in $F_3$ families in which the *Ml-a6* line was the original female parent. A maternally acting recombination suppressor/enhancer is unlikely, since the hordein genes recombined in approximately equal frequencies in reciprocal crosses. A cytoplasmic resistance suppressor is plausible; however, it should have been expressed in backcrosses to the parental lines possessing *Ml-a6* and *Ml-a13*. All seedlings in this test were resistant (data not shown). A closely linked resistance suppressor would not be expected to segregate in progeny of the recombinants. Resistant progeny were recovered at a significant frequency, however (Table 4).

Some *Ml-a6/Ml-a13* revertant plants were tested with race A27 of *E. graminis* f. sp. *hordei*. Race A27 is avirulent on *Ml-a13* and virulent on *Ml-a6*. A27 produces a type 2-3n infection on *Ml-a14*, a presumptive second gene in the *Ml-a6* isoline (GIESE 1981). However, additional experiments indicate that *Ml-a14* is not present in these recombinant plants (R. P. WISE, unpublished results). This allows *Ml-a13* resistance to be scored. These *Ml-a6/Ml-a13* revertants had a type 0 reaction with A27. This suggests that *Ml-a13* resistance was restored by the reversion event. Inoculations of additional progeny of the *Ml-a6/Ml-a13* recombinants were carried out with race MK 24-76. Race MK 24-76 is avirulent on *Ml-a6* lines and virulent on *Ml-a13* lines. These tests showed that *Ml-a6*-specific resistance is restored in progeny of at least four of the recombinants. The frequency of reversion of *Ml-a6* resistance in these recombinants ranged from 0.013 to 0.077 (J. H. JORGENSEN, personal communication).

The original crossing scheme was designed to select susceptible segregants arising from crossing over between recessive, closely linked genes *or* intragenic recombination between either dominant or recessive alleles. The pattern of flanking marker exchange in the *Ml-a6/Ml-a13* recombinants might lead one to conclude that selection in this cross had been for intergenic recombination between recessive genes. However, *Ml-a6*- and *Ml-a13*-specific resistance were identified in revertant progeny of these susceptible recombinants. This suggests that the dominant variants *Ml-a6* and *Ml-a13* were present in most of these recombinant plants but were repressed. Furthermore, if recombination did occur between *Ml-a6* and *Ml-a13* at a percentage of 0.93, we would expect to recover susceptibles that were exchanges between *ml-a6* and *ml-a13*. No such segregants were recovered, suggesting that this did not occur. Therefore, 0.93 is probably not an accurate representation of the recombination distance between these two variants.

There are many anomalies associated with the *Ml-a6/Ml-a13* recombinants that cannot be simply explained: (1) Susceptible segregants were observed only in $F_3$ families in which *Ml-a6* was the original female parent. (2) If each recombinant is the result of an independent event, then based on the twelve 3R:1S families we would expect less than one totally susceptible $F_3$ family. Nine of these susceptible families were observed (Table 2). (3) Resistant revertants were recovered in high frequencies showing variation in infection type among progeny from the same homozygous susceptible recombinants. These revertants retained the original exchanged outside marker relationship. Furthermore, resistant revertants appeared in the $F_5$ and $F_6$ generations, and not all of these revertants are stable.

The occurrence of a significant number of completely susceptible $F_3$ families and homozygous $F_2$ recombinants implies that not all of these events are independent. The clustering of these events suggests that some may have occurred prior to meiosis. The observation of these events in four different crosses, however, indicates that some of these events occurred more than once.

*Susceptible phenotypes may be due to transposable elements*: The reciprocal cross differences, possible premeiotic events and reversion to wild-type activity of *Ml-a6* and *Ml-a13* display similarities to hybrid dysgenesis in Drosophila (KIDWELL, KIDWELL and SVED 1977). Hybrid dysgenesis is due to a family of transposable elements termed *P* factors (RUBIN, KIDWELL and BINGHAM 1982). *P* factor-induced rearrangements seem to occur prior to and at meiosis (ENGELS and PRESTON 1984). The apparent ability to make *bz*-mutable alleles in maize with *Spm* was easier using a male *Spm*-containing stock rather than a female *Spm* stock (NELSON and KLEIN 1984). The rates of reversion of the *Spm*-controlled *bz-m13*-mutable allele and another *Spm* insertion mutant of the *A1* allele of maize, *a1-m (pa-pu)* (PETERSON 1970), are comparable to the reversion rates seen in the *Ml-a* system.

These properties suggest to us that the initial recombination in crosses with *Ml-a6* and *Ml-a13* lines may have been associated with a transposition event. Modulator (*Mp*), a transposable element in maize, increases the frequency of crossing over in chromosomal segments adjacent to its position but not within the interval containing *Mp* (GREENBLATT 1981). This could explain the differences in recombination frequencies between *Hor-1* and *Hor-2*. Recombinant 168-1, which has the *Ml-a13* hordein pattern, reverted to *Ml-a13*-type resistance, but not *Ml-a6* type. This suggests that the element is coming into the cross from the *Ml-a13* line. This does not, however, exclude the possibility that the element originated within *Ml-a6*. Integration into or near specific *Ml-a* alleles could lead to complete or partial loss of the gene product. Accurate excision would lead to normal function, whereas inaccurate excision may lead to partial or modified function. This could explain the observed intermediate infection types seen in many of the resistant progeny of the *Ml-a6/Ml-a13* susceptible recombinants (SUTTON *et al.* 1984).

This study began with the question of whether the variants of the *Ml-a* region were allelic or nonallelic. Except for *Ml-a1* and *Ml-a10* the data have

not answered that question. The data have provided a model in which the possibility of a transposable element is presented.

Based on the recombination estimates, flanking marker constitution and interpretation of reversion events, a model of the *Ml-a* locus is presented in Figure 5. The flanking marker constitution in progeny of the *Ml-a10/Ml-a1* susceptible recombinant was *Hor-1*(a10) and *Hor-2*(a1). In addition, it was possible to recover *Ml-a1*-type resistance in progeny of a cross between *Ml-a10/Ml-a1* recombinants and a Manchuria isoline. *Ml-a10*-type resistance may also have been recovered, but it may have been masked by the more resistant *Ml-a1* type. For these reasons, *Ml-a10* is positioned between *Hor-1* and *Ml-a1*. In progeny of the *Ml-a1/Ml-a15* susceptible recombinant the flanking marker constitution was *Hor-1*(a15) and *Hor-2*(a1). *Ml-a15*-type resistance was recovered in these progeny. The reasons for this are unclear. The other variants are positioned based on their recombination estimates.

Unstable *Ml-a6/Ml-a13* susceptible recombinants occurred at a percentage of 0.93. The flanking marker constitution of most of these recombinants was *Hor-1*(a13) and *Hor-2*(a6). Recombination along chromosome *5* in *Ml-a6* and *Ml-a13* lines may have positioned a previously stable transposable element into a genetic background where it could transpose, specifically into or near *Ml-a6* or *Ml-a13*.

These results show the complex nature of the *Ml-a* region. Transposable elements have been shown to be associated with other complex loci (KERMICLE 1980; GREENBLATT 1981), although at present this may be the only example of a locus conditioning resistance to an obligate parasite.

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