References and Notes

1. Apollo Lunar Geology Investigation Team, *Science* **175**, 407 (1972).
2. The members of the Lunar Sample Analysis Planning Team are A. J. Calio, M. B. Duke, A. L. Burlingame (University of California, Berkeley), D. S. Burnett (California Institute of Technology, Pasadena), B. Doe (U.S. Geological Survey, Denver, Colorado), D. E. Gault (NASA Ames Research Center, Moffett Field, California), L. A. Haskin (University of Wisconsin, Madison), W. G. Melson (U.S. National Museum, Smithsonian Institution, Washington, D.C.), J. Papike (State University of New York, Stony Brook), R. O. Pepin (University of Minnesota, Minneapolis), P. B. Price (University of California, Berkeley), H. Schnoes (University of Wisconsin), B. Tilling (NASA headquarters, Washington, D.C.), N. M. Toksoz (Massachusetts Institute of Technology, Cambridge), and J. A. Wood (Astrophysical Observatory, Smithsonian Institution). Those members listed with no institution are from the NASA Manned Spacecraft Center, Houston, Texas.
3. L. H. Ahrens and H. Von Michaellis, *Earth Planet. Sci. Lett.* **5**, 395 (1969).
4. Lunar Sample Preliminary Examination Team, *Science* **173**, 681 (1971).
5. M. H. Carr and F. El Baz, *U.S. Geologic Survey Map 1723* (U.S. Geological Survey, Washington, D.C., 1971).
6. G. D. O'Kelley, J. S. Eldridge, E. Schonfeld, P. R. Bell, *Geochim. Cosmochim. Acta* (Suppl. 2) **2**, 1747 (1971).
7. Lunar Sample Preliminary Examination Team, *Science* **165**, 1211 (1969).
8. C. B. Moore, E. K. Gibson, J. W. Larimer, C. F. Lewis, W. Nichiporuk, *Geochim. Cosmochim. Acta* (Suppl. 1) **2**, 1375 (1970).
9. K. Norrish and J. T. Hutton, *ibid.* **33**, 431 (1969).
10. K. Norrish and B. W. Chappell, in *Physical Methods in Determinative Mineralogy*, J. Zussman, Ed. (Academic Press, New York, 1967), p. 161.
11. KREEP (potassium, rare-earth elements, and phosphorus) is discussed by C. Meyer, Jr., R. Brett, N. J. Hubbard, D. A. Morrison, D. S. McKay, F. K. Aitken, H. Takeda, and E. Schonfeld [*Geochim. Cosmochim. Acta* (Suppl. 2) **1**, 393 (1971)].

Genes Conferring Specific Plant Disease Resistance

Differences in behavior may reflect structure differences with important general implications.

K. W. Shepherd and G. M. E. Mayo

Although it is well known that major genes control the resistance of plants to a wide variety of parasites and that these genes are of value when producing resistant forms of crop plants (*1*), the detailed structure and function of these host genes remain obscure. For example, it has frequently been reported that genes conferring resistance to an obligate parasite are grouped together in a small segment of a host chromosome, but in most cases it is not known whether these genes are closely linked or functionally allelic.

One of the best examples of such groups of host genes comes from the work of Flor (*2*) with flax (*Linum usitatissimum* L.) and its rust [*Melampsora lini* (Ehrenb.) Lév.] where genes dominant for conferring resistance have been assigned to five "loci" named *K*, *L*, *M*, *N*, and *P*, with 1, 11, 6, 3, and 4 "alleles," respectively. Other good examples include the Rp_1 locus in maize (*Zea mays* L.) and the Ml_a locus in barley (*Hordeum vulgare* L.), where 14 different genes conferring resistance to maize rust (*Puccinia sorghi* Schw.) (*3*) and at least 7 different genes conferring resistance to powdery mildew (*Erysiphe graminis* D.C. f.sp *hordei* E. M. Marchal) (*4*) have been identified, respectively.

Initially, the genes within these groups were considered allelic when they failed to show recombination among F_2, F_3, or a limited number of testcross progeny (*5*). Recently, however, more critical data on the structure of some of these groups have been obtained by testing much larger testcross families.

For example, both Flor (*6*) and Shepherd (*7*) detected rare recombination between genes from each of the *L*, *M*, and *N* groups in flax. In the more extensive studies of Flor, the expected reciprocal products of recombination were recovered with the *M* and *N* genes, but only the double recessive phenotypic class was detected with the *L* genes tested. Also, Saxena and Hooker (*3*) showed that several of the genes in the Rp_1 group of maize could be recombined with low frequency and reciprocal products were recovered whenever the testcross progeny were tested with an appropriate combination of rust strains.

From these results it was concluded that each of the *M*, *N*, and Rp_1 groups are complex regions of chromosome possessing several closely linked genes that can be recombined reciprocally (*3*, *6*). Because the reciprocal products of recombination were not detected with *L* genes, Flor was unable to decide whether these genes are "mutually exclusive alleles" or closely linked genes. However, we shall show that when his results are compared with the expectations from a modified *cis-trans* test for allelism, the *L* genes appeared to behave as functional alleles.

In our work we have set out to test critically the possibility that some groups of genes conferring disease resistance consist of closely linked genes, whereas others consist of a series of functional alleles. The practical importance of deciding between allelism and close linkage has been stated by other workers (*3*, *6*), but such a decision could contribute to an understanding of the origin and mode of action of genes conferring disease resistance. Consequently we have chosen flax and its rust to make a detailed study of recombination between two gene groups, *L* and *M*, that seem to have different structures. Our results are reviewed in this paper and more detailed treatments are forthcoming (*8*, *9*).

Concept of Allelism and a Modified Cis-Trans Test

Before referring to our experiments, it is necessary to choose an operational definition of the gene, and hence allelism, appropriate for genes controlling disease resistance. The modern definition of the gene is as a unit of function, the cistron, which has been shown to be composed of numerous mutant sites separable by recombination [see, for example, Fincham (*10*)]. Thus to

Dr. Shepherd is senior lecturer in the department of agronomy at Waite Agricultural Research Institute. Dr. Mayo is senior lecturer in the department of genetics at the University of Adelaide, North Terrace, Adelaide, South Australia 5001.

demonstrate that genes controlling similar phenotypes are closely linked, rather than allelic, it is not sufficient to show that they can be recombined. Instead it is necessary to show that they control different, but possibly related, primary functions. On the other hand, if these genes control the same function, they are considered to be functionally allelic.

Since the primary products of most genes have not been identified, empirical methods are necessary to determine whether genes are functionally identical. The *cis-trans* test has been widely used for this purpose with mutant strains possessing a defective and usually recessive phenotype compared to the normal or wild type (*10*). In this test, if the *trans* arrangement of two mutant genes produces a mutant phenotype, and the *cis* arrangement a normal, or nearly normal phenotype, it is concluded that these genes are defective in the same function and therefore are functional alleles. Alternatively, if both *trans* and *cis* arrangements produce a normal phenotype, it is concluded that functionally different genes are involved, though, in this case, it has been shown that interallelic complementation in *trans* could occasionally lead to an incorrect conclusion (*11*). In practice, because of the difficulty of producing and recognizing the *cis* arrangement, the test for functional allelism is usually restricted to producing the *trans* arrangement and observing whether it has a mutant phenotype or exhibits complementation.

However, as shown below, the *cis-trans* test devised for defective mutants is not directly applicable to certain groups of genes associated with polymorphic populations in nature. Some examples are genes controlling blood groups and histocompatibility in animals (*12, 13*), reproductive incompatibility in plants (*14*), and, of immediate relevance, disease resistance in plants. These groups are characterized by the absence of a dominant wild type; and, if a standard form is available for comparison, it is equivalent to Fisher's (*15*) "universal recessive." This form has a null phenotype, whereas the other variants within a group produce distinctive phenotypes dominant to the "universal recessive," but showing no dominance among themselves.

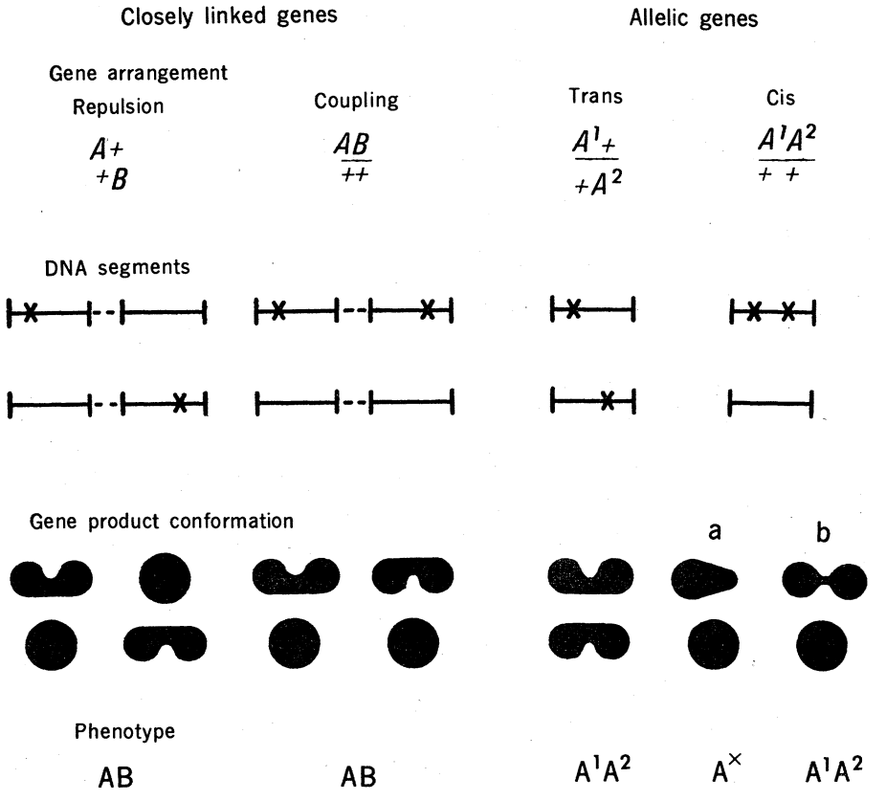

Fig. 1. Modified *cis-trans* test for allelism with codominant genes. The closely linked (*A* and *B*) and allelic (A^1 and A^2) codominant genes each differ from their standard gene (+) by a single DNA base change (×) and produce primary products with single amino acid substitutions. These substitutions, through effects on conformation, convert the inactive standard gene products (●) into the different active forms shown (◒, ◓). With the closely linked genes involving separate DNA segments, the repulsion and coupling arrangements produce the same array of gene products and thus they have the same phenotype (AB when the standard gene produces a null phenotype). With the allelic genes involving the same DNA segment, the *trans* phenotype is predictably additive (A^1A^2), but because the *cis* arrangement produces a product with two amino acid alterations it is not possible to predict its phenotype. If an interaction product is produced (a), the *cis* phenotype ($A^\times$) will be unrelated to that of the parent genes; and the *cis-trans* test will give positive evidence for allelism. If no interaction occurs (b), its phenotype could be additive, and then the test would be equivocal.

This test is illustrated by the examples in Fig. 1, alternatively involving closely linked (*A* and *B*) and allelic (A^1 and A^2) codominant genes. It is assumed, for simplicity, that each of these genes differs from its standard gene (+) by a single DNA base change such that they each produce a gene product (polypeptide chain) which differs from the standard gene product by a single amino acid substitution. To account for the distinctive phenotypes of genes *A*, *B*, A^1, and A^2 compared to the null phenotype of the standard gene, it is assumed that these single amino acid changes, through their effect on protein conformation, confer qualitatively different functional properties on the standard gene products.

On this model, it is expected that the repulsion and coupling arrangements of closely linked genes will both exhibit the same additive phenotype (AB, Fig. 1). The *trans* arrangement of allelic genes is also expected to produce an additive phenotype, but it is proposed that the phenotype of the *cis* arrange-

ment will be nonadditive, in some cases at least, because it has been shown that the properties of a protein with two amino acid alterations cannot necessarily be predicted from the properties of the singly altered parent proteins (*17*). Moreover, if the specificities of genes A^1 and A^2 depend on different surface configurations in the same or partly overlapping regions of the three-dimensional structure of the standard gene product, even though it might be possible to combine these two genes in *cis*, it would not be possible for the *cis* gene product to express both A^1 and A^2 specificities simultaneously. On the other hand, if genes A^1 and A^2 produce amino acid alterations in regions of independent function, which are known to exist in some single gene products (*18*), it is expected that both A^1 and A^2 effects could be expressed in the *cis* as well as the *trans* arrangement.

Thus the modified *cis-trans* test for functional allelism with codominant genes may be stated as follows. If the *cis* arrangement of these genes produces an interaction such that the *cis* phenotype differs from that of the *trans* arrangement, it is inferred that the genes are functionally allelic. In practice, at least with disease resistance genes, we would not necessarily expect to identify this *cis* arrangement because its precise phenotype is not predictable. Rather the occurrence of an interaction in *cis* would be suggested if only one (the homozygous recessive) of the two reciprocal recombinant products was detected among testcross progeny. However, the test is equivocal when the genes exhibit an additive phenotype when present in different strands and also when introduced into the same strand. In this case the genes may be either closely linked or functionally allelic with alterations in regions of independent function within the single gene product.

Experimental Program

Analysis of testcross progeny. Testcross progeny were produced in order to detect recombination between genes within the *L* and *M* groups and, hence, to obtain possible *cis* gene arrangements for comparison with the *trans* forms. The procedure is illustrated by the model, in Table 1, involving rust strains 1 and 2 and two members of the same group of host genes, A^1 and A^2. Rust strains 1 and 2 are used as specific testers for the presence of host genes A^1 and A^2, respectively. For example, host plants possessing gene A^1 are resistant (R_1) to strain 1 whereas all other plants, including those with A^2 alone, are susceptible (S_1) to this strain. By determining the reaction of each plant to rust strains 1 and 2, the genotypes of all testcross progeny may be identified, including both recombinant classes, namely plants phenotypically susceptible (S_1 S_2) or resistant (R_1R_2) to both strains of rust, provided that A^1 and A^2 do not interact in *cis* arrangement.

Our results, together with those of Flor (*6*) for the same *L* and *M* gene combinations, are included in Table 1. Rare recombinants were observed with each gene pair tested; but, whereas both of the expected recombinant classes occurred with approximately equal frequency among genes of the *M* group, only the S_1 S_2 class was observed among those of the *L* group. Although absence of phenotypically R_1 R_2 plants among progeny of $L^2+/+L^6$ and $L^6+/+L^{10}$ heterozygotes is not individually significant, such an absence among progeny of $L^2+/+L^{10}$, in either Flor's or our own observations, cannot be ascribed to chance. Flor attempted to explain his results by suggesting that the rare S_1 S_2 plants observed were derived from chromosomal aberrations or deletions rather than from reciprocal crossing over. However, adopting the modified *cis-trans* test for functional allelism, we postulated that the different behavior of genes in the *L* and *M* groups may reflect a fundamental difference in their structure. Consequently, we have studied these genes in more detail.

F_2 analysis of M *genes.* Since genes *M* and M^3 behaved as independent functional units in the test crosses, it is assumed tentatively that they are closely linked genes rather than allelic genes. On this basis, the R_1 R_2 recombinants (Table 1) are coupling double heterozygotes with genotype $MM^3/++$ and such a gene arrangement suggests a new procedure for determining the structure of the *M* group.

The expectation of detectable recombinants in the F_2 from coupling double heterozygotes is equal to $p - (p^2/2)$, where p is the recombination fraction, so that, for values of p close to 0.002 observed in the testcross analysis, this F_2 expectation closely approximates to p, the equivalent expectation for testcross progeny. Since flax is naturally

Table 1. Origin of recombinants among testcross progeny from within Flor's *M* and *L* groups. R, resistant (R_1 means resistant to strain 1); S, susceptible.

Types	Generation							
	Parents		F_1 × susceptible (testcross)		Testcross progeny			
					Parentals		Recombinants*	
	[1]	[2]			[1]	[2]		
			Model					
Genotypes	$\frac{A^1+}{A^1+}$	$\frac{+A^2}{+A^2}$	$\frac{A^1+}{+A^2}$	$\frac{++}{++}$	$\frac{A^1+}{++}$	$\frac{+A^2}{++}$	$\frac{++}{++}$	$\frac{A^1A^2}{++}$
Phenotypes Reaction to rust strain 1.	R_1	S_1	R_1	S_1	R_1	S_1	S_1	R_1
2.	S_2	R_2	R_2	S_2	S_2	R_2	S_2	R_2
			Experimental					
Gene group								
M	$\frac{M+}{M+}$	$\frac{+M^3}{+M^3}$	$\frac{M+}{+M^3}$	$\frac{++}{++}$	1169 8049†	1129 8112	0 11	2 16
L	$\frac{L^2+}{L^2+}$	$\frac{+L^{10}}{+L^{10}}$	$\frac{L^2+}{+L^{10}}$	$\frac{++}{++}$	1593 1903†	1533 1943	6 12	0 0
	$\frac{L^6+}{L^6+}$	$\frac{+L^{10}}{+L^{10}}$	$\frac{L^6+}{+L^{10}}$	$\frac{++}{++}$	1182	1259	1	0
	$\frac{L^2+}{L^2+}$	$\frac{+L^6}{+L^6}$	$\frac{L^2+}{+L^6}$	$\frac{++}{++}$	4350 1643†	4260 1663	4 0	0 0

* In our work identification of recombinants has been verified by either progeny tests or, when available, by the use of marker genes. † Observations of Flor (*6*). He does not distinguish between parental progenies [1] and [2].

Table 2. Recombination between M and M^3 in F_2 progeny of heterozygotes.

Coupling heterozygote	F_2 progeny				
	Number observed of indicated phenotype*				Total
	Parentals		Recombinants		
	MM^3	$++$	$M+$	$+M^3$	
$\frac{MM^3}{++}$	5498	1875	10	10	7393
$\frac{MM^3}{M^4}$	9074	3292	15	18	12399

* Phenotypes determined by their reaction to rust strains 1 and 2, namely

Rust strain	Host gene		
	M	M^3	M^4
1	R	S	S
2	S	R	S

where R is resistant; and S is susceptible.

self-fertilizing it is an advantage to be able to use F_2 progeny rather than progeny from laborious testcrosses. Consequently, additional plants with the expected genotype $MM^3/++$ were produced from the original R_1R_2 recombinants, and their F_2 progeny were tested with appropriate strains of rust to detect recombinant phenotypes. The results (Table 2), not only confirm the genotype of the original R_1R_2 plants, but also the reciprocal nature of recombination between the genes and its frequency of occurrence (*19*).

In subsequent studies, a third member of the M group, for example M^x, representing any one of the other members of the same group, was introduced to form a triple heterozygote of the constitution, $MM^3\ +/++M^x$, on the assumption that M^x is distal to M and M^3. Provided that the F_2 progeny of this heterozygote are tested with rust strains to which M^x is susceptible so that M^x remains "silent," it is possible to detect recombination between M and M^3 as before. The advantage of including a third gene in repulsion is that the event leading to recombination between M and M^3 is expected simultaneously to produce new combinations of M genes in coupling, and hence to provide additional information on the relationship of the M genes. For example, a single reciprocal crossover between M and M^3 would produce $M+M^x$ and $+M^3+$ recombinant strands if M^x is distal, and M^x+M^3 and $+M+$ recombinant strands if M^x is proximal. However, if M^x is located between M and M^3, all four of these recombinant strands could be produced by single exchanges between M and M^3. Thus the relative positions of the three genes can be determined.

Since in practice recombination between genes M and M^3 is detected in F_2 progeny of the triple heterozygote, all the selected recombinants are expected to possess the common parental strand carrying M^x alone (that is, $++M^x$, if M^x is distal) in combination with the recombinant strand. Thus, to determine whether M^x is present in the selected recombinant strand, it is sufficient to progeny test the selected recombinant to ascertain whether it is homozygous rather than heterozygous for M^x.

We are using M^1, M^4, and M^5, in turn, as the third gene M^x, in combination with the strand carrying M and M^3 in coupling, but only the analysis with M^4 has been completed. Thirty-three recombinants with respect to M and M^3 were detected in the progeny of the $MM^3\ /\ M^4$ triple heterozygote with M^4 segregating but "silent" (Table 2). Twenty-six of these recombinants (13 M + and 13 + M^3) survived to maturity, and, when their progeny were tested with a rust strain specific for detecting gene M^4, it was found that all 13 of the + M^3 and one of the M + recombinants were heterozygous for M^4 and that the remaining 12 M + recombinants were homozygous. The occurrence of 12 recombinants with M and M^4 in coupling and which express separate M and M^4 specificities provides another example of M genes that are functionally independent. Furthermore, the new coupling arrangement provides a starting point for additional F_2 analyses and more precise mapping of the M genes. For example, we have already analyzed progeny from MM^4/M^3 triple heterozygotes, and the results, in conjunction with those given above, suggest a gene order $M\ M^4\ M^3$ (*8*).

F_2 analysis of L *genes.* In contrast to the M genes, the results obtained with the L genes were not consistent with reciprocal crossing over occurring between closely linked genes (Table 1). With L^2 and L^{10}, the failure to detect among testcross progeny individuals resistant to both rust strains (R_1R_2) could have been due to the nonreciprocal nature of the events (for example, gene mutation or conversion) producing the 18 progeny susceptible to both rust strains (S_1S_2). Alternatively, it is possible that both of the expected recombinant classes, namely *cis* heterozygotes ($L^2L^{10}/++$) and recessive homozygotes ($++/++$) were produced by reciprocal crossing over, but the former were not recognized because of an interaction between the genes in *cis* leading to a null phenotype as predicted in the modified *cis-trans* test for functional allelism.

The second hypothesis can be directly tested since, if correct, the phenotypically S_1S_2 plants would consist of genotypes $L^2L^{10}/++$ and $+\ +/++$ in equal numbers. Therefore, L^2 and L^{10} genes should be separately recoverable by rare recombination among progeny of $L^2L^{10}/++$ when self-fertilized, but not from $++/++$.

The six S_1S_2 recombinant plants (Table 1) detected in testcross progeny were allowed to self-fertilize in isolation, and their progeny were tested with a rust strain that would reveal resistant plants possessing either an L^2 or L^{10} gene. Resistant plants did not occur among the progeny of five of these recombinants, but the family size was too small to be conclusive, except possibly with recombinant No. 1 (Table 3). However, with recombinant No. 6 maintained as two offsets in separate pots, five resistant plants occurred among more than 2700 fully susceptible progeny.

In tests with additional rust strains it was shown that all of the resistant plants possessed L^{10} and not L^2 specificity, but the degree of resistance expressed was somewhat less than that of the parental L^{10} gene. Since, on the allelic interaction hypothesis, both L^2 and L^{10} genes are expected with equal frequency, additional progeny from recombinant No. 6a were produced and tested for L^2 and L^{10} specificity. Seven

of the 1597 susceptible plants from this recombinant were grown in separate pots and allowed to self-fertilize in isolation. The progeny from these plants were tested with the same rust strain used before, and the results are included in Table 3. Rare resistant plants occurred in five out of the seven progeny groups, but in further tests it was found that, once again, they all possessed L^{10} specificity.

All these results, taken together, clearly indicate that some form of suppression of L^{10} specificity must have occurred during the formation of the female gamete giving rise to recombinant No. 6, and that this effect is reversible.

The allelic interaction hypothesis predicts mutual suppression of L^2 and L^{10} expression when combined in *cis* arrangement, which is reversible by recombination; but the failure to recover any L^2 genes among 20 resistant progeny from recombinant No. 6 makes this hypothesis untenable, at least in its simplest form.

In our further studies (*9*) of this phenomenon it was shown that the strand carrying suppressed L^{10} specificity does not affect the expression of the parental L^{10} gene when combined with it in *trans* arrangement, indicating that the suppressor is recessive and located within the L^{10} gene. Furthermore, whereas plants heterozygous for suppressed L^{10} produced rare resistant progeny, plants homozygous did not, thus indicating that reversion to L^{10} specificity occurs by recombination rather than mutation. Consequently, by combining the chromosome carrying the suppressed L^{10} gene in *trans* with another L gene, L^x, and selecting for L^{10} specificity in F_2 progeny keeping L^x "silent," it should be possible to carry out an F_2 analysis with L genes analogous to that used for M genes. Such an F_2 analysis may be expected to provide a more critical test of the allelic interaction hypothesis since, if this interaction does not occur, it should be possible to produce some $L^{10}L^x$ combinations in *cis* in which both L^{10} and L^x specificities are expressed.

Table 3. Incidence of resistant (R) plants among progeny of phenotypically S_1S_2 members of $L^2 + / + L^{10}$ testcross families.

Parent plants (No.)	Progeny from selfing		
	Number tested	R plants	
		Number	Phenotype
Six S_1S_2 recombinants from testcrosses			
1	1504	0	
2	727	0	
3	12	0	
4	806	0	
5	452	0	
6a*	1597	2	L^{10}
b	1158	3	L^{10}
Seven plants from selfing of recombinant No. 6a			
1	948	4	L^{10}
2	1356	2	L^{10}
3	621	1	L^{10}
4	1368	5	L^{10}
5	1220	3	L^{10}
6	815	0	
7	766	0	

* Grown as offsets in separate pots a and b.

Discussion

The aim of our study was to determine the structure of groups of genes in plants conferring disease resistance, and particularly, whether the genes in a group are closely linked or whether they are functional alleles. Genes from the L and M groups in flax were used in the recombination tests, and, although the L and M genes behaved differently, it is still not clear from our criteria of allelism whether this behavior reflects a fundamental difference in structure. However it is now possible to suggest critical experiments, based on F_2 analyses, that should resolve this question.

It is evident from the phenotype of the coupling genotypes that genes M^3 and M^4 are both functionally different from gene M. The practical implication is that two or more different M specificities can be combined in a homozygote. However, it has not been possible to decide whether these M genes are closely linked, producing different primary products, or whether they are alleles possessing alterations in regions of independent function within a single gene product. This question could be decided by producing the other 13 possible arrangements of M genes in pairs and observing their phenotypes. If the M group consists of six different closely linked genes, then the phenotypes of all the coupling and repulsion arrangements are expected to be the same. A different behavior would be expected of a group of allelic genes, because it is unlikely that six different alterations of a single gene product would occur in regions of mutually independent function. Thus, if some coupling combinations of M genes exhibited phenotypic interaction, it would be concluded that at least some of the M genes are allelic. These data are being obtained with the use of the F_2 method of analysis to generate additional combinations of M genes in coupling.

Testcross results, particularly those of Flor, emphasize that genes in the L group behave differently from M genes, as well as from those of Rp_1 in maize (*3*). In addition to the data in Table 1, Flor (*6*) used 11 other combinations of L genes in testcrosses; and three of these, L and L^4, L^2 and L^3, and L^2 and L^8, produced a total of nine possible recombinant progeny, and again all had the S_1S_2 phenotype. Since this behavior corresponds to that predicted by the allelic interaction hypothesis, we proposed that these genes are functionally allelic rather than closely linked.

An attempt to confirm this hypothesis by showing that at least one of the phenotypically S_1S_2 progeny possesses the parental L genes in *cis* arrangement has not yet succeeded. Nevertheless, we did obtain a strikingly positive result in that one of the S_1S_2 recombinants from the $L^2 + / + L^{10}$ heterozygote produced rare resistant plants with L^{10}, and not L^2, specificity among otherwise fully susceptible progeny. Clearly, Flor's suggestion that the S_1S_2 phenotype could represent a chromosome deletion does not apply to this particular recombinant. Instead, it was inferred that the recombinant possesses suppressed L^{10} specificity (*su* L^{10}) and further tests (*9*) have shown that the suppressor must be located within the L^{10} gene and that reversion to L^{10} occurs by recombination, rather than by mutation.

The critical question of whether the suppression of L^{10} is due to the presence of L^2, as required by the allelic interaction hypothesis, remains unanswered. Various models based on reciprocal and nonreciprocal recombination have been considered to explain the failure to recover L^2 specificity from the presumed $L^2L^{10}/++$ heterozygote. One possibility is that the supposedly homozygous recessive parent used in the testcross possesses an L specificity which is located distally to L^{10} and remains "silent" with all of the rust strains used. On this hypothesis the genotype of the recombinant would be $L^2L^{10}+/++(L)$ and if L^{10} progeny arose from rare reciprocal recombination, the other product would be a new *cis* arrangement, $L^2+(L)$, which would not be detected if allelic interaction occurred once again.

A test of this hypothesis, and others

not requiring suppression of L^{10} by L^2, is expected to come from a comprehensive study of reversion to L^{10} or L^2 specificity in progeny of heterozygotes of the general constitution *su* L^{10}/L^x, where L^x represents any one of the L genes. If suppression of L^{10} is due to L^2, it should be possible to recover L^2 from heterozygotes of the type $+L^2L^{10}/L^x++$, where L^x is located proximally. On the other hand, if L^{10} specificity is recovered in the progeny from *su* L^{10}/L^2 heterozygotes, this would indicate that L^{10} suppression does not depend on L^2. Such a result would imply that allelic interaction does not occur, and then it should be possible to recover from *su* L^{10}/L^x heterozygotes some $L^{10}L^x$ combinations in coupling in which both L^{10} and L^x specificities are expressed.

In considering the structure of the L and M groups it is useful to consider the possible origin of these, and other, groups of genes conferring resistance to disease. The most direct origin of such groups would be mutation occurring at different sites within a single gene, resulting in a series of alleles with qualitatively different phenotypes for rust reaction. If further tests show that L genes are functional alleles, it follows that they must have arisen in this way.

Alternatively, it is possible that the genes in some of these groups arose from unequal crossing over, similar to the origin of the α and β components of the A locus controlling anthocyanin pigmentation in maize (*20*). Beginning with a single host gene controlling disease reaction, rare unequal crossing over could have produced serial duplications of this gene; and, if the originally identical genes diverged in function, they would appear as closely linked genes with different but related functions. This would mean that genes in this type of group arose in basically the same way as those in allelic groups, namely, from mutational changes occurring in a single ancestral gene or its duplicate descendants, and therefore it would be expected that several alleles might occur at each of these duplicate loci just as at a locus where duplication has not occurred. It has been suggested that the Rp_1 genes in maize represent serial duplications but, so far, there is no evidence indicating that any of the 14 genes are allelic (*3*).

Finally, there is the possibility that, beginning with genes controlling disease reaction located at separate unlinked or loosely linked loci, there has been an evolution toward close linkage between these loci as suggested by Mode (*21*). In practice it would be difficult to distinguish between this process and that involving duplication of loci. However, if host genes occur as tandem repeats produced by unequal crossing over, these genes themselves would be expected to exhibit unequal crossing over and this could be detected if marker genes closely linked with the genes conferring disease resistance were available. It is interesting that lines homozygous for Rp_1 genes, in crosses to susceptible lines, produced a relatively high frequency (4 out of 17,749) of susceptible progeny (*3*); but since marker genes were not used, it is not known whether they arose from unequal crossing over.

In general terms, we may speculate, then, on the nature of the primary products of the different host genes within these groups. Since it is suggested that allelic and closely linked duplicate genes have the same basic origin, it follows that in both cases the primary products of these genes would represent variants of a common product, even though this product may be very different in the separate groups. However, it is difficult to visualize the structure and mode of action of such a gene product, since in the case of the Rp_1 group it would have to exist in at least 14 different active forms to produce 14 specificities.

On the other hand, if evolution of close linkage had occurred, the genes concerned would most likely have controlled different primary products initially and they would have retained their original functions on becoming closely linked. Thus the primary products of these closely linked genes would be unrelated; and, in this case, there is no a priori expectation for some of the loci to possess several alleles controlling different specificities.

Though it is clear that more information is required on the "fine structure" of the host genes conferring resistance, that which is available raises important implications in the theory and practice of specific host-parasite interactions.

Summary

Genes conferring host resistance to an obligate parasite, grouped together in complex loci provide opportunities to study their structure. By means of an appropriate operational definition of these genes, a modified *cis-trans* test was used to interpret the position effects of codominant genes mutually recombined within each of two complex loci of flax, with the use of a specially developed method of analysis among F_2 segregants. The different behavior of genes in the M and L groups may reflect a difference in their structure sufficient to raise important implications in the theory of specific host-parasite interactions.

References and Notes

1. P. R. Day, *Sci. Progr.* **56**, 357 (1968).
2. H. H. Flor, *Advan. Genet.* **8**, 267 (1956).
3. K. M. S. Saxena and A. L. Hooker, *Proc. Nat. Acad. Sci. U.S.* **61**, 1300 (1968).
4. J. G. Moseman, *Annu. Rev. Phytopathol.* **4**, 269 (1966).
5. H. H. Flor, *J. Agric. Res.* **63**, 369 (1941); *ibid.* **74**, 241 (1947); H. B. Kerr, *Proc. Linnean Soc. N.S. Wales* **85**, 273 (1960); A. L. Hooker and W. A. Russell, *Phytopathology* **52**, 122 (1962); C. W. Schaller and F. N. Briggs, *Genetics* **41**, 421 (1955).
6. H. H. Flor, *Crop Sci.* **5**, 415 (1965).
7. K. W. Shepherd, thesis, University of Adelaide (1963).
8. G. M. E. Mayo and K. W. Shepherd, in preparation.
9. K. W. Shepherd and G. M. E. Mayo, in preparation.
10. J. R. S. Fincham, *Genetic Complementation* (Benjamin, San Francisco, 1966).
11. J. R. S. Fincham and J. A. Pateman, *Nature* **179**, 741 (1957).
12. R. R. Race and R. Sanger, *Blood Groups in Man* (Blackwell, Oxford, ed. 5, 1968).
13. W. H. Stone and M. R. Irwin, *Advan. Immunol.* **3**, 315 (1963).
14. D. Lewis, *Advan. Genet.* **6**, 235 (1954).
15. R. A. Fisher, *Amer. Nat.* **64**, 385 (1930).
16. G. Pontecorvo, *Proc. Roy. Soc. London Ser. B* **144**, 171 (1955).
17. B. D. Maling and C. Yanofsky, *Proc. Nat. Acad. Sci. U.S.* **47**, 551 (1961); D. R. Helinski and C. Yanofsky, *J. Biol. Chem.* **238**, 1043 (1963).
18. D. M. Bonner, J. A. DeMoss, S. E. Mills, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 305.
19. Estimates of recombination fraction (p) between M and M^3 and a discussion of its use in subsequent mapping of the M locus are included in a later paper (*8*).
20. J. R. Laughnan, *Amer. Natur.* **89**, 91 (1955).
21. C. J. Mode, *Evolution* **12**, 158 (1958).
22. We thank Dr. Jean Mayo for advice and Heather Stock for technical assistance.