"STUDIES OF THE GENETICS OF HOST-PATHOGEN INTERACTIONS, WITH FLAX AND ITS RUST"

A Thesis presented

by

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CONTENTS

SUMMARY

A combined study has been made of the linkage relationships between genes controlling reaction to Melampsora lini, in Flor's (1954) differential varieties of Linum usitatissimum, and the inheritance of pathogenicity in two strains of M. lini collected in New Zealand.

Test cross progeny were produced from four flax hybrids heterozygous for one or other of the pairs of genes L^2 and L^6, M and M^3, N and N^1, and P and P^3, and the progeny were screened for recombinants to obtain more critical data on Flor's postulate of allelism between these genes.

No recombinants were detected among 439 progeny in the P family. Two double-susceptible recombinants occurred in each of the L and N families, among 1,968 and 1,152 progeny, respectively. Two double-immune recombinants occurred among 2,300 progeny in the M family.

It is concluded that the genes in each pair, if not hetero-alleles of a single locus, must occur at separate loci which are very closely linked.

Test cross progeny were produced from three flax hybrids to measure the amount of recombination occurring between the N and P loci. Values ranging from $9.76 \pm 2.38\%$ to $21.53 \pm 1.84\%$ were obtained.

Some aberrant segregation ratios for rust reaction occurred in these studies and an attempt was made to find their cause. These aberrations are apparently restricted to the segregation of genes at the N or P locus and occur among progeny from relatively few combinations of flax varieties. Furthermore, they occur among the female gametes, but not the male gametes, of hybrids. However, it was not possible to find a cytological basis for this behaviour.

Inheritance studies show that the aberrant ratios are not caused by properties of alleles at the N locus. It is suggested that they are controlled by two or more genetic factors which are linked with both the N and P loci.

Tests were made to find if the aberrant ratios for rust reaction are related to aberrant segregation ratios for flower colour, also known to occur in flax. Although both aberrations are restricted to the female gametes of hybrids, it is shown that they are caused by separate events.

In the study of pathogenicity, thirteen first generation progeny were produced from the two parental strains of M. lini, and four of these progeny were used in crosses to produce a total of 143 test cross progeny. Segregation for pathogenicity occurred on a total of nine single host varieties or variety pairs. It is concluded that a single gene controls the pathogenicity of the parental strain with

a narrow range of virulence, on each of the host varieties Bombay, Cass, Dakota, Kenya, Stewart and Victory A, and a single gene or closely linked genes, on each of the variety pairs Abyssinian and Leona, Wilden and Birio, and Bowman and Clay. Each of these host varieties possesses a single gene controlling reaction to this parental rust strain. Therefore, the combined results are consistent with Flor's gene-for-gene hypothesis.

There is no indication of linkage between any of the single genes or presumed closely linked gene pairs which segregated among rust progeny.

The significance of the results from the present study, and those from Flor's studies, for the nature of the genetical control of host-pathogen interactions with flax and its rust, is discussed.

DECLARATION

I hereby declare that this dissertation comprises my own work, except where specifically stated to the contrary, and that it is not substantially the same as any dissertation which has already been submitted to any other University.

K. W. Shepherd.

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PREFACE

Following Biffen's (1907) demonstration that the resistance of wheat to yellow rust is controlled by Mendelian genes, numerous studies have been made on the genetical control of host plant resistance to a wide variety of pathogens. In many of these examples the resistance of the host plant is determined by major genes (see review: Stevenson and Jones, 1953).

Although the existence of physiological specialization (Stakman and Piemeisel, 1917) in the pathogens infecting these host plants was widely recognized, relatively few studies have been made on the genetical control of pathogenicity* on host varieties (see review: Johnson, 1960). However, Flor (1942) has emphasized that a complete study of the genetic basis of the phenotype resulting from pathogen infection of host plants, involves a parallel investigation of the genetics of reaction in both the host and the pathogen.

The most comprehensive combined study of the genetics of resistance in the host and pathogenicity in the pathogen has been made with flax (Linum usitatissimum L.) and its

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* This term is used in a general sense to include both the virulent and the avirulent phenotype.

rust (Melampsora lini (Ehrenb.) Lév.), due largely to the work of Flor (see review: Flor, 1956a). Flor's extensive studies have not only contributed much to the understanding of the genetics of this host-pathogen system, but have served as a model for studies with other combinations of hosts and pathogens.

In this work, Flor identified many different genes controlling rust reaction in a world collection of flax varieties and he showed that each of these genes interacted with a specific gene controlling pathogenicity in the rust. However, his studies did not provide critical data on the linkage relationships between the genes in the host or those in the pathogen. The present study was made, primarily, to obtain these data in both flax and its rust.

These investigations were not independent, since some of the same flax and rust materials were used in both studies, but, following Flor (1946, 1947), the results from host and pathogen investigations have been reported separately (Sections I and II, respectively). However, the significance of the combined results from these separate studies, for the genetics of host-pathogen interaction with flax and its rust, is discussed in Section III.

I. INVESTIGATION OF HOST GENE SEGREGATIONS

1. INTRODUCTION

Henry (1930) was the first to show that major genes control the immunity of flax plants to rust and he identified four different genes conferring immunity in three host varieties. Subsequent workers investigated a much wider range of host material and identified at least twenty-one additional genes conferring either resistance or immunity to rust (Myers, 1937; Flor, 1941, 1947, 1951; Kerr, 1954).

It has been reported that immunity to rust is dominant to susceptibility (Henry, 1930), high resistance is dominant to low resistance (Myers, 1937; Flor, 1947), but resistance may be incompletely dominant over susceptibility (Flor, 1941). Furthermore, genes controlling immunity or a high degree of resistance were found to be epistatic to genes controlling low resistance located at a different locus (Myers, 1937).

A striking feature of this literature is that most of the genes in flax conferring resistance (or immunity) to rust, have been reported to occur as multiple alleles at a restricted number of loci. For example, Myers (1937) studied the inheritance of reaction to two stocks of rust, in thirty-seven crosses involving seventeen varieties of flax, and accounted for his results by assuming that the six different genes identified occur in two allelic series,

named L and M.

Also, Flor (1941) determined the rust reaction of F_2 and F_3 progeny from crosses between the flax varieties Buda and J.W.S. and concluded that one of the two genes conferring resistance in Buda (L^1) is allelic with the gene conferring immunity in J.W.S., since no recombinants were detected among these progeny. Later, Flor (1947) studied the inheritance of reaction to rust in a wide range of host material and, using the selective pathogenicity reactions of several rust stocks, identified nineteen different genes among the sixteen differential host varieties, then in use. He reported that sixteen of these genes occur in three series, named L, M and N; genes in each of the L and M series were assumed to be allelic, whereas genes in the N series were assumed to be linked, since some of the latter genes could be recombined.

Subsequently, Kerr (1954) made a more detailed study of the linkage relationship between the genes in the N series and he concluded that these genes occur as multiple alleles at two linked loci, which he named the 2-N and 1-N loci. Later, Flor (1955) renamed these loci N and P, respectively.

More recently, Flor (1956a) reported that at least twenty-five different genes controlling reaction to rust have been identified in flax and he postulated that these are located at five loci described as the K, L, M, N and P

loci with one, eleven, six, three and four alleles, respectively.

In all of these studies, host genes controlling rust reaction were assumed to be allelic if they failed to show recombination when combined in a heterozygote and analysed in F_2 (Flor, 1947, 1951; Kerr, 1954), F_3 (Myers, 1937; Flor, 1941) or test cross progeny (Kerr, 1954). However, as emphasized by Mayo (1956), the failure to detect recombinants in a segregating family cannot be taken as evidence for allelism exclusively, but it is also consistent with a certain amount of recombination between the genes under test, the upper limit of which depends on the type, and the number, of progeny tested.

Flor's (1941) most critical evidence for allelism was obtained from an analysis of the 584 F_3 families derived from crosses between Buda and J.W.S. No recombination was detected between genes L^1 (from Buda) and L^2 (from J.W.S.) in this study and these data exclude, with 95% certainty, recombination percentages greater than 0.3% between genes L^1 and L^2 (Mayo, 1956). However, Mayo (1955, 1956) showed that the remainder of Flor's evidence for allelism, obtained from F_2 families, and that of Myers, obtained from a small number of F_3 families, were consistent with relatively loose linkage between the genes controlling rust reaction. Mayo (1955) suggested that more critical data on this hypothesis of allelism

could be obtained from an analysis of test cross progeny, because, as shown by Mather (1936), these progeny are much more efficient than F_2 progeny, for detecting rare recombinants from repulsion phase heterozygotes.

Kerr (1954) analysed some test cross progeny in his study, but he did not observe recombination between host genes postulated by Flor to be allelic. However, he tested relatively few progeny in each test cross family and the data from the family providing the most critical test for recombination between supposedly allelic genes, excluded only recombination percentages greater than 6.5%, with 95% certainty.

It is important to decide whether genes controlling rust reaction are either allelic or closely linked, because these alternatives present different implications for the mode of action and origin of these genes. Consequently, one aspect of the present study was concerned with obtaining more critical data on Flor's postulate of allelism at the L, M, N and P loci. In this, a large number of test cross progeny were produced from appropriate parents (Table 3) and these progeny were screened for recombinants.

Kerr (1960) reported that Flor's (1955) N and P loci

were linked with 9.6%* recombination between them. He observed recombination between genes at the N and P loci in several different families of test cross progeny and in some families it was possible to estimate p/2 directly, and in the others, p/4 only, where p represents the recombination percentage. However, he used an inefficient method for obtaining the above combined estimate of p from these families. The more efficient maximum likelihood method for combined estimation (Mather, 1935) was applied to his data, and this gave a recombination value of 10.1%, with a large standard error of 2.2%. Therefore, appropriate test cross progeny (Table 3) were also produced in the present study, to obtain a more reliable estimate of the degree of recombination between the N and P loci.

Finally, during the course of the present studies, designed to obtain these more critical data on postulated allelism between genes at the L, M, N and P loci and the degree of recombination between the N and P loci, aberrant segregation ratios were observed among some families of F_2 and test cross progeny (Tables 5, 6 and 8). An attempt was made to find the nature of the process causing these aberrations and the details of this are given in Section I.5.v.

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* Earlier, Kerr (1954) had reported from these same data that approximately 26% recombination occurred between the N and P loci. However, there was an error in this calculation, which was corrected subsequently.

2. THE CONCEPT OF ALLELISM

Before describing the experiments designed to test for close linkage between genes conferring rust resistance in flax, it is necessary to decide on an operational definition of the gene, and hence allelism, appropriate for the present study. The concept of allelism has been greatly modified in the last twenty years from studies with organisms suitable for a detailed analysis of gene structure and function. There are numerous review articles summarising this current information (for example, Carlson, 1959; Demerec and Hartman, 1959; Beadle, 1960; Yanofsky and St. Lawrence, 1960) and consequently the present discussion will be restricted to a brief account of the more relevant findings from these studies.

Beginning with the work of Oliver (1940) and Lewis (1945) with lozenge and Star-asteroid mutants, respectively, in Drosophila melanogaster, and followed by numerous other investigators with maize, Drosophila, silkworm, Neurospora, Aspergillus, yeast, Escherichia coli, Salmonella and bacteriophage ('phage), it has been found that many mutant factors of independent origin, although behaving functionally as alleles, could be recombined with a low frequency (see reviews by Carlson, 1959; Demerec and Hartman, 1959). The method of recombination varied in these examples and orthodox

crossing over (for example Green, 1959), unequal crossing over (for example Judd, 1961), and gene conversion (for example Mitchell, 1957) have been implicated in different examples.

The occurrence of rare recombinants was not consistent with the classical concept of the gene, which postulated that each gene was at the same time the unit of mutation, recombination and function. Consequently, new definitions had to be introduced.

The phenomenon of position pseudo-allelism* (Lewis, 1951) or the "Lewis effect" (Pontecorvo, 1955), where the trans or repulsion configuration of two mutant factors produced a mutant phenotype, whereas the cis or coupling arrangement gave a normal, or nearly normal phenotype, was widely encountered in these studies. This phenomenon now provides the basis for an operational definition of the gene as a unit of function. This has been

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* The original definition of this phenomenon was appropriate for diploid organisms only, where the mutant factors occur in the same nucleus. However, special techniques have been used to allow a similar type of comparison to be made in haploid organisms; namely, heterokaryon formation in Neurospora, abortive transduction in bacteria, and mixed infection in 'phage.

specifically elaborated by Benzer (1955, 1957) from his elegant studies of the rII region in 'phage T4. The unit of function was defined as that segment of chromosome within which defective genetic elements produce a defective phenotype in the trans configuration, but a non-defective phenotype in cis arrangement, and this unit was termed the cistron. However, the general applicability of this test was reduced when it was found that some mutant factors which affected the production of the same enzyme, and consequently had the same primary function, exhibited complementation in trans arrangement, giving an apparently normal phenotype (Fincham and Pateman, 1957).

Besides the above definition of the gene as a unit of function, Benzer (1955) also gave separate definitions to the genetic unit of recombination (the recon) and the unit of mutation (the muton).

The most significant features of Benzer's (1955, 1957) studies were that a single cistron in 'phage T4 contained numerous mutant sites (at least thirty-eight and more recently, many more) and, furthermore, it was possible to arrange these sites in a strictly linear array.

These findings, together with the postulated structure of desoxyribose nucleic acid (DNA) (Watson and Crick, 1953) and protein (Sanger and Smith, 1957), have led to a theory of gene action at the level of the chemical structure of

DNA, and the presumed primary product of the gene. This molecular theory of gene action (Crick, 1958) states that specific base sequences in DNA are associated with the selection of a specific amino acid for incorporation into a polypeptide chain and, furthermore, that the order of these base sequences determines the order of amino acids in the polypeptide chain. This model allows the gene, as a functional unit, to be precisely defined as that segment of the genetic material which carries the information necessary to completely specify the amino acid sequence of a particular protein or polypeptide chain.

We may conclude from the above, that an appropriate definition of the gene is the unit of function (cistron) and this unit may be operationally defined by means of the cis-trans test, providing that inter-allelic complementation (Fincham and Pateman, 1957) can be recognized if it occurs. The term locus may be used to describe the location of the functional unit (cistron) on the genetic map, and the term mutant site (muton) to describe the location of altered genetic units within the locus. The term allele may be used, in a general sense, to describe any one of the many forms a cistron may take by virtue of different alterations within the cistron. More specifically, we may recognize hetero-alleles (Roman,

1956) as cistrons altered at different mutant sites, and homo-alleles* as cistrons possessing different alterations at the same mutant site. These latter could be termed 'true' alleles in the classical sense, since they would not recombine and would behave as alternatives in inheritance. These homo-alleles were apparently found by Benzer (1955) in the rII region of °phage T4, and by Maling and Yanofsky (1961) in the A cistron of E. coli.

On the basis of these definitions, the demonstration that genetic factors controlling similar phenotypes are closely linked, rather than allelic, does not depend on the occurrence of recombination alone, but depends on finding whether the factors control different, but possibly related, primary functions; that is, whether they belong to separate cistrons. However, the observed recombination frequency can be used to indicate the likelihood of functional non-identity if it is above a rather indefinite lower limit. For example, Pontecorvo (1958) cites the level of recombination (0.5%) found by Koske and Maynard (1954) between two ar alleles in Drosophila subobscura, as the maximum

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* This use of the term homo-allele is different from that originally proposed by Roman (1956) in that he used this term to describe mutants which possessed the same alteration at a given site.

amount so far reported between any two factors apparently belonging to the same cistron. However, the problem remains of deciding on adequate criteria for determining the functional identity, or otherwise, of two factors which recombine with a frequency of less than, say, 1%.

The cis-trans comparison has been widely used as a test of functional identity in those studies where it was possible to recognize the 'wild' or normal phenotype, if it was produced by complementation in the trans arrangement. However, the occurrence of inter-allelic (or intra-locus) rather than inter-locus complementation could lead to incorrect conclusions in these tests. This group of mutants includes most of those that have been used in extensive studies of allelism, namely many of the mutant strains of Drosophila, Aspergillus, Neurospora, bacteria and 'phage, possessing a deleterious and usually recessive phenotype compared with 'wild' type.

However, there is a large group of examples, including genes conferring rust resistance in flax, in which it is not possible to apply the conventional cis-trans test for functional allelism. These examples are characterized by the absence of a dominant 'wild' type and if a standard form is available for comparison, it is always the "universal recessive" (Fisher, 1930). The "universal recessive" usually produces no recognizable distinctive

phenotype and may be considered an amorph in Muller's (1932) terminology. In the present study, the universally susceptible flax plant corresponds to the "universal recessive" form. The other forms within these groups produce distinctive phenotypes and they are all dominant to the "universal recessive", but show no dominance *inter se*. That is, the individual phenotypes of any two of these forms are expressed in *trans* combination. Furthermore, inter-locus complementation could not be detected even if it occurred, since it would be expected to produce the phenotype of the "universal recessive", which is not distinctive. Numerous examples of this situation have been described (Komai, 1950; Sheppard, 1953; Huxley, 1955) and they are often associated with polymorphic populations in nature, for example, colour pattern polymorphism in insects and higher animals, antigen differences including blood groups of birds and mammals, and some self-sterility alleles in higher plants.

Although the conventional *cis-trans* test for functional identity of the genes controlling the phenotype of the different forms within a group cannot be applied with these examples, the following modified form of it is proposed as a substitute. This is based on the Crick (1958) hypothesis of gene action and is similar to a proposal made by

Pontecorvo (1955, 1958).

The *trans* configuration of two of the different genetic factors, from members within the above group, produces a phenotype which represents a combination of the individual phenotypes of the two genes expressed when homozygous. This phenomenon is consistent with the existence of either closely linked genes or allelic genes, since with the former we infer that the phenotype of the heterozygote results from the production of two different gene products, whereas in the latter we infer that the phenotype results from the production of two different forms of the same gene product. However, if the *cis* arrangement of these genes produces an interaction, such that the *cis* phenotype is not the same as that of the *trans* arrangement, we may infer from Crick's (1958) hypothesis of gene action that the genes control the same function and are consequently allelic. More specifically, we would infer that the *cis* arrangement produces a doubly defective single gene product and an interaction occurs between the two altered parts of the same gene product, giving a phenotype different from that of the *trans* arrangement. In practice we would not expect to identify this *cis* arrangement because of its unknown phenotype, but the occurrence of such an interaction would be indicated if only one of the two reciprocal recombinant products was

recovered among test cross progeny (see p. 15). Conversely, if the *cis* arrangement produces the same phenotype as the *trans* arrangement this would be consistent with the assumption that the particular genes control different functions and are consequently non-allelic. However, this latter comparison cannot be considered exclusive evidence for non-allelism, because it is possible that a single gene product may possess some regions of independent action, allowing the doubly altered product to exhibit the sum of the properties of the singly altered products (see Discussion p. 96).

A comparison between *cis* and *trans* arrangements of genes conferring rust resistance in flax will be used as an operational test for functional non-identity in the present study, with the reservation that any conclusions drawn from it must remain tentative, until further information is obtained on the possible relationships between singly and doubly altered gene products.

3. THE TEST FOR CLOSE LINKAGE

The general method used to search for recombination between genes in flax conferring resistance to rust is illustrated by the hypothetical example set out in Table 1. It is assumed that each parental flax variety possesses a different single dominant gene controlling resistance (A^1 or A^2). The test cross involves crossing these parental varieties, and subsequently crossing the F_1 plants with a flax variety which is homozygous for the recessive alleles of these genes, the so-called "universally susceptible" variety.

The expectations in the test cross progeny may be set out assuming these genes to be either closely linked or allelic.

If it is assumed that genes A^1 and A^2 occur at separate loci (Table 1) then they will have different primary functions (p. 9) and, consequently, it will be possible to detect the reciprocal products of crossing over when they occur. The joint segregation column of Table 1 shows that these reciprocal products may be detected by determining the rust reaction of the test cross progeny with the two rust strains α^1 and α^2. These rust strains are characterized by their reactions with the parental host varieties. Rust strain α^1 interacts specifically with host plants possessing gene A^1, giving a

TABLE 1

MODEL FOR ANALYSIS OF TEST CROSS PROGENY*

	PARENTAL CROSS PARENT 1 X PARENT 2		TEST CROSS F_1 X SUSCEPT.≠		TEST CROSS PROGENY						
					JOINT SEGREGATION				INDIVIDUAL SEGREGATION EXPECTED RATIO R : S		
Genotype	$\frac{A^1a^2}{A^1a^2}$	$\frac{a^1A^2}{a^1A^2}$	$\frac{A^1a^2}{a^1A^2}$	$\frac{a^1a^2}{a^1a^2}$	$\frac{A^1a^2}{a^1a^2}$	$\frac{a^1A^2}{a^1a^2}$	$\frac{A^1A^2}{a^1a^2}$	$\frac{a^1a^2}{a^1a^2}$			
Phenotype	A^1a^2	a^1A^2	A^1A^2	a^1a^2	A^1a^2	a^1A^2	A^1A^2	a^1a^2			
Reaction$^\lambda$ of indicated plants with:											
Rust strain											
α	R	R	R	S	R	R	R	S	$\frac{200-p}{2}$	:	$\frac{p}{2}$
α^1	R	S	R	S	R	S	R	S	1	:	1
α^2	S	R	R	S	S	R	R	S	1	:	1
Expected ratio‖					$\frac{100-p}{2}$	$\frac{100-p}{2}$	$\frac{p}{2}$	$\frac{p}{2}$			

* Assuming host genes A^1 and A^2 are closely linked.

λ R = resistant S = susceptible (see Table 4).

≠ 'Universally susceptible' flax variety.

‖ p = recombination percentage.

resistant host reaction, but is able to grow freely in host plants possessing gene A^2 only, giving a susceptible reaction. Thus, gene A^1 is assumed to be present in all progeny plants which are resistant to strain α^1, and absent in those plants susceptible to this rust. Similarly, rust strain α^2 interacts specifically with plants possessing gene A^2, giving a resistant host reaction and, consequently, may be used as a specific indicator for the presence of gene A^2. The individual segregation column of Table 1 shows that the above testing procedure also allows the segregation ratio of each gene to be determined.

However, if rust strains of type α^1 and α^2 are not available, the double recessive recombinant may be detected with rust strain α. This strain is non-specific and host plants with genes A^1, or A^2, or both, will be resistant to this rust (see Table 1). Unlike the previous test with strains α^1 and α^2, this test does not give information on the individual segregation ratios. Therefore, in these tests it is necessary to obtain independent evidence on whether the parental varieties possess one or more genes conferring resistance to rust strains of the α type.

The number of operative genes present in the parental varieties can be best determined by crossing each of these varieties with the "universally susceptible" variety and producing F_2 progeny. The expected F_2 ratio of plants

resistant and susceptible to strain α will be 3:1, 15:1 or 63:1, depending on whether 1, 2 or 3 genes conferring resistance are segregating. This procedure is more efficient than backcrossing, since the corresponding ratio in backcrosses will be 1:1, 3:1 or 15:1, and more plants are required to distinguish between these latter, at a given level of certainty, than the F_2 ratios. Furthermore, flax is naturally self-fertilizing and a large number of F_2 progeny can be produced relatively easily.

Alternatively, if it is assumed that genes A^1 and A^2 are allelic instead of closely linked, and represent hetero-alleles, it would be expected in some cases at least that the double dominant recombinant would not be detected (p. 75). However, the double recessive recombinants (that is, plants susceptible to rust strain α , or strains α^1 and α^2, of Table 1) would be expected to occur. On the other hand, if genes A^1 and A^2 represent mutations at the same site within a locus (homo-alleles), recombinant plants would not be expected to occur, no matter how many test cross progeny were tested.

4. MATERIALS AND METHODS

i. Host Parental Stocks

The parent material of L. usitatissimum used in the present study included the eighteen differential varieties used by Flor (1954). According to Flor, each of these varieties, except Bison, possesses a single gene conferring resistance to some North and South American stocks of rust. The variety Hoshangabad was used as the "universally susceptible" parent in test crosses. A list of these varieties, together with their postulated genotype with respect to genes conferring rust resistance, is given in Table 2.

ii. Host Progeny Material

Late in 1957 a large number of crosses were made between the parental flax varieties to produce a wide range of F_1 progeny material. Three groups of F_1 seeds were produced, namely:

a. Progeny from crosses between the differential varieties and Hoshangabad.

b. Progeny from crosses between differential varieties postulated to possess genes conferring resistance situated at the same locus.

c. Progeny from crosses between selected pairs of varieties possessing genes conferring resistance at different

TABLE 2

REACTIONS OF DIFFERENTIAL VARIETIES AND HOSHANGABAD TO FOUR STOCKS OF *MELAMPSORA LINI*

VARIETY OF *LINUM USITATISSIMUM*	C.I.≠ NUMBER	FLOR'S (1955) GENOTYPE	REACTION* TO *M. LINI* STOCK			
			P-1	P-2	3	4
Abyssinian	701	nP^2/nP^2	R	S	S	S
Akmolinsk	515	nP^1/nP^1	S	S	S	S
Birio	1085	L^6L^6	I	S	S	S
Bison	389	L^9L^9	S	S	S	S
Bombay	42	Np/Np	I	S	I	I
Bowman	1184	≠	R	S	S	R
Cass	1182	M^3M^3	I	S	S	S
Clay	1188	KK	R	S	S	R
Dakota	1071	MM	I	S	S	I
Kenya	709	L^4L^4	IR	SR	SR	SR
Koto	842	nP/nP	I	I	I	I
Leona	836	nP^3/nP^3	I	SR	SR	SR
Ottawa 770B	355	LL	I	I	I	S
Polk	1191	N^1p/N^1p	IR	IR	SR	IR
Stewart	1072	L^2L^2	I	S	S	S
Victory A	1170	M^4M^4	R	SR	SR	SR
Wilden	1193	L^5L^5	IR	S	S	S
Williston Brown	803	M^1M^1	S	S	S	S
Hoshangabad Y4633	Ø	∤	S	S	S	S

* I = immune IR = immune to resistant R = resistant SR = semi-resistant S = susceptible (see Table 4).

≠ Refers to accession number of Division of Cereal Crops and Diseases, U.S. Department of Agriculture.

≠ Genotype not reported.
Ø Hoshangabad obtained from Waite Agricultural Research Institute, accession number 89.
∤ Possesses no known genes controlling reaction to rust.

loci. This group includes crosses between pairs of varieties, one of which possessed a gene conferring resistance at the N locus and the other a corresponding gene at the P locus.

The F_1 seeds in the first group above were grown to produce F_2 seeds. The rust reaction of these F_2 progeny was determined to find the number of genes conferring rust resistance present in each differential variety.

A selected range of F_1 hybrids from the second group of parental crosses was used to produce test cross progeny. The rust reaction of these progeny was determined, in order to search for recombination between the genes postulated by Flor (1956)[a] to exist as multiple alleles.

The particular parental variety combinations used in test crosses (Table 3) were selected on the basis of their reactions with the rust stocks available at that time, and also on their expected reaction with progeny to be produced in the concurrent pathogen breeding programme. As far as possible, parental combinations were chosen where it was expected that rust stocks of the type α^1 and α^2 (p. 15) would be produced among these progeny, hence permitting a more efficient analysis of host test cross progeny. In any case, a rust stock of the α type was available, initially, for testing each of these families

TABLE 3

SUMMARY OF TEST CROSSES MADE WITH DIFFERENTIAL FLAX VARIETIES AND HOSHANGABAD, AND RUST STOCKS AVAILABLE FOR TESTING PROGENY

LOCUS	TEST CROSS* (1) (2) (3)	FLOR'S (1955) GENOTYPE OF PARENTS (1)	(2)	(3)	TESTER RUST STOCKS AVAILABLE ≠
L	(Stewart-Birio) x Hosh≠	L^2L^2	L^6L^6	ll	α, α^1, α^2
M	(Dakota-Cass) x Hosh	MM	M^3M^3	mm	α, α^1, α^2
N	(Polk-Bombay) x Hosh	N^1N^1	NN	nn	α, α^1, α^2
P	(Koto-Leona) x Hosh	PP	P^3P^3	pp	α, α^1
N and P	(Leona-Bombay) x Hosh	nP^3/nP^3	Np/Np	np/np	α, α^1, α^2
	Hosh x (Leona-Bombay)	np/np	nP^3/nP^3	Np/Np	α, α^1, α^2
N and P	(Koto-Polk) x Hosh	nP/nP	N^5p/N^5p	np/np	α, α^1

* Female parent in test cross listed first. The separation of parental varieties with a hyphen indicates reciprocal parental crosses were made.

≠ Correspond to types α, α^1 and α^2 of Table 1.

≠ Hosh = Hoshangabad Y4633.

of test cross progeny.

Test cross progeny were produced by crossing the F_1 plants with Hoshangabad. This variety was chosen not only as the "universally susceptible" parent in these crosses, but also because its flower colour could be used as a genetic marker. Hoshangabad has white flowers with a tinge of blue, whereas all of the parents used in these crosses possessed blue flowers. Since blue flower colour is dominant to white (Tammes, 1928), all test cross progeny were expected to be blue-flowered and also their progeny were expected to show segregation for flower colour.

Initially, the F_1 plants were used only as the female parent in the test crosses with Hoshangabad. If Hoshangabad had been used as the female parent, self fertilization or fertilization with stray pollen from another susceptible plant would have produced susceptible progeny plants and these could have been incorrectly classified as recombinant plants. If these susceptible plants survived to maturity, their flower colour would indicate whether they arose from self fertilization or not, but it would not be possible to distinguish between fertilization with stray pollen from a blue-flowered susceptible plant and with pollen representing a recombinant gamete from the F_1 hybrid.

Later, it was found necessary to make reciprocal test crosses with Polk x Bombay hybrids as part of a study of aberrant segregation ratios (Section I. 5. v.).

The third group of parental crosses included material which was used in test crosses to obtain estimates of the degree of recombination between the N and P loci. Leona-Bombay* and Polk-Koto hybrids were crossed with Hoshangabad to produce test cross progeny. Reciprocal test crosses were made with the Leona-Bombay F_1 plants so that the degree of recombination occurring during microsporogenesis and megasporogenesis could be compared. In this case, occasional undetected selfing or pollen contamination of Hoshangabad, used as the female parent, would have little effect on the recombination value measured, since this was expected to be of the order of 10% (Kerr, 1960)

iii. Pathogen Stocks

All collections of M. lini used in this study have been referred to non-specifically as stocks. However,

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* The separation of two host varieties with a hyphen indicates that reciprocal crosses were made between these varieties. That is, Leona-Bombay indicates plants produced from Leona x Bombay and Bombay x Leona crosses.

stocks giving different pathogenicity reactions on the differential host varieties may be classified as separate strains.

Five different collections of rust were utilized in the present study and two of these were selected as parents in a breeding programme to study the inheritance of pathogenicity (Section II). These five stocks together with many of the progeny stocks, were used to detect the segregation of host genes conferring resistance to rust.

Since the majority of the rust stocks maintained were products of the breeding programme, a system of nomenclature was adopted which related progeny rust stocks to their parents.

The five rust stocks originally collected in the field were numbered from 1 to 5, and the two used in the breeding programme were given the prefix 'P' to indicate their use as parents. Both of these stocks came from New Zealand and had been classified as New Zealand strains 1 and 10 (Cruickshank, 1956). These have been referred to as stocks P-1 and P-2, respectively, in this study. The other three stocks were obtained specifically for testing host gene segregations, and included New Zealand strain 5 (Cruickshank, 1956), a rust stock collected in the field at Colac, Victoria, and another stock collected

at Boyup Brook, West Australia. These have been referred to as stocks 3, 4 and 5, respectively.

All other rust stocks were derived from parental crosses between P-1 and P-2, test crosses between some of the first generation progeny and P-2, and self fertilization of P-2.

Thirteen first generation progeny were obtained from crosses between P-1 and P-2 and each of these was given the prefix F (indicating first generation rather than F_1), followed by a different lower case letter. That is, these progeny were named F-a through to F-m.

Test cross progeny were given the prefix T followed by the lower case letter of the first generation stock used in test crosses with P-2, and Arabic numerals for different progeny. For example, Ta-19 and Ti-152 represent two of these test cross progeny; the former was derived from a test cross between F-a and P-2, and the latter from a test cross between F-i and P-2.

Ten progeny were produced from selfing stock P-2 and these were designated S(P-2)-1 through to S(P-2)-10.

With this system of nomenclature the designation of a rust stock indicated its origin and, consequently, some of its pathogenicity reactions. The conventional method of assigning Arabic numerals to different rust stocks,

regardless of their origin (Flor, 1946), was considered less useful for the present study than the above system.

The reactions of stocks P-1, P-2, 3 and 4 on the differential host varieties are given in Table 2. Stock 5 was not obtained until late in the present study and was not tested on these varieties. The reactions of the progeny rust stocks on the differential varieties are given in the Appendix.

iv. Host Culture

All host plants were grown in a potting compost (made up of approximately four parts of loam, two of washed sand and one of sheep manure leaf mould), in unglazed earthenware pots.

Sets of the eighteen differential host varieties were planted for testing of rust stocks and these plantings were made in pots of six-inch internal diameter. The soil surface was divided into eighteen equal areas in a definite pattern, and three seeds of each differential variety were planted in the separate areas so that a complete set of differentials was included in a single pot. This procedure allowed an economy of space for growing the differentials and this was particularly useful when space was limited, for example in the plant growth cabinet.

Test cross progeny seeds were planted in four-inch pots with ten seeds per pot. These seeds were planted in a definite pattern so that each plant could be identified by its position and related to a particular test cross, if necessary.

Twenty seeds of F_2 progeny were planted in each six-inch pot initially, but later when space was limited, forty seeds were planted.

All plants, except those required for culturing rust stocks, were grown in the glasshouse, but as many as space permitted were later removed to a plant growth cabinet for rust reaction tests. Plants were first inoculated with rust when they were two to four inches high, and possessed a rosette of unfolded leaves around the terminal bud. In order to reduce the risk of contamination infected plants were either cut back to remove infected parts (Flor, 1947) or immediately destroyed, after scoring, depending on whether they were required for further tests. Removal of the apical meristem stimulated the development of lateral buds at the base of the plant and these provided new shoots for further rust inoculations. However, many plants died after cutting back during hot weather.

v. Pathogen Culture

M. lini is an obligate parasite and, consequently, all stocks of this rust have to be grown on living host plant tissue. Furthermore, all rust stocks used were indistinguishable morphologically. Consequently, special precautions were taken to reduce the risk of contamination of rust stocks during their growth on plants.

a. Wherever possible, a rust stock was inoculated on a host variety which was immune to other rust stocks being cultured at the same time. For example, stock P-2 was initially grown on host varieties Dakota and Bombay but later, when stock 3 and test cross stocks were obtained, this procedure was less efficient since some of these stocks could also attack Dakota and Bombay. Stocks 4 and 5 were grown on Ottawa 770B, since no other rust attacked this variety.

However, those host varieties susceptible to stock P-1 were susceptible to all other rusts and therefore the above procedure could not be used. This stock was cultured on Hoshangabad, because this variety supported dense uredospore growth. Although stocks F-a and F-i attacked Abyssinian and Leona in addition to those varieties attacked by P-1, these progeny were also grown on Hoshangabad, since the former varieties gave a low yield of uredospores.

b. Stocks of rust, including parental and first generation strains, grown to produce teleutospores for use in the breeding programme were cultured in separate glasshouses.

c. The plants to be used for culturing rust stocks were grown in an area which was free of rust-infected plants, prior to inoculation.

d. Immediately after the host plants used for growing rust stocks had been inoculated and incubated, they were completely enclosed within a wire frame covered with colourless "cellophane". These cages were removed from the plants only when uredospores were collected.

e. Uredospore collections from infected plants were made in a room isolated from the area in which the plants were grown. Care was taken not to touch the infected plants during collection.

f. After collection the uredospores were placed in small tightly stoppered glass phials and stored at temperatures of 0-4°C. The longevity of these stored spores was not great and frequently they were completely inviable after six months storage. Consequently, stocks used for repeated tests were regularly cultured on host plants. However, some important rust stocks, for example P-1, were lost because of their premature death while in storage.

g. The homogeneity of rust stocks used in tests of host progeny was checked by determining their reactions on the differential varieties.

Despite these precautions, some rust stocks became contaminated and single uredospore infections were made to re-isolate the original stock.

vi. Inoculation and Incubation

The method used to inoculate plants with uredospores was similar to that described by Flor (1935). The spores were suspended in water in a watch glass and the suspension was applied to the terminal bud and surrounding leaves of plants, with a camel's-hair brush. The inoculated plants were sprayed with water and placed in chambers, kept at high humidity and at a temperature below 24°C. After twelve to eighteen hours they were removed to the glasshouse, or plant growth cabinet, for the remainder of the incubation period.

Whenever a group of host progeny was to be tested with more than one rust stock, the stock expected to have the mildest reaction on the host plants was used first (cf. Flor, 1947).

vii. Scoring

The first sign of rust growth on host plants showed as a faint chlorosis of leaves, six to seven days after

inoculation. Orange pustules of uredospores appeared after eight days and these erupted through the leaf epidermis on the ninth or tenth day. The majority of the inoculated plants were classified prior to the stage of pustule eruption and the infected areas of the plant were removed to prevent the spread of uredospores to other plants in the glasshouse or plant growth cabinet.

The system of scoring adopted was similar to that described by Mayo (1955) and was based on the amount of growth made by the rust on host plants. This is analogous to the system used to describe the growth of auxotrophic mutants of non-parasitic, and facultatively parasitic fungi, on artificial media. Furthermore, it recognizes that a single phenotype, the amount of growth of the pathogen, describes at the same time the phenotype of the host and the pathogen. However, for descriptive purposes, following Flor (1947) and Kerr (1960), it is convenient to refer to this phenotype in terms of the degree of host resistance, when describing breeding experiments with the host. Conversely, when describing the phenotype of progeny derived from breeding experiments with the rust it is convenient to describe this in terms of degree of rust virulence.

A description of the degrees of rust growth recognised in this study, together with the corresponding classes of host resistance and pathogen virulence, are given in Table 4. These ranged from free growth (+), through restricted growth (- and +/-), to no growth (--), and examples of these reactions are shown in Figure 1. The presence of necrosis, chlorosis and leaf distortion was also noted,but the main criterion for classification was the degree of pustule development. The occurrence of necrotic flecks characteristic of no growth, interspersed with large uredospore pustules, was considered evidence for heterogeneity in the rust stock,since the mesothetic reaction characteristic of wheat rust (Stakman and Levine, 1922) apparently does not occur with flax rust (Flor, 1935).

The analysis of host gene segregations depended on determining whether a particular gene was present or absent in host progeny. In most tests,this involved distinguishing between free growth and some degree of restricted growth of the rust. However,in test cross progeny from Bombay-Polk and Polk-Koto hybrids it was possible to distinguish the three phenotypes of free growth, restricted growth and no growth (see Figures 2 & 4).

At first,it was difficult to distinguish the lower limit of the semi-resistant reaction from the susceptible

TABLE 4

INTERACTION PHENOTYPES RESULTING FROM INFECTION OF *L. USITATISSIMUM* WITH *M. LINI*

INTERACTION PHENOTYPE						
CLASS*	SYMBOL	DESCRIPTION	FROM VIEW POINT OF:			
			HOST/		PATHOGEN	
			CLASS	SYMBOL	CLASS	SYMBOL
Free growth	+	Large, often compound uredospore pustules, on stems and leaves. Older leaves also produce large pustules.	Susceptible	S	Virulent	V
	+/-	Some restriction of pustule development, especially on older leaves. Numerous pustules, but single and smaller than above. Infected leaves often show marked necrosis if left for fourteen to fifteen days after inoculation.	Semi-resistant	SR	Semi-virulent	SV
Restricted growth	-	Marked restriction of pustule development. Often many pustules on young leaves, but small and frequently associated with the boundary of large necrotic areas in the leaf. Older leaves develop few pustules and show marked necrosis.	Resistant	R		
No growth	- -	No sign of pustule development. Variable degree of leaf necrosis but usually very small flecks or necrotic spots on leaf.	Immune	I	Avirulent	A

* Adapted from Mayo (1955).

/ IR, used in text, signifies reaction class between immune and resistant.

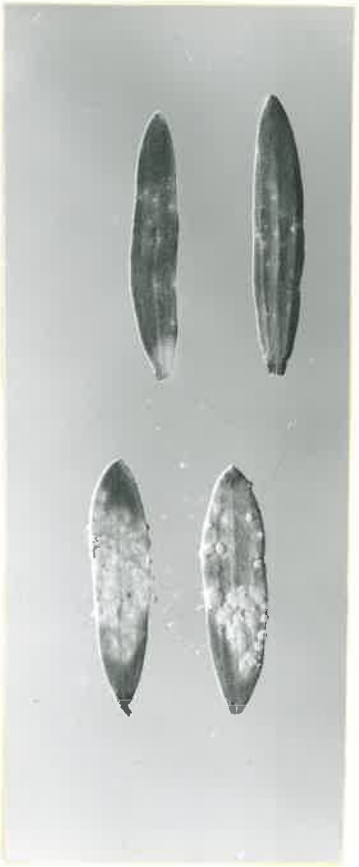
FIGURE 1.

Classes of interaction phenotypes resulting from infection of *Linum usitatissimum* with stock F-1 of *Melampsora lini*.

Left to right - Top row - Free growth on Hoshangabad (+); restricted growth on (Clay x Hoshangabad) F_1 plants (+/-)

Bottom row - Restricted growth on Victory A (-); no growth on Dakota (- -)

First leaf of each pair, lower surface; second leaf, upper surface.



reaction when these occurred as alternatives in a segregating family (p.36). However, as reported by Kerr (1960), it was found that discrimination was aided by leaving these plants for fourteen to fifteen days after inoculation, before scoring them. The leaves of semi-resistant plants then showed marked necrosis and eventual death, whereas the leaves of susceptible plants showed increased pustule development and little necrosis.

viii. Control of Environment

In some cases, the host reaction to a particular rust stock was very sensitive to the environmental conditions occurring during the test period. For example, when plants which were normally susceptible to rust were inoculated and incubated at a temperature of 32°C or higher they developed few, if any, uredospore pustules. Consequently, it was not possible to use the glasshouse for rust reaction tests during the period November to April, inclusive, when the temperature was frequently above 32°C and occasionally over 37°C.

A plant growth cabinet, built to the design of Dr. G.M.E. Mayo to provide suitable conditions for rust reactions tests, became available for use early in 1958. Light corrected high pressure mercury vapour lamps and fluorescent tubes provided the light and the light intensity at plant height was of the order of 4,500 foot candles, which supported vigorous growth of flax plants. Tempera-

tures were controlled between 21°C to 26°C during the ten hour light period and at, or below, 16°C during the dark period.

These environmental conditions were very suitable for making rust reaction tests and, furthermore, these tests could be made throughout the year. In addition, this control over the environment gave greater consistency to rust reaction classifications made at different times of the year. However, there was not sufficient space in the cabinet to accomodate all inoculated plants and a large number of tests were made in the glasshouse, when environmental conditions permitted.

5. OBSERVATIONS AND THEIR ANALYSIS

1. Rust Reaction of Host Parent Material

The reactions of the eighteen differential varieties, and Hoshangabad, with four rust stocks are given in Table 2. These tests give, at the same time, information on the pathogenicity of the rust stocks and the range of reactions of host varieties.

Each rust stock gave a different pattern of pathogenicity on these varieties and, consequently, the stocks may be classified as separate rust strains, namely P-1, P-2, 3 and 4. Another stock (5) was obtained late in this study to replace stock 4 for a specific test (p. 72), but its reaction on the differential varieties was not determined.

Stocks P-1 and P-2 differed widely in their pathogenicity range on the host varieties and for this reason they were chosen for use in the pathogen breeding programme (Section II).

Host varieties Akmolinsk, Bison, Williston Brown and Hoshangabad were fully susceptible to the four rust stocks used. However, the remaining host varieties restricted the growth of at least one of these stocks, but the degree of restriction varied, depending on the particular host variety - rust stock combination tested

(Table 2).

The host varieties restricting growth of stock P-1 were grouped into three categories depending on whether they completely restricted growth (immune reaction), gave a high degree of restriction (immune to resistant reaction) or moderate restriction (resistant reaction). Varieties Birio, Bombay, Cass, Dakota, Leona, Ottawa 770B, Stewart and Koto were placed in the first category, Kenya, Polk and Wilden in the second and Abyssinian, Clay, Bowman and Victory A in the third. The rust reactions of the varieties in the first and second categories varied little with change in environment, but the reactions of the varieties in the third group were sensitive to environmental conditions occurring during the test period. It was assumed that the gene, or genes, controlling rust reaction in the former varieties exhibited a higher degree of penetrance than those in the latter, and consequently the parents used in the host breeding programme (Table 3) were chosen from the first two groups of varieties.

ii. Genes Conferring Rust Resistance in Differentials

Although Flor (1954) reported that each of the differential varieties used in this study possess a single gene conferring resistance to North and South American stocks of rust, it is possible that more than one gene

controls the resistance of some of these varieties to other rust stocks. Therefore, tests were made to find the number of genes controlling the resistance of the differential varieties to stocks P-1 and P-2.

Each immune, or resistant, differential variety was crossed with Hoshangabad and F_2 progeny were produced. Initially, only sufficient seeds of each F_2 family were planted to distinguish between a 3:1 and a 15:1 ratio, with 96% certainty. However, after the occurrence of aberrant segregation ratios, many more F_2 progeny plants from some of these families were tested, and the combined results from all tests are given in Table 5.

Stock P-1 was lost during storage before some of these later tests were made and stock F-1, producing similar reactions to P-1 on all differential varieties except Abyssinian and Leona (see Appendix), was used in its place. Stock Tk-47 reacted similarly to P-1 on Abyssinian and Leona (see Appendix) and was used when testing additional F_2 progeny from these two host varieties.

These substitute rust stocks gave results which were homogeneous with those obtained with P-1. At least two, and often four, F_1 plants in each family were used to produce these F_2 progeny and the segregation ratios among the progeny from each F_1 plant were also homogeneous. Consequently, all data within each F_2 family were grouped

TABLE 5

REACTION TO STOCK P-1[1] OF THE DIFFERENTIAL HOST VARIETIES RESTRICTING GROWTH OF THIS STOCK, AND THE F_1 AND F_2 PROGENY FROM EACH CROSSED WITH THE SUSCEPTIBLE VARIETY HOSHANGABAD

IMMUNE OR RESISTANT PARENT	REACTION[2] OF INDICATED PLANTS					x_1^2 (3:1)	x_1^2 (15:1)
	PARENT LISTED	F_1 PROGENY	F_2 PROGENY				
			I	R or SR	S		
Abyesinian	R	SR		89	25	0.57	
Birio	I	I	47		18	0.25	
Bombay	I	I	357		116	0.06	
Bowman	R	SR		164	46	1.07	
Cass	I	I	28		9	0.09	
Clay	R	SR		72	26	0.12	
Dakota	I	I	30		8	0.32	
Kenya	IR	R		72	29	0.74	
Koto	I	I	125		42	0.002	
Leona	I	I	208		35	14.55***	27.57***
Ottawa 770B	I	I	171		67	0.56	
Polk	IR	R		181	15	31.46***	0.66
Stewart	I	I	28		6	0.98	
Victory A	R	SR		184	48	2.30	
Wilden	IR	R		113	34	0.27	

[1] Some of the F_2 progeny were tested with stocks P-1 and Tk-47, after P-1 was lost.

[2] I = immune IR = immune to resistant R = resistant SR = semi-resistant S = susceptible (see Table 4).

*** x^2 significant at 0.001 level of probability.

(Table 5).

The reactions of the F_1 and F_2 progeny with P-1 showed two general features similar to those reported by Flor (1941, 1947). Immune parents crossed with Hoshangabad gave F_1 progeny which were also immune, and F_2 progeny plants which could be classified unambiguously as either immune or susceptible. However, with near-immune or resistant parents crossed with Hoshangabad, the reaction of the F_1 plants showed a shift towards greater susceptibility and the non-susceptible class of F_2 progeny showed a wide range of reactions. These F_2 reactions varied from near-immune to semi-resistant in a given family and it was not possible to group progeny plants of similar reaction, since the variation was not discrete. At first, it was difficult to distinguish the lower limit of the semi-resistant from the fully susceptible phenotype in the F_2 progeny derived from Abyssinian, Bowman, Clay and Victory A, but later this difficulty was overcome by leaving the plants for fourteen or fifteen days before scoring them (see p. 31).

Table 5 shows that in tests with stock P-1 (or equivalent strain), with two exceptions, the F_2 segregation ratios of non-susceptible to susceptible plants agreed with the 3:1 ratio expected if a single gene was responsible for the immunity, or resistance, of the parent differential

variety. The exceptions occurred among the F_2 progeny derived from Leona and Polk. The results obtained with Polk x Hoshangabad F_2 progeny agreed with a 15:1 ratio, suggesting that Polk possessed two genes controlling rust reaction. However, subsequent data contradicted this hypothesis and showed that Polk possessed a single gene which conferred near immunity to rust (p.46).

The results from Leona x Hoshangabad F_2 progeny did not agree with either a monohybrid or a dihybrid segregation ratio. However, it was shown subsequently that Leona also possessed a single gene controlling reaction to rust (p.48).

At least thirty-four F_2 progeny from each of Polk, Ottawa 770B, and Koto crossed with Hoshangabad, were tested with stock P-2, as well as P-1. The reactions of all progeny plants to these two stocks corresponded plant by plant, indicating that the same gene, or separate closely linked genes, controlled the resistance of these differential varieties to both stocks.

iii. Tests of Close Linkage at L, M, N and P Loci

The results obtained from experiments designed to obtain more critical data on Flor's postulate of multiple allelism at the L, M and N and P loci in flax are given in Table 6.

TABLE 6

TESTS OF CLOSE LINKAGE AT L, M, N AND P LOCI – REACTION OF PARENTAL FLAX VARIETIES AND TEST CROSS PROGENY WITH SELECTED RUST STOCKS

LOCUS	PEDIGREE[1] (1) (2) (3)	NUMBER OF F_1 PLANTS USED IN TEST CROSSES	TESTER RUST STOCK	REACTION[1] OF INDICATED PLANTS TO TESTER RUST STOCKS: PARENTS (1) (2) (3)	PROGENY: JOINT SEGREGATION	PROGENY: INDIVIDUAL SEGREGATION: OBSERVED FREQUENCY	EXPECTED RATIO	x^2_1	HETEROGENEITY df	HETEROGENEITY x^2
P	(Leona-Koto) x Hosh	6	P-1	I I S	I I S	439 0	$\frac{2-2}{2}$: $\frac{2}{2}$			
			F-i	SR I S	I S S	42 53	1 : 1	1.27		ø
					Observed frequency: 42 53 0					
						I S	I : S			
L	(Birio-Stewart) x Hosh	22	Ta-1(9-1)	I S S	I S I S	987 1002	1 : 1	0.11	21	12.38
			F-k	S I S	S I I S	987 988	1 : 1	0.00		
					Observed frequency: 980 986 0 2					
						I S	I : S			
M	(Cass-Dakota) x Hosh	16	Ti-15	I S S	I S I S	1140 1178	1 : 1	0.62	15	12.22
			Ti-58	S I S	S I I S	1172 1131	1 : 1	0.73		
					Observed frequency: 1129 1169 2 0					
						I R S	I:(R+S)			
N	(Polk-Bombay) x Hosh	12	F-i	IR I S	R I I S	192 839 2	1 : 1	403.5***	11	10.23
			Tk-41	IR S S	R S R S	320 69	1 : 1	165.4***		
					Observed frequency: 320 67 0 2		R : S			

[1] I = immune IR = immune to resistant R = resistant S = susceptible (see Table 4).

[2] Hosh = Hoshangabad Y4633, the 'universally susceptible' parent. The parental varieties separated by a hyphen were used in reciprocal crosses.

*** x^2 significant at 0.001 level of probability.

ø Not possible to calculate heterogeneity x^2, because of low number of plants tested with F-i.

Each of the four families of test cross progeny (L, M, N and P) was derived from several F_1 plants (including progeny from reciprocal parental crosses), used as female parents in crosses with Hoshangabad. Within the L, M and N families the results obtained from the separate F_1 plants were homogeneous and, consequently, these within family data were pooled (Table 6). It was not possible to test the P family results for heterogeneity, since too few test cross progeny from separate F_1 plants were tested with stock F-1, but for convenience these results were also grouped.

The pairs of rust stocks used to screen the L, M and N families for recombinants were expected to detect both recombinant classes if the genes segregating in these families were closely linked rather than allelic. However, both recombinant classes could be detected in less than half of the test cross progeny in the N family. These progeny were planted in two groups and the majority of the plants in one group, after testing with stock F-1, died when cut back during a period of high temperature in the glasshouse. Consequently, only the double-susceptible recombinant class could be detected in this group. The other group of progeny was grown during a more favourable time of the year and was tested with both stocks F-1 and Tk-41, allowing both recombinant classes to be detected.

The double recessive recombinant class only, could be detected in the P family of test cross progeny, since no rust stocks were available which attacked Koto but not Leona.

The progeny plants in the L, M and P families were classified, unambiguously, as either immune or susceptible to rust. However, three clear cut reaction classes, namely, immune, resistant and susceptible were recognized among the progeny in the N family when tested with stock F-1. The contrast between the immune and resistant reactions is shown in Figure 2. Tests on a sample of these progeny with stock 3 and Tk-41 showed that these three reactions correspond to the presence of the gene conferring immunity from Bombay, the gene conferring resistance from Polk, and the absence of both of these genes, respectively.

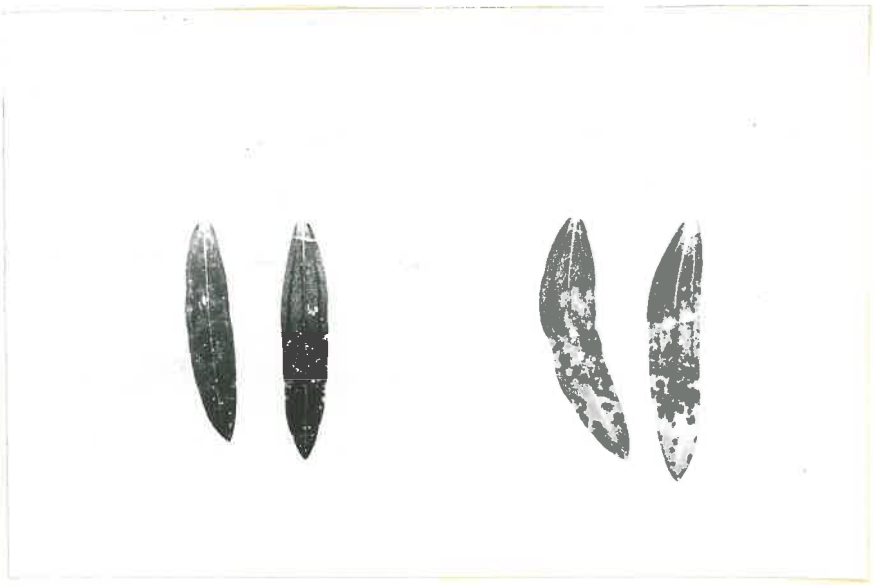
Table 6 shows that recombinant phenotypes occurred in the L, M and N families of test cross progeny, but not in the P family. Both Koto and Leona apparently possess a single gene conferring resistance to stock P-1 (Tables 5 and 8), and since no recombination occurred between these genes among 439 test cross progeny (Table 6), it was assumed that these genes correspond to Flor's (1955) genes P and P^3, respectively. The non-occurrence

FIGURE 2.

Reaction of test cross progeny from Hoshangabad x (Polk-Bombay) crosses to rust stock F-1.

Left to right - Immune reaction of plants with gene N_b from Bombay; resistant reaction of plants with gene N^5, from Polk.

First leaf of each pair, upper surface; second leaf, lower surface. Photographed with transmitted light to show necrotic flecks.



of detectable recombinants in this family cannot be taken as exclusive evidence in favour of allelism, but is consistent with a certain degree of recombination between genes P and P^3, the upper limit of which depends on family size. These data gave an upper limit of 'p', the recombination frequency between these genes, equal to 1.39%, with 95% certainty.

Two types of recombinant plants were detected in tests of the L, M and N families of test cross progeny. Two plants susceptible to both tester rust stocks (double-susceptible) were observed among the progeny from each of the L and N families. These plants will be referred to as Lr-1, Lr-2 and Nr-1, Nr-2, respectively. Plants immune to both rust stocks (double-immune), possibly representing the reciprocal product of crossing over, were not detected in these families, but two such plants, referred to as MR-1 and MR-2, were obtained in the M family of test cross progeny. These exceptional plants occurred very infrequently (approximately 1 in 1,000) and before classifying them as true recombinants, resulting from equal crossing over, all other possible origins must be considered.

The double-susceptible plants could have arisen by means of mutation, either at the resistance locus itself or at a suppressor locus, from seed contamination, or from unequal or equal crossing over between these genes.

The possibility that these plants arose by mutation of a dominant gene conferring rust resistance to the recessive form cannot be excluded from the data available. However, if mutation was the sole cause these genes must mutate with a relatively high frequency of 1 in 1,000. Unfortunately, there are no data available on the spontaneous or induced mutation rate of genes conferring rust resistance in flax, for comparison.

The progeny seed from plants Lr-1, Lr-2 and Nr-1 were grown and the rust reaction of the seedlings was determined, in an attempt to decide between some of the other possible origins. No progeny were obtained from plant Nr-2, since it died before maturity. Thirty-nine and nineteen progeny plants derived from self fertilisation of plants Lr-1 and Lr-2, respectively, were tested with rust stocks P-k, Ta-1 and P-1. In addition, nineteen progeny from crosses between Lr-1 and Hoshangabad were tested with these stocks. All progeny plants were susceptible, indicating that the original classification of the rust reaction of plants Lr-1 and Lr-2 was correct. Furthermore, if the rust reaction of these two plants was due to mutation at a suppressor locus, not linked with the L locus, we would expect that some of their progeny would be resistant to stock P-1 when the suppressor gene

segregated from the gene conferring resistance. There were not sufficient progeny to exclude the possibility that a suppressor mutation had occurred at a locus closely linked with the L locus. However, this latter explanation would require two occurrences of an extremely unlikely event, namely, mutation from a recessive to a dominant gene which was restricted to loci closely linked with the L locus.

Forty-six progeny plants derived from self fertilization of Nr-1 were tested with rust stocks F-1 and Tk-41, and these plants were all susceptible. From the above considerations, it was concluded that the susceptible reaction of plant Nr-1, as well as that of plants Lr-1 and Lr-2, was most likely not due to mutation at a suppressor locus.

Plants Lr-1, Lr-2 and Nr-1 had blue flowers and their progeny segregated for blue and white flowers, as expected if the former plants were derived from a test cross rather than from seed contamination. Blue-flowered susceptible plants originating from seed contamination were expected to be homozygous for genes controlling flower colour.

These double-susceptible plants could also have arisen from either equal or unequal crossing over between genes conferring rust resistance. However, it is impossible to distinguish between these two processes unless closely linked marker genes are present (p.91)

The double-immune plants could have been produced by self fertilization of the F_1 plants, by mutation or by some form of crossing over.

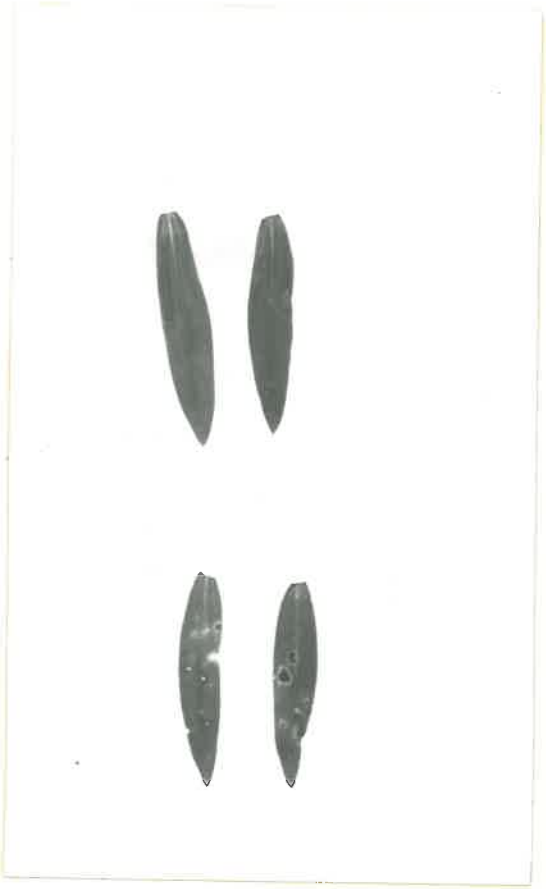
It is unlikely that the double-immune phenotype of plants MR-1 and MR-2 arose from mutation at the M locus, since the new phenotype represented an advantageous, rather than a deleterious change. It would be very unusual if two advantageous mutations had occurred without any of the deleterious changes (double-susceptible phenotype), which are expected to be much more frequent.

The immune phenotype of Dakota, with gene M, could be distinguished from that of Cass, with gene M^3 (Figure 3). Dakota was immune to stocks F-1 and Ti-58, and the infected leaves exhibited small necrotic flecks. Cass was immune to stocks F-1 and Ti-15, but in this case the infected leaves had larger necrotic areas with surrounding chlorosis. Furthermore, test cross progeny plants immune to stock Ti-58, and those immune to stock Ti-15, showed this same contrast in reaction phenotype. Therefore, this difference in phenotype was due to the properties of the M and M^3 genes themselves. Plants MR-1 and MR-2, when infected with rust stocks Ti-58 and Ti-15, also showed this contrast in reaction phenotype. This was taken as additional

FIGURE 3.

Immune reactions of Dakota (top) and Cass (bottom) to stock F-1. Note that large areas of necrosis occur on Cass, but only faint flecks on Dakota.

First leaf of each pair, upper surface; second leaf, lower surface.



evidence against the mutation hypothesis, since it is extremely unlikely that random mutation would have produced a change at the M locus as specific as that observed in these two plants.

Tests were made with the progeny of plants MR-1 and MR-2, to find whether these plants arose from self fertilization of the Dakota-Cass hybrid used in the test cross. As shown in Table 7, progeny plants immune to stock Ti-58 were also immune to stock Ti-15, whereas the remaining plants were susceptible to both stocks, and these plants occurred in an approximate 3:1 ratio. Furthermore, the immune progeny plants produced the distinctive reaction phenotypes characteristic of genes M and M^3, when tested with stocks Ti-58 and Ti-15, respectively. The absence of recombinants among these progeny showed that plants MR-1 and MR-2 possessed genes M and M^3 closely linked in coupling phase, and therefore did not arise from self fertilization of the F_1 hybrid.

However, it was not possible to decide whether equal or unequal crossing over had occurred in the F_1 hybrids to produce the gametes possessing genes M and M^3 in coupling phase.

TABLE 7

REACTION OF PARENTS, RECOMBINANT TEST CROSS PLANTS MR-1 AND MR-2, AND THEIR PROGENY TO TWO RUST STOCKS

TESTER RUST STOCK	REACTION* OF INDICATED PLANTS															
	PARENTS		RECOMBINANTS			MR-1 PROGENY				x_1^2 (3I:1S)		MR-2 PROGENY				x_1^2 3I:1S
	CASS	DAKOTA	MR-1	MR-2												
Ti-15	I	S	I	I		I	S	I	S			I	S	I	S	
Ti-58	S	I	I	I		I	S	S	I			I	S	S	I	
			Observed frequency		MR-1 selfed	411	136	0	0	0.03	MR-2 selfed	134	41	0	0	0.28
					(MR-1 x Hosh/) selfed	40	9	0	0	1.15						

* I = immune S = susceptible (see Table 4)

/ Hosh = Hoshangabad Y4633

The recombinant plants provided favourable material for investigating the recombination percentage between genes M and M^3, because, with very close linkage in coupling phase, F_2 progeny are almost as efficient as test cross progeny for detecting rare recombinants (Mather, 1936). Flax is naturally self fertilizing and, therefore, there is no difficulty in producing a very large number of F_2 progeny.

Although no recombinant plants were observed in the progeny of plants MR-1 and MR-2 (Table 7), these data provide information on the upper limit of the recombination value between genes M and M^3. The best estimate of this upper limit was obtained by pooling the data from the three progeny groups and this gave a value of 0.39%, with 95% certainty. The observed recombination value between genes M and M^3 from test cross progeny was 0.087%, with 95% confidence interval* from 0.011% to 0.310%. Thus, although no recombinants occurred in the progeny of plants MR-1 and MR-2 to confirm the test cross findings, the data obtained were, nevertheless, consistent with the test cross results.

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* All confidence intervals reported in this study were calculated using the method of Stevens (1942). See Fisher and Yates (1953) Table VIII.

As shown in Table 6, all individual segregation ratios observed, except those in the N family, agreed with the 1:1 ratio expected if each resistant parent possessed a single gene conferring resistance. With the N family, there was a large excess of plants resistant to stock F-i, compared with those immune, and the ratio approximated 4.5:1 instead of the 1:1 expected. This aberrant ratio was not due to misclassification of host reaction to stock F-i, since supplementary tests of some of these progeny with stocks 3 and Tk-41 confirmed the original classification. It was postulated earlier (p. 37) that Polk possesses two genes conferring resistance to stocks P-1 and F-i. However, if this hypothesis is correct some of the test cross progeny plants possessing the gene conferring immunity from Bombay (N), should also posseas the non-allelic gene conferring resistance from Polk, and consequently be jointly immune to stock F-i and resistant to stock Tk-41. The absence of such plants proved conclusively that Polk possesses one gene, not two, conferring resistance to the tester rust stocks. This gene is presumably Flor's (1955) N^1 gene.

Later, additional progeny from Polk-Bombay hybrids were tested, during a study of these aberrant segregation ratios, and the results are given in Section I. 5.v.

iv. Recombination Between the N and P Loci.

The results from an analysis of the progeny derived from Leona-Bombay and Polk-Koto hybrids, and designed to measure the amount of recombination between the N and P loci, are given in Table 8.

a. Progeny from Leona-Bombay hybrids

Several F_1 plants, derived from reciprocal crosses between Leona and Bombay, were used to produce the test cross progeny. All F_1 plants used as the female parent, were also used as the male parent in test crosses with Hoshangabad, but some F_1 plants were used only as the male parent in these crosses.

Two suitable rust stocks, corresponding to types α^1 and α^2 (see Table 1) were available for determining the rust reaction of the progeny and, consequently, both recombinant classes could be detected. In these tests, it was found, unexpectedly, that reciprocal test crosses gave rise to different segregation ratios in their progeny. However, within each type of test cross the segregation ratios from individual F_1 plants (including reciprocal parental crosses) were homogeneous. Therefore, the data within each group were pooled and the combined results are given in Table 8.

When the F_1 plants were used as the male parent in

TABLE 8

RECOMBINATION BETWEEN N AND P LOCI - REACTION OF PARENTAL FLAX VARIETIES AND PROGENY TO SELECTED RUST STOCKS

PEDIGREE[Ø]	NUMBER OF F_1 PLANTS USED IN CROSSES	TESTER RUST STOCK	REACTION[≠] OF INDICATED PLANTS													
			PARENTS			PROGENY										
						JOINT SEGREGATION					INDIVIDUAL SEGREGATION					
											OBSERVED FREQUENCY		EXPECTED RATIO	x^2_1	HETEROGENEITY df	HETEROGENEITY x^2
			Leona	Bombay	Hosh[≠]						I	S	I : S			
Hosh x (Leona-Bombay)	13	Tk-47	I	S	S		I	I	S	S	253	244	1 : 1	0.16	12	17.98
		F-i	SR	I	S		I	S	I	S	253	244	1 : 1	0.16	12	4.64
						Observed frequency	58	195	195	49						
											I	S	I : S			
(Leona-Bombay) x Hosh	9	Tk-47	I	S	S		I	I	S	S	141	66	1 : 1	27.17***	6	11.87
		F-i	SR	I	S		I	S	I	S	82	208	1 : 1	54.74***	8	12.12
						Observed frequency	12	129	43	23						
											I	S	I : S			
(Leona-Bombay) F_2	3	Tk-47	I	S			I	I	S	S	219	26	3 : 1	27.05***	2	1.42
		F-i	SR	I			I	SR	I	S	163	82	3 : 1	9.37**	2	1.35
						Observed frequency	138	81	25	1						
			Koto	Polk	Hosh						I	R	S	I :R+S		
(Koto-Polk) x Hosh	6	F-1	I	IR	S		I	R	S		61	123	7	1 : 1	24.93***	5	9.55
		3	I	SR	S		I	S	S		9		29			
						Observed frequency	9	26	3							

≠ I = immune IR = immune to resistant R = resistant SR = semi-resistant S = susceptible (see Table 4).

Ø The parental varieties separated by a hyphen were used in reciprocal crosses.

*** x^2 significant at 0.001 level of probability.

** x^2 significant at 0.01 level of probability.

≠ Hosh = Hoshangabad Y4633.

crosses with Hoshangabad, the individual segregation ratios in the progeny agreed with the 1:1 ratio expected if Leona contributed a single gene conferring immunity to rust stock Tk-47, and Bombay contributed a single gene conferring immunity to stock F-1. The joint segregation showed that these genes did not segregate independently ($\chi^2_1 = 159.2, P < 0.001$) and the estimated amount of recombination between them was 21.21%, with 95% confidence interval 17.69% to 25.08%. The possibility that some of the plants classified as double-susceptible recombinants arose from undetected selfing of the Hoshangabad female parent was checked by growing these plants to maturity and scoring for flower colour. All test cross progeny were expected to be blue-flowered (p. 20) and the double-susceptible plants which survived to maturity (forty out of forty-nine) possessed blue flowers, showing that these plants, at least, did not originate from self fertilization.

It was assumed that the genes conferring immunity to rust in Leona and Bombay corresponded to Flor's (1955) genes P^3 and N, respectively, since the genes in Leona and Bombay were found to be linked in the present study and previous tests had shown that they were allelic (or closely linked) with genes controlling reaction to rust in Koto and Polk, respectively (Table 6).

In contrast with the above, when the F_1 plants were used as the female parent in crosses with Hoshangabad aberrant segregation ratios occurred. There was a significant excess of plants immune to rust stock Tk-47 and a significant deficiency of plants immune to stock F-1. Again, the individual classifications were not independent ($x_1^2 = 71.05$, $P < 0.001$) and the estimate of the recombination percentage between genes N and P^3 was 16.91%, with 95% confidence interval from 12.07% to 22.72%. There were more double-susceptible recombinants than the double-immune type, but the difference was not significant ($x_1^2 = 3.46$, $P = 0.05$-0.1). Undetected selfing of the F_1 female parents would not have contributed to this trend, since such an event would increase the number of apparent double-immune recombinants.

Although this estimate of the recombination percentage was not significantly different from the previous one ($x_1^2 = 1.44$, $P = 0.2$-0.3), the two values were not pooled, because it was not known whether the process responsible for the aberrant segregation ratios, also affected the recombination percentage.

F_2 seeds from three of the F_1 plants used in the test cross were grown and the plants were tested with rust stocks F-1 and Tk-47 to find whether aberrant segregation ratios also occurred in these progeny. The results are

given in Table 8 and these show that aberrant ratios occurred, which were similar to those observed in progeny from the F_1 plants used as female parents in crosses with Hoshangabad. That is, there was a significant excess of plants immune to stock Tk-47, and a significant deficiency of plants immune to stock F-i, from expected.

It is possible that these aberrant ratios were due to differential viability of zygotes. However, this was considered unlikely, since the aberrant segregation ratios occurred only when the Leona-Bombay hybrids were used as the female parent in test crosses with Hoshangabad. That is, if zygotic inviability was the cause we would have to postulate that this inviability occurred when the test cross zygote developed in nucellar tissue of the F_1 plant, but not when they developed in the nucellar tissue of Hoshangabad. It appears more likely that the aberration occurred at the gametic, rather than the zygotic stage and, in particular, at some stage during the development of female gametes.

b. Progeny of Polk-Koto hybrids

The F_1 plants derived from reciprocal crosses between Koto and Polk were used, initially, as the female parent only, in the test cross. All of the test cross progeny were tested with rust stock F-i and some of them were tested

with stock 3 also. In the former test, three distinctive host phenotypes corresponding to immune, resistant and susceptible reactions, were observed (Figure 4). The limited tests with stock 3 confirmed the classifications made with stock F-i.

The segregation ratios of immune to non-immune plants among progeny groups from individual F_1 plants were homogeneous and the pooled data are given in Table 8.

A 1:1 ratio of immune to non-immune plants was expected among these progeny, since it had been shown that Koto possessed a single gene conferring immunity to stock F-i (p.36) and immunity is epistatic to resistance (Myers, 1937). However, there was a significant deficiency of immune plants in the progeny and the test with stock 3 showed that this was not due to misclassification. Evidently the same aberrant process was occurring, which had been encountered in previous crosses involving the variety Polk (Tables 5 and 6).

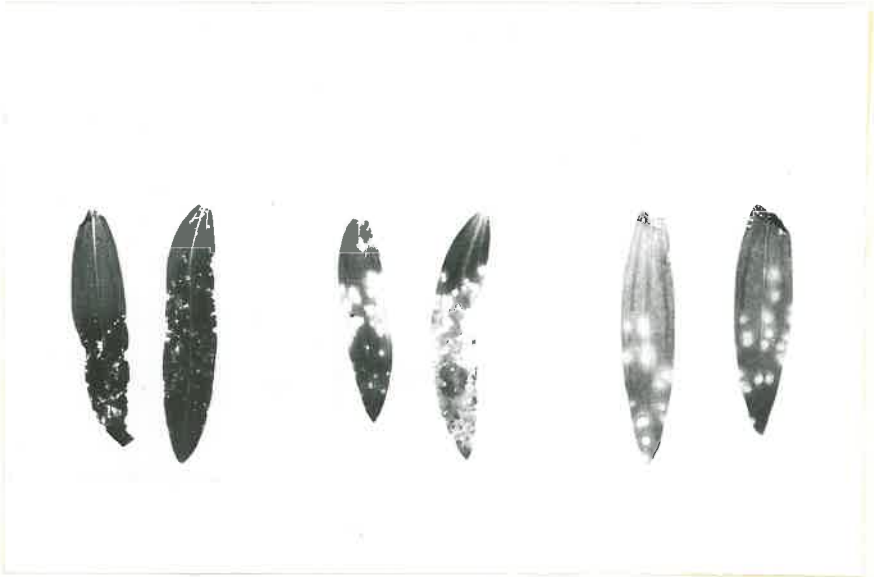
The seven plants susceptible to stock F-i were classified as recombinants, but, because of the aberrant segregation ratio, it is considered unlikely that this group represents one half of the recombination fraction between the N and P loci (see Discussion, p.109). Therefore, no attempt was made to calculate the recombination value between the N and P loci from these data.

FIGURE 4.

Reactions of test cross progeny from Hoshangabad x (Polk-Koto) crosses to stock F-1.

Left to right - Immune reaction of plants with gene P, from Koto; resistant reaction of plants with gene N^5, from Polk; susceptible reaction of plants with neither gene.

First leaf of each pair, upper surface; second leaf, lower surface.



c. Additional crosses were made, subsequently, which provided further information on recombination between the N and P loci, but, since these crosses were made primarily to investigate the nature of aberrant segregation ratios, the results will be reported in Section I. 5. vi. (Tables 12 and 13).

v. The Nature of Aberrant Segregation Ratios for Rust Reaction

The initial experiments of the present study were not designed to investigate the nature of aberrant segregation ratios, because the possible significance of Flor's (1947, 1951) previous reports of aberrant ratios had been overlooked. This was due, mainly, to the lack of evidence in the literature confirming the reality of these aberrant ratios.

Flor reported that "Some crossing over and irregular segregation ratios were obtained in hybrids between varieties having rust conditioning factors in the NN series" (Flor, 1947, p. 261), and a "departure from the theoretical ratio has occasionally been encountered in hybrids of varieties having rust conditioning genes in the Bombay linkage group " (Flor, 1951, p. 528), but he did not report any attempt to find the cause of these aberrant ratios. Furthermore, Kerr (1954) and Mayo (1955) did not report the occurrence of similar aberrant segregation

ratios in their studies.

However, after the occurrence of aberrant segregation ratios in the present investigation, an attempt was made to find the nature of the process causing these aberrations. The aberrant ratios were observed only among progeny of test crosses analysed late in the present study and there was insufficient time to investigate their nature in detail. Therefore, the additional studies were restricted to the following:

a. The literature on the genetics of rust reaction in flax was re-examined to find whether there is a consistent pattern in the occurrence of the aberrant ratios, and these data were related to the observations made in the present study.

b. Additional groups of host progeny were produced to find which combinations of parental varieties produce aberrant ratios and whether these aberrations are always restricted to the products of megagametogenesis. These data also gave further information on recombination between the N and P loci.

c. Experiments were made to find whether there is any relation between the occurrence of aberrant segregation ratios for rust reaction and the aberrant ratios for flower colour, also reported to occur in flax (Tammes, 1914, Kappert, 1924).

d. Studies were initiated to determine the mode of inheritance of these aberrant ratios for rust reaction.

e. Limited studies of megagametogenesis and microsporogenesis were made in certain flax hybrids in an attempt to find a cytological basis for the aberrant ratios.

a. Analysis of published data

Chi-square values, as a measure of deviation from expected, were calculated on data from Flor's (1947, 1951) F_2 families, which gave a two class segregation of immune (or resistant) and susceptible plants when tested with a single rust stock. The expected ratio of immune (or resistant) to susceptible plants in these families was 3:1, 15:1 or 63:1 depending on whether 1, 2 or 3 dominant, and independent genes, respectively, were segregating and interacting with the tester rust stock. All two and three gene segregation ratios, with one exception (see below), agreed with expected, but, since less than 200 F_2 plants were tested in most families, only large deviations from expected would have been detected. Consequently, only the exception in this group, involving F_2 progeny from Morye x Ottawa 770B, has been included in the following analysis.

Altogether, Flor detected 98 monohybrid segregation

ratios, 32 involving genes conferring resistance located at either the N or P locus, and 66 involving corresponding genes located at either the L or M locus. A list of the twelve combinations of parental varieties which produced significantly deviating monohybrid segregation ratios (at the 0.05 level of significance) in their progeny, together with the genes involved in these segregations, is given in Table 9. Only one monohybrid segregation ratio was detected in most F_2 families, but where two were observed, both have been included in the table.

It is difficult to interpret these data because:
(1) In a total of 98 monohybrid segregation ratios an average of five will be expected to deviate significantly from expected, at the 0.05 level of significance, from the influence of chance alone.
(2) It is possible that, besides chance, more than one process was responsible for the significant deviations.

However, the data in Table 9 show agreement with some of the general properties of the aberrant ratios suggested from the present study (Table 10).

Eleven of the twelve significant deviations reported by Flor, involved genes located at either the N or P locus, and only one involved a gene at either the L or M locus, whereas approximately two and three, respectively, were

TABLE 9

FLOR'S (1947, 1951) F_2 FAMILIES IN WHICH THE SEGREGATION RATIO FOR RUST REACTION DEVIATED SIGNIFICANTLY FROM EXPECTED

PARENTAL FLAX VARIETIES		GENES CONTROLLING RUST REACTION DETECTED IN PARENT		F_2 PROGENY		
				NUMBER TESTED	RELEVANT[Ø] SEGREGATION RATIO DETECTED	x_1^2
(1)	(2)	(1)	(2)			
Bombay	Victory A	N	M^4	124	N : $\underline{n}$[/]	6.19*
"	Victory C	N	M	128	N : $\underline{n}$	24.00**
					M : m	0.18
"	Victory B	N	MN^1	203	$\underline{N}^1$: N	4.27*
"	Leona	N	P^3	190	N : $\underline{n}$	9.61**
					P^3 : p	1.36
"	Rio	N	$L^6M^6N^3$	192	N : $\underline{n}$	23.36***
"	Italia Roma	N	$M^3(P)$	48	N : $\underline{n}$	5.44*
"	Billings	N	M^4	207	$\underline{N}$: n	4.17*
					M^4 : m	2.98
Punjab	Bolley Golden	N	KM^3	550	N : $\underline{n}$	4.48*
Bison	Victory D	≠	M^4N^1	176	$\underline{N}^1$: n	8.76**
					M^4 : m	0.27
Ottawa 770B	Leona	L	P^3	184	$\underline{P}^3$: p	4.9*
					L : l	0.12
"	Morye	L	KL^6N^1P	754	L : l	0.78
					N^1 and/or K:$\underline{nk}$	35.14***
"	Abyssinian	L	P^2	200	L : $\underline{l}$	4.5*

Ø With one exception, dihybrid segregation ratios were not included because of the small size of F_2 families.
/ Allele underlined occurred in excess of expected.
≠ Bison possesses no known genes conferring resistance to the tester rust stocks used by Flor.
* x_2^2 significant at the 0.05 level of probability.
** x_2^2 significant at the 0.01 level of probability.
*** x^2 significant at the 0.001 level of probability.

TABLE 10

PROGENY GROUPS IN WHICH THE SEGREGATION RATIO OF GENES AT EITHER THE N OR P LOCUS WAS DETECTED IN THE PRESENT STUDY

PARENTAL FLAX VARIETIES		GENES CONTROLLING RUST REACTION DETECTED IN PARENT:		PEDIGREE	PROGENY			
					F_1 PARENT IN TEST CROSS	NUMBER TESTED	ALLELIC PAIR SEGREGATING	x_1^2
(1)	(2)	(1)	(2)					
Polk	Bombay	N^1	N	Test cross	Female	1033	$\not\underline{N^1}$: N	403.5^{***}
"	Koto	N^1	P	Test cross	Female	191	P : p	24.93^{***}
"	Hoshangabad	N^1	-	F_2		196	$\underline{N^1}$: n	31.46^{***}
Leona	Bombay	P^3	N	Test cross	Female	207	$\underline{P^3}$: p	27.17^{***}
						290	N : n	54.74^{***}
"	Bombay	P^3	N	Test cross	Male	497	P^3 : p	0.16
						497	N : n	0.16
"	Bombay	P^3	N	F_2		245	$\underline{P^3}$: p	27.05^{***}
						245	N : n	9.37^{**}
"	Hoshangabad	P^3	-	F_2		243	$\underline{P^3}$: p	14.55^{***}
"	Koto	P^3	P	Test cross	Female	95	P^3 : p	1.27
Koto	Hoshangabad	P	-	F_2		167	P : p	0.00
Bombay	Hoshangabad	N	-	F_2		473	N : n	0.06

$\not$ Allele underlined occurred in excess of expected.

** x^2 significant at the 0.01 level of probability.

*** x^2 significant at the 0.001 level of probability.

expected by chance. If the segregation ratio of genes L:l in the F_2 progeny from Ottawa 770B-Abyssinian hybrids represents a chance deviation, as seems likely (p.60), then it may be postulated that the aberrant ratios are restricted to the segregation of genes at the N or P locus. The normal segregation of genes at either the L or M locus in F_2 families showing simultaneously an aberrant segregation ratio of genes at the N or P locus (Table 9), gave added support to this postulate.

Aberrant ratios occurred in the progeny from Leona-Bombay hybrids in both studies and, furthermore, there was a significant deficiency of the N gene from Bombay in both groups of progeny. However, whereas there was a significant excess of the P^3 gene from Leona in the test cross progeny (Table 10), there was only a trend in this direction in Flor's data (Table 9).

The variety Polk was derived from a line of Victory, and has the same gene conferring resistance (N^1) as Victory B and Victory D (Flor, 1954). The occurrence of aberrant segregation ratios in the test cross progeny from Polk-Bombay hybrids (Table 10), and in Flor's F_2 progeny from Bombay crossed with each of Victory A, Victory B and Victory C (Table 9), showed that Polk and Victory were behaving in a similar manner in these crosses. This similarity in behaviour of the two varieties indicates

that the aberrant ratios observed by Flor in the progeny from crosses between Victory and Bombay represent real effects, rather than chance deviations.

A more general feature of the aberrant segregation ratios is indicated from the data given in Tables 9 and 10. Table 10 shows that in those progeny giving an aberrant ratio, and where the segregation of the relevant alleles was detected, there was a significant excess of the N and P alleles derived from Polk and Leona, and a significant deficiency of the corresponding alleles from the other parents, namely, Bombay, Koto and Hoshangabad. Similarly, in Flor's data (Table 9) there was a significant deficiency of the N gene from Bombay in all progeny groups, except two (see below), in which the segregation of this gene was detected. Also, there was a significant excess of the P^3 gene from Leona, and the N^1 gene from Victory B and Victory D, in F_2 progeny from crosses between these varieties and Ottawa 770B, Bombay and Bison, respectively.

One of the exceptions noted above, in the F_2 progeny from Billings x Bombay, may have been a chance deviation. However, the deviation observed in the other exception, with F_2 progeny from Morye x Ottawa 770B, was much too large to be reasonably ascribed to chance and the significance of this aberrant segregation ratio of genes N^1 and/or K:nk is not clear.

Flor's data, which included segregations of genes at the L, M, N and P loci, indicate that the aberrant ratios are most likely restricted to the segregation of genes at the N or P locus. However, any hypothesis invoked to explain the occurrence of these aberrant ratios must take account of the large number of segregations involving genes at either of these loci, which do not show any abnormality. Besides those found in the present study (Table 10), Flor observed many normal monohybrid segregation ratios of genes located at the N or P locus and these are listed in Table 11.

In an attempt to explain the different types of segregation ratios observed with genes at the N or P locus (Tables 9, 10 and 11), we may speculate that host parental varieties exist in two groups, and that only parental combinations involving a variety from each group, have the potential to produce aberrant segregation ratios in their progeny. For example, from the data in Tables 9 and 10, we may assign the varieties Bombay, Hoshangabad, Koto, Bison and possibly Ottawa 770B to a common group (A), because there was, characteristically, a significant deficiency of the N or P alleles derived from these varieties in progeny showing an aberrant ratio. Conversely, varieties Polk, Leona, Victory, Rio and possibly Italia Roma and Bolley Golden, contributing a significant excess

TABLE 11

FLOR'S (1947, 1951) F_2 FAMILIES IN WHICH APPARENTLY NORMAL MONOHYBRID SEGREGATION RATIOS OF GENES LOCATED AT THE N OR P LOCUS, WERE OBSERVED

PARENTAL FLAX VARIETIES		GENES CONTROLLING RUST REACTION DETECTED IN PARENT:		F_2 PROGENY		
				NUMBER TESTED	RELEVANT* SEGREGATION RATIO DETECTED	x_1^2
(1)	(2)	(1)	(2)			
Bombay	Williston Golden	N	L^5M^1	188	N : n	3.43
"	C.I. 438	N	L^7X	172	N : n	0.03
"	C.I. 438	N	L^7	188	N : n	1.39
"	Williston Brown	N	M^1	184	N : n	2.90
"	Akmolinsk	N	P^1	160	N : n	0.53
					P^1: p	0.83
"	Abyssinian	N	P^2	64	N : n	0.75
"	Ottawa 770B	N	L	194	N : n	0.17
"	Newland	N	M	192	N : n	0.44
"	Bison	N	≠	189	N : n	2.41
"	Kenya	N	L^4	64	N : n	0.75
Punjab	Buda	N	L^1M^2	192	N : n	2.25
"	Tammes Pale Blue	N	N^4P	192	N : n	0.00
					N^4 and/or P:np	≠
Bison	Victory B	≠	MN^1	178	N^1: n	0.37
"	Akmolinsk	≠	P^1	192	P^1: p	0.00
"	Abyssinian	≠	P^2	192	P^2: p	0.69
Ottawa 770B	Victory B	L	MN^1	192	N^1: n	1.78
Newland	Leona	M	P^3	192	P^3: p	0.69
Williston Golden	Akmolinsk	L^5M^1	P^1	192	P^1: p	0.03
"	Abyssinian	L^5M^1	P^2	164	P^2: p	1.17

* Dihybrid segregation ratios were not included because of the small size of F_2 families.

≠ Bison possesses no known genes conferring resistance to the tester rust stocks used by Flor.

≠ Flor (1947) originally interpreted this as an aberrant monohybrid segregation ratio, but later (Flor, 1954), he found that both of the linked genes N^4 and P were effective against the tester rust stock. Because of this linkage, there is no simple expectation for this segregation ratio and therefore x^2 could not be calculated.

of their N or P alleles to the progeny, may be considered to belong to the other group (B).

The hypothesis predicts that parental varieties giving normal segregation ratios in their progeny belong in the same group. Therefore, it is postulated that Newland belongs in the same hypothetical group as Bombay (Group A), since the N gene segregated normally in the F_2 progeny from Bombay x Newland (Table 11).

From these classifications, we would expect aberrant ratios to occur in the F_2 progeny from Victory B x Bison, Victory B x Ottawa 770B and Leona x Newland, but their progeny gave normal segregations of genes at the N or P locus (Table 11). However, these results do not necessarily invalidate the original hypothesis, because it is possible that some of these varieties were misclassified (see p.55). Alternatively, some of these parental combinations may have possessed the potential to produce aberrant ratios in their progeny, but this was not expressed in the small numbers of progeny tested.

Kerr (1954) used many of Flor's flax varieties as parents in his study and his data were also examined to obtain additional information on the variety combinations producing aberrant ratios. In his study, thirty-two monohybrid segregation ratios of genes at the N or P locus were detected in F_2 and/or test cross progeny, derived from

crosses between thirty-one different pairs of parental varieties. However,none of these progeny provided strong evidence for the occurrence of aberrant segregation ratios.

Only two of Kerr's numerous parental crosses were directly comparable with those found by Flor (1947) to give segregation ratios deviating significantly from expected. These were Ottawa 770B x Morye and Ottawa 770B x Abyssinian. Although Kerr did not report any aberrant ratios in the progeny from the former cross, this result did not necessarily conflict with that of Flor, because it was not possible to decide,unambiguously, from Kerr's data, which of the four genes conferring resistance in Morye were effective against the tester rust stocks. Kerr found that the L gene from Ottawa 770B segregated normally in the progeny from Ottawa 770B x Abyssinian and this supported the hypothesis that the deviation observed in Flor's corresponding family was due to chance.

It is apparent from the data given in Tables 9, 10 and 11, and Kerr's (1954) study, that the aberrant ratios are restricted not only to the segregation of genes at the N or P locus, but to progeny from relatively few combinations of host varieties. However,it is likely that some parental combinations had the potential to produce aberrant ratios,but this was not expressed due

to small family size, especially in Kerr's studies.

b. Additional pedigrees tested

The analysis of published data shows that the aberrant segregation ratios reported by Flor (1947, 1951) have features similar to those found in the present study. However, additional information was required to find whether all parental flax varieties could be classified into either hypothetical group A or B (p.58).

A striking feature of the present study is the difference between the segregation ratios in progeny derived from reciprocal test crosses between Leona-Bombay hybrids and Hoshangabad (Table 8). These results indicate that the aberrant ratios most likely arise at the gametic, rather than the zygotic stage of development, and are restricted to the products of megagametogenesis. Additional information was required to find whether the other hybrids producing aberrant segregation ratios (Table 10), also show this different behaviour in reciprocal test crosses.

Additional host progeny were tested to obtain information on both of these aspects of the aberrant segregation ratios. However, limited time was available and this additional study had to be restricted to the use of existing F_1 and F_2 seed.

F_1 seeds were available from Leona x Hoshangabad, Polk x Bombay, Polk x Koto and Polk x Hoshangabad parental crosses and these combinations had previously produced aberrant ratios in test cross, or F_2 progeny (Table 10). The F_1 plants were crossed reciprocally with Hoshangabad to find whether the aberrant ratios are restricted to the products of megagametogenesis.

F_1 seeds from Bombay x Koto and Leona x Polk parental crosses were also available and, from previous classifications (p.58), these two combinations represent crosses between two parents from the hypothetical A group of varieties and between two parents from the B group, respectively. Consequently, the F_1 plants were crossed reciprocally with Hoshangabad to find whether these combinations produce normal segregation ratios in their progeny, as predicted earlier (p.59).

On the basis of the above hypothesis and variety classifications, an aberrant segregation ratio was expected to occur in the test cross progeny from (Leona-Koto) x Hoshangabad, but an apparently normal ratio was observed in these progeny (Table 10). However, only a small sample of these progeny was tested and supplementary data were required. F_1 seeds from Leona x Koto crosses were not available, but F_2 seeds derived from the F_1 plants used

earlier (Table 6), were grown and tested.

F_2 seeds from Polk x Wilden, Polk x Dakota and Polk x Clay crosses were grown and tested, to find whether the parental variety Polk is frequently associated with the occurrence of aberrant segregation ratios.

The F_1 plants used in test crosses were grown during the summer months, because of the limited time available, and they began flowering during mid-autumn. High glasshouse temperatures (often above 32°C) occurred during flowering and these greatly reduced the number of seeds obtained, and in some families no viable test cross seeds were produced on the F_1 plants. Later in the season, environmental conditions improved, and although the F_1 plants were then too old to be used as female parents, they were used successfully, in most families, as male parents in the crosses with Hoshangabad. Where no test cross seeds were produced on the F_1 plants, the limited number of F_2 seeds produced were analysed, to find whether the particular F_1 plants used possessed the potential to produce aberrant ratios.

The results from rust reaction tests made on these additional groups of progeny, except the F_2 progeny of Leona x Koto, are given in Tables 12 and 13.

The parental variety combinations listed in Table 12, which had previously given aberrant segregation ratios,

TABLE 12

ADDITIONAL CROSSES MADE INVOLVING COMBINATIONS OF PARENTAL FLAX VARIETIES PREVIOUSLY GIVING ABERRANT SEGREGATION RATIOS AND REACTION OF PARENTS AND PROGENY TO SELECTED RUST STOCKS

PEDIGREE	NUMBER OF F_1 PLANTS USED IN CROSSES	TESTER RUST STOCK	REACTION[≠] OF INDICATED PLANTS					
			PARENTS	PROGENY INDIVIDUAL SEGREGATION				
				OBSERVED FREQUENCY	EXPECTED RATIO	x_1^2	HETEROGENEITY df	HETEROGENEITY x^2
			Hosh[≠] Polk Bombay					
Hosh x (Polk x Bombay)	2	F-1	S IR I	I R S	I:R+S			
				64 55 0	1:1	0.68	1	0.00
(Polk x Bombay) F_2	2	3	- SR I	I SR	I:SR			
				144 71	3:1	7.38**	1	0.50
			Hosh Polk Koto					
Hosh x (Polk x Koto)	1	F-1	S IR I	I R S	I:R+S			ø
				108 71 19	1:1	1.64		
(Polk x Koto) x Hosh	1	F-1	S IR I	I R S				
				26 54 2	1:1	10.98***		ø
			Hosh Polk					
Hosh x (Polk x Hosh)	2	F-1	S IR	R S	R:S			
				39 29	1:1	1.47	1	0.00
(Polk x Hosh) F_2	2	F-1	S IR	IR S	IR:S			
				124 23	3:1	6.63**	1	0.35
			Hosh Leona					
Hosh x (Leona x Hosh)	3	Tk-47	S I	I S	I:S			
				15 12	1:1	0.33		ø
(Leona x Hosh) F_2	4	Tk-47	S I	I S				
				88 15	3:1	5.98*		ø

≠ I=immune IR=immune to resistant R=resistant SR=semi-resistant S=susceptible (see Table 4) ≠ Hosh = Hoshangabad Y4633
ø Not possible to calculate x^2 because of small number of progeny tested. * x^2 significant at 0.05 level of probability.
** x^2 significant at 0.01 level of probability. *** x^2 significant at 0.001 level of probability.

again produced aberrant ratios in their F_2 progeny, or the test cross progeny derived from the F_1 plants used as the female parent in crosses with Hoshangabad. Furthermore, all of these segregation ratios deviated in the same direction from expected, as observed previously (Table 10). In contrast with the above, apparently normal monohybrid segregation ratios occurred in all test cross progeny groups, when the same F_1 plants were used as the male parent in crosses with Hoshangabad. That is, all of the results were consistent with the hypothesis that the aberrant segregation ratios, previously observed among certain progeny from these hybrids, do not occur among the male gametes, but are apparently restricted to the female gametes.

The results obtained from the progeny of the other parental combinations are given in Table 13. The segregation ratio of alleles at either the N or P locus was detected in each of these families, and parental variety combinations possessing the potential to produce aberrant segregation ratios were expected to express this in their F_2 progeny, or in the test cross progeny produced on the F_1 plants. Normal segregation ratios of genes at the N or P locus occurred in all families, except the F_2 progeny from Polk x Dakota hybrids; there was a significant excess of the N^1 gene, derived from Polk, in this family. However,

TABLE 13

ADDITIONAL CROSSES MADE WITH NEW COMBINATIONS OF PARTIAL FLAX VARIETIES POSSESSING GENES AT EITHER THE N OR P LOCUS, AND REACTION OF PARENTS AND PROGENY WITH SELECTED RUST STOCKS

PEDIGREE	NUMBER OF F_1 PLANTS USED IN CROSSES	TESTER RUST STOCK	REACTION[1] OF INDICATED PLANTS											
			PARENTS			PROGENY								
						JOINT SEGREGATION				INDIVIDUAL SEGREGATION				
										OBSERVED FREQUENCY		EXPECTED RATIO	x^2_1	HETEROGENEITY df / x^2
			Hosh[2]	Bombay	Koto					I	S	I : S		
Hosh x (Koto x Bombay)	4	F-i	S	I	I		I	I	S	71	3	1 :p/2		
			S	S	I		I	S	S	40	34	1 : 1	0.49	ø
						Observed frequency	40	31	3					
(Koto x Bombay) x Hosh	4	F-i	S	I	I		I	I	S	241	13	1 :p/2		
		Tk-41	S	S	I		I	S	S	135	119	1 : 1	1.01	3 / 2.57
						Observed frequency	135	106	13					
			Hosh	Polk	Leona					I	S	I : S		
Hosh x (Polk x Leona)	3	Tk-47	S	IR	I		I	I	S	25	1	1 :p/2		
		F-i	S	IR	SR		I	S	S	15	11	1 : 1	0.62	ø
						Observed frequency	15	10	1					
										IR	SR	IR:SR		
(Polk x Leona) F_2	3	F-i		IR	SR					151	47	3 : 1	0.15	2 / 1.34
			Polk	Dakota						I	S	I : S		
(Polk x Dakota) F_2	4	F-i	IR	I			I	I	S	214	9	15 : 1	1.87	
		Ti-15	IR	S			IR	S	S	184	39	3 : 1	6.77**	3 / 3.43
						Observed frequency	184	30	9					
			Polk	Wilden						IR	S	IR: S		
(Polk x Wilden) F_2	3	F-i	IR	IR			IR	IR	S	184	13	15 : 1	0.04	
		F-k	IR	S			IR	S	S	155	42	3 : 1	1.42	2 / 1.09
						Observed frequency	155	29	13					
			Polk	Clay						IR	S	IR: S		
(Polk x Clay) F_2	4	F-i	IR	R			IR	IR	S	150	10	15 : 1	0.00	
		F-k	IR	S			IR	S	S	121	39	3 : 1	0.03	3 / 0.33
						Observed frequency	121	29	10					

[1] I = immune IR = immune to resistant R = resistant SR = semi-resistant S = susceptible (see Table 4).
[2] Hosh = Hoshangabad Y4633.
ø Not possible to calculate x^2 because of small number of progeny tested.
** x^2 significant at 0.01 level of probability.

although sufficient progeny from (Bombay x Koto) x Hoshangabad test crosses were tested to detect relatively small disturbances from the 1:1 segregation ratio expected, only large deviations from expected would be detected in the F_2 families tested.

The normal segregation ratios in the progeny from Bombay x Koto and Leona x Polk hybrids are consistent with the prediction made from the hypothesis proposed to explain why only certain combinations of parental varieties produced aberrant ratios (p.58 and 59). Furthermore, it was predicted from this same hypothesis that an aberrant segregation ratio would occur among the F_2 progeny of Leona x Koto hybrids and, out of 266 F_2 plants tested with stock F-1, 181 were immune and 85 were semi-resistant, which is significantly different from the 3:1 ratio expected ($\chi^2_1 = 11.97$, $P < 0.001$), if the Koto gene (P) conferring immunity segregates normally.

The data obtained from the reciprocal test crosses with Polk x Koto (Table 12) and Bombay x Koto (Table 13) hybrids, also provide further information on recombination between the N and P loci.

The frequencies of immune, resistant and susceptible plants in the progeny derived from (Polk x Koto) x Hoshangabad test crosses, were similar to those found previously (Table 8) and these two sets of data were

pooled. A total of 9 plants out of 273 tested were susceptible to rust stock F-1 and these plants presumably resulted from recombination between the N^1 gene (from Polk) and the P gene (from Koto), in the hybrid. However, when the F_1 plant was used as the male parent in the test cross a significantly greater proportion (19 out of 198) of these susceptible plants occurred ($\chi^2_1 = 7.06$, $P < 0.01$). It is unlikely that undetected self-fertilization of the Hoshangabad female parent contributed significantly to the larger number of susceptible plants in this family, because all of the susceptible plants which survived to maturity (10 out of 19) possessed blue flowers, rather than the white flowers expected, if they were derived from self-fertilization of Hoshangabad.

The relative frequency of susceptible plants in the (Polk-Koto) x Hoshangabad progeny probably represents less than one-half of the recombination fraction (p/2), due to the occurrence of the aberrant segregation ratio (see p.109). However, it was assumed that the relative frequency of susceptible plants in the progeny from the reciprocal test cross represents p/2, because these progeny showed a normal segregation ratio. The recombination percentage between genes N^1 and P, calculated from these latter data, is 19.19%, with 95% confidence interval

from 11.76% to 29.16%. This recombination value is of the same order as that obtained earlier for recombination between genes N and P^3, in Leona-Bombay hybrids (p.48).

From the data given in Table 13, the recombination percentage between genes P and N in Bombay x Koto hybrids was estimated to be 9.76%, with 95% confidence interval from 5.63% to 15.60%, assuming that the proportion of plants susceptible to stock F-1 represents p/2 in both groups of test cross progeny. This particular recombination value is much lower than the other estimates of recombination between genes at the N and P loci. Although it is not significantly different from the estimate obtained from Polk x Koto hybrids ($x_1^2 = 3.70$, $P = 0.05-0.1$), it is significantly less than that obtained from Hoshangabad x (Leona-Bombay) progeny ($x_1^2 = 7.92$, $P < 0.01$).

c. Relation of aberrant segregation ratios for rust reaction and flower colour.

During a search of the literature on the genetics of L. usitatissimum, reports of aberrant segregation ratios for flower colour were noted. Some of the characteristics of these aberrant ratios for flower colour are similar to those for rust reaction, and therefore, an

attempt was made to find the relationship, if any, between these two phenomena.

Tammes (1914) and Kappert (1924) reported that a significant deficiency of white-flowered plants, from expected, occurred in the F_2 progeny from crosses between certain pairs of white and blue-flowered flax varieties. They used different pairs of varieties in their studies, but in both cases, the white-flowered parent possessed narrow petals with crimped edges, yellow anthers and greenish seed and the blue-flowered parent had broad flat petals, blue anthers and brown seed.

Tammes (1922) postulated that two genes, B and C, are necessary for production of basal colour in the petals of flax, but Kappert (1924) showed later that three genes (B_1, B_2 and C) are required, and he assigned genotypes $b_1b_1B_2B_2CC$ and $B_1B_1B_2B_2CC$ to the above white and blue-flowered parents, respectively.

Tammes (1914) accounted for her results by assuming that the observed deficit of white-flowered F_2 plants was due to lower viability of this class of zygotes. However, this assumption was inadequate to explain the results obtained by Kappert (1924, 1930, 1935) in his more extensive studies of aberrant segregation ratios for flower colour.

Kappert (1930) found that the white-crimped flax variety Stockholm, giving an aberrant F_2 segregation ratio

when crossed with the blue-flowered variety Weimar, gave a normal F_2 ratio when crossed with another blue-flowered variety, Pskow. He concluded, from this, that the observed deficiency of white-flowered F_2 plants from the Stockholm x Weimar cross could not be due to lower zygotic viability of the b_1b_1 genotype per se. Instead, he assumed that Weimar possesses a "disturbance" factor (or factors) which caused the aberrant segregation ratio.

Although Kappert (1930, 1935) reported that the white-flowered and heterozygous blue-flowered plants produced less seeds per capsule than the homozygous blue-flowered plants, he showed that the difference was not sufficient to account for the total observed deficit of white-flowered plants in the F_2 progeny. That is, he showed this deficit could not be due, wholly, to a pre-natal inviability of zygotes with genotype b_1b_1.

Furthermore, he showed, in contrast to Tammes (1914), that among F_2 progeny with a deficiency of white-flowered plants, there was also a significant deficiency of heterozygous blue-flowered plants. This observation suggested that the aberration was occurring at the gametic rather than the zygotic stage, and that the F_1 plant produced less gametes with gene b_1, than with its allele, B_1. Kappert (1930, 1935) made reciprocal crosses between

blue-flowered Weimar x Stockholm F_1 plants and the white-flowered parent, Stockholm, and found that the aberrant segregation ratio occurred only when the F_1 plant was used as the female parent.

These results proved that the F_1 plants produced a deficiency of gametes with gene b_1, but only among the female gametes. Kappert concluded that this deficiency was not due to inviability of the female gametes with gene b_1, since, like zygotic inviability, this would have affected the number of seeds developed per capsule on the F_1 plants.

Instead, he suggested that competition occurred between megaspores in the same ovule such that those with gene B_1, were selected more often than those with gene b_1, for development into the embryo-sac. However, he was not able to provide conclusive cytological evidence for this postulate (see Section f below).

Henry (1930), Myers (1936) and Barnes et al (1960) have also reported a deficiency of white-flowered plants in the F_2 progeny from crosses between pairs of white and blue-flowered flax varieties, differing with respect to gene B_1. However, only Myers made an attempt to analyse the cause of the observed aberrant segregation ratio. He found that white-flowered plants were less viable than blue-flowered plants in F_3 families, but the

difference was not sufficient to account for the total deficiency of white-flowered plants observed in the F_2 progeny. Barnes et al did not refer to Kappert's extensive studies and postulated that the observed deficiency of white-flowered plants in the F_2 was due to lower viability of the b_1b_1 zygotic genotype, as suggested by Tammes (1914).

There is a striking similarity between the aberrant segregation ratios for rust reaction, observed in the present study (Tables 8 and 12), and the above aberrant segregation ratios for flower colour, in that both aberrations are apparently restricted to the female gametes. This similarity suggested that both examples of aberrant ratios may have a common cause: if so, it could be that the observed results were due to linkage between the N, P and B_1 loci.

It was possible to test this hypothesis with F_2 progeny from Koto x Ottawa 770B and Bombay x Ottawa 770B crosses. Ottawa 770B possesses white-crimped petals, yellow anthers and yellow seed and has given a deficiency of white-flowered plants in the F_2 progeny from crosses with several blue-flowered flax varieties (Henry, 1930; Myers, 1936; Barnes et al, 1960). These reports indicate, as stated by Plonka (1957), that Ottawa 770B possesses the basal flower colour genotype $b_1b_1B_2B_2CC$.

Koto and Bombay have blue flowers and therefore both must possess the dominant genes B_1, B_2 and C (Kappert, 1924).

Previous tests showed that Koto and Bombay each possess a single gene conferring immunity to rust stocks F-1 and P-1 (Table 5), and these were assumed to be Flor's (1955) P and N genes, respectively (p.39+48). Rust stock 5 was obtained specifically for testing the rust reaction of these F_2 progeny, since Ottawa 770B is susceptible and both Bombay and Koto are immune to this stock. However, it was not known whether the N gene in Bombay and the P gene in Koto are responsible for this immunity. Consequently, sixty F_2 progeny plants from each of Bombay x Hoshangabad and Koto x Hoshangabad crosses, were tested jointly with rust stocks F-1 and 5. Progeny plants gave the same reactions with both stocks, indicating that gene P in Koto, and N in Bombay, are effective against stock 5, as well as stock F-1.

Therefore, Ottawa 770B x Bombay and Ottawa 770B x Koto hybrids are heterozygous for genes at the N and B_1 loci, and the P and B_1 loci, respectively, and the joint classification of their F_2 progeny for flower colour and rust reaction to stock 5, was expected to provide data suitable for detecting linkage between these loci.

For convenience, following Kappert (1930, 1935), the

F_2 progeny plants were classified for hypocotyl colour rather than flower colour, since there is a complete association between the presence of the B_1 gene for flower colour and the occurrence of a red-brown (anthocyanin) pigment in the hypocotyl of flax seedlings (Sylven, 1925). Seedlings without the B_1 factor do not produce this pigment and, consequently, have green hypocotyls (Figure 4B).

F_2 seeds derived from three Ottawa 770B x Koto F_1 plants and four Ottawa 770B x Bombay F_1 plants were germinated on moist filter paper in petri dishes. After four to five days growth, the hypocotyl colour of the seedlings was recorded and the seedlings were then planted in soil in earthenware pots. Approximately four weeks later, the rust reaction of these plants to stock 5 was determined.

The data obtained from each F_1 plant within a family were homogeneous and the pooled results from these joint classifications are given in Table 14.

The individual segregation ratios in the Ottawa 770B x Koto F_2 progeny agreed with the 3:1 ratios expected if a single gene difference controlled hypocotyl colour, and another single gene controlled rust reaction. Furthermore, there was no evidence of any correlation between these two classifications ($\chi^2 = 0.17$, $P = 0.5\text{-}0.7$). Therefore,

FIGURE 4A.

Seedlings of flax plants showing presence of anthocyanin pigment in hypocotyls of Bombay (left), and absence in Ottawa 770B (right).

TABLE 14

HYPOCOTYL COLOUR AND REACTION TO RUST STOCK 5, OF PARENTS AND F_2 PROGENY DERIVED FROM OTTAWA 770B X KOTO AND OTTAWA 770B X BOMBAY CROSSES

PHENOTYPE	PARENTS		F_2 PROGENY								
			JOINT SEGREGATION					INDIVIDUAL SEGREGATION			
								OBSERVED FREQUENCY	EXPECTED RATIO	x^2_1	HETEROGENEITY≠ x^2 / df
	Ottawa 770B	Koto									
Hypocotyl colour	Green	Red-Brown		Red-Brown		Green		RB: Gr = 439:143	3:1	0.06	4.44 / 3
Rust reaction≠	S	I		I	S	I	S	I : S = 445:137	3:1	0.66	5.26 / 3
			Observed frequency	338	101	107	36				
	Ottawa 770B	Bombay									
Hypocotyl colour	Green	Red-Brown		Red-Brown		Green		RB: Gr = 455:39	3:1	60***	0.02 / 1
Rust reaction	S	I		I	S	I	S	I : S = 360:134	3:1	1.19	0.04 / 2
			Observed frequency	331	124	29	10				

≠ Heterogeneity x^2 between groups of F_2 progeny from individual F_1 plants.

≠ I = immune S = susceptible (see Table 4).

*** x^2 significant at 0.001 level of probability.

it may be assumed that the gene conferring rust reaction (P) segregates independently from the gene controlling hypocotyl pigmentation (B_1). The segregation for rust reaction in the Ottawa 770B x Bombay F_2 progeny agreed with a monohybrid segregation ratio, but the segregation for hypocotyl colour did not. Instead, this agreed with a 15:1 ratio ($\chi_1^2 = 2.28$, $P = 0.1-0.2$), suggesting that duplicate genes control pigment colour in the hypocotyl of the Bombay parent. Again, there was no evidence of any correlation between the two classifications ($\chi_1^2 = 0.001$, $P = 0.9-1.0$).

Duplicate genes controlling flower colour have not been reported in the extensive literature on the genetics of flower colour in flax. Therefore, it is possible that the observed approximate 15:1 segregation ratio represents an aberrant monohybrid ratio, similar to that described by Kappert (1924).

An attempt was made to distinguish between these two hypotheses by classifying the hypocotyl colour of the F_3 progeny from sixteen blue-flowered plants in the Ottawa 770B x Bombay F_2 progeny. Also, five of these blue-flowered F_2 plants were used as the male parent in crosses with their white-flowered sibs, and the hypocotyl colour of the progeny was recorded. The results of these classifications are given in Table 15.

TABLE 15

HYPOCOTYL COLOUR OF F_3 AND SIB CROSS PROGENY SEEDLINGS DERIVED FROM BLUE-FLOWERED (OTTAWA 770B X BOMBAY) F_2 PLANTS

F_2 PLANT NUMBER	SEEDLING HYPOCOTYL COLOUR							
	F_3 PROGENY				SIB CROSS[1] PROGENY			
	OBSERVED FREQUENCY		χ^2_1 ON EXPECTATION OF		OBSERVED FREQUENCY		χ^2_1 ON EXPECTATION OF	
	Red-Brown	Green	3:1	15:1	Red-Brown	Green	1:1	3:1
1	76	15	3.52	16.26***	13	14	0.04	10.38**
2	89	15	6.21*	11.86***	22	23	0.02	16.36***
3	87	18	3.46	21.26***				
4	106	18	7.27**	14.46***				
5	82	11	8.61**	4.94*				
6	92	16	5.98*	13.52***				
7	62	9	5.75*	5.00*				
8	67	8	8.22**	2.50				
9	43	4	6.82**	0.41				
10	117	0			27	1 ≠		
11	53	0			48	0		
12	36	0			25	0		
13	111	0						
14	91	0						
15	117	0						
16	43	0						

≠ Blue-flowered F_2 plants used as male parent in crosses with white-flowered sibs.

≠ Probably due to self fertilization of white-flowered female parent.

* χ^2 significant at 0.05 level of probability.

** χ^2 significant at 0.01 level of probability.

*** χ^2 significant at 0.001 level of probability.

The segregation ratio in five of the nine F_3 families which segregated for hypocotyl colour, did not agree with either the 3:1 or 15:1 ratio expected on a duplicate gene hypothesis.

On the other hand, the F_3 results showed a feature similar to that found by Kappert (1935) in his studies. He reported that when the percentage of seedlings with green hypocotyls was much less than the expected 25% in a segregating family (e.g. 11.9%), the average percentage in the progeny from selfed heterozygous plants in this family was much closer to 25% (e.g. 19.1%). In the present study, the percentage of F_2 seedlings with green hypocotyls was 7.89% (Table 14), whereas the average percentage among the segregating F_3 families was 16.64% (Table 15).

Furthermore, F_2 plant No. 2 (Table 15) produced an aberrant monohybrid segregation ratio for hypocotyl colour in F_3 progeny, but this same plant gave a normal 1:1 ratio among progeny when used as the male parent in crosses with white-flowered sibs. This result is consistent with one of the observations made by Kappert (1930) in his study, namely that the aberrant segregation ratios for flower colour occur only among the female gametes of heterozygotes.

It is inferred, from the above three features of

the F_3 results, that the approximate 15:1 ratio for hypocotyl colour is not due to the segregation of duplicate genes, but represents an aberrant monohybrid segregation ratio, similar to that reported by Kappert (1924).

The present study showed that the aberrant segregation ratios for rust reaction (Table 10), and those for flower (or hypocotyl) colour, must be controlled by completely separate events, since:

(1) There was no evidence of linkage between the B_1 locus and the N and P loci.

(2) The occurrence of an aberrant monohybrid segregation ratio for hypocotyl colour was accompanied by a normal 3:1 ratio for rust reaction in the Ottawa 770B x Bombay F_2 progeny.

(3) Whenever an aberrant segregation ratio occurred for rust reaction in progeny derived from Bombay, there was a deficiency of the N gene derived from Bombay (Table 10). However, there was an excess of the B_1 gene derived from Bombay in the aberrant F_2 segregation ratio for hypocotyl colour (Table 14).

(4) Bombay and Koto behaved similarly in producing aberrant segregation ratios for rust reaction in crosses with other varieties (Table 10), whereas Bombay, and not Koto, was associated with an aberrant segregation ratio

for hypocotyl colour, in crosses with Ottawa 770B.

d. Inheritance of aberrant segregation ratios for rust reaction

Although Kappert (1935) made an extensive study of the 'heritability' of the aberrant segregation ratios for flower colour in flax, there is no information in the literature on the inheritance of aberrant segregation ratios for rust reaction.

Kappert concluded from his studies that:

(1) In crosses between white-flowered Stockholm and blue-flowered Weimar, the Weimar parent contributes several "disturbance" factors to the F_1 plants and these factors interact with the b_1 gene from Stockholm, to produce an aberrant segregation ratio of genes B_1 and b_1, among the female gametes.

(2) These "disturbance" genes are inherited independently from the b_1 gene.

(3) The degree of aberration observed in the progeny is greatly affected by the environment.

However, when it was found that aberrant segregation ratios for flower colour and rust reaction are controlled by separate events (Section c above), a study was initiated to determine the mode of inheritance of the latter aberration.

This investigation was made with test cross progeny derived from crosses between Polk-Bombay F_1 plants and Hoshangabad. Polk crossed with Hoshangabad gave an aberrant F_2 segregation ratio for rust reaction, whereas Bombay crossed with Hoshangabad gave a normal monohybrid ratio (Table 5). That is, crosses between Polk and Bombay involved one parental variety giving an aberrant F_2 segregation ratio, and the other a normal F_2 ratio, in crosses with Hoshangabad. Therefore, it was expected that the genetic factor (or factors) responsible for the aberrant ratio would segregate in the gametes of Polk-Bombay hybrids and that, in test crosses with Hoshangabad, those progeny plants which received this factor would produce, in turn, an aberrant segregation ratio for rust reaction among their progeny. On the other hand, test cross progeny which did not receive this disturbance factor would be expected to give normal segregation ratios for rust reaction among their progeny.

It was shown earlier that Bombay and Polk each possess a single gene (genes N and N^1, respectively) controlling reaction to tester rust strains, and that Hoshangabad possesses the recessive form (n) of these genes (Tables 5 and 6). Furthermore, it was shown that the great majority of progeny plants, from crosses between Polk-Bombay hybrids and Hoshangabad, possess

either gene N, or N^1, in combination with the recessive gene 'n' from Hoshangabad (Table 6). Therefore, the presence of the factor (or factors) causing the aberrant ratio in the female gametes could be detected by determining the segregation ratio for rust reaction among the progeny derived from self-fertilization of the test cross plants.

Forty-two progeny seeds from each of (Polk x Bombay) x Hoshangabad and Hoshangabad x (Polk x Bombay) test crosses were planted, with three seeds per six-inch pot. The plants were tested with rust stock F-i to find whether they possessed genes N^1 or N, and were then left to produce seed.

In the limited time available, progeny seed of only fifteen plants from the Hoshangabad x (Polk x Bombay) test crosses were grown, and tested with rust stock F-i. A small sample of the progeny from each plant were also tested with stock 4, as an independent check on whether genes N (immune reaction) or N^1 (semi-resistant reaction) were segregating. These fifteen plants were taken from the group of test cross plants described earlier (Table 12) and nine of them possessed gene N from Bombay, and the remainder, gene N^1 from Polk. The results of the rust reaction tests with stock F-i are given in Table 16.

TABLE 16

REACTION OF FIFTEEN HOSHANGABAD X (POLK X BOMBAY) TEST CROSS PLANTS AND THEIR PROGENY DERIVED FROM SELF FERTILIZATION TO RUST STOCK F-1

GENE CONTROLLING RUST REACTION IN TEST CROSS PLANT	TEST CROSS PLANT NUMBER	REACTION[/] OF INDICATED PLANTS			x_1^2 (3I:1S)	% SUSCEPTIBLE PLANTS (25% EXPECTED)	
		TEST CROSS	PROGENY I	PROGENY S		FROM INDIVIDUAL TEST CROSS PLANTS	FROM EACH GROUP OF TEST CROSS PLANTS
N (from Bombay)	1	I	122	50	1.52	29.07	
"	2	I	127	48	0.55	27.43	
"	3	I	158	41	2.05	20.60	
"	4	I	142	46	0.03	24.47	
"	5	I	277	65	6.55*	19.01	
"	6	I	147	53	0.24	26.50	
"	7	I	162	50	0.23	23.58	
"	8	I	273	63	7.00**	18.75	
"	9	I	135	30	4.09*	18.18	
			R	S	(3R:1S)		18.7
N[1] (from Polk)	10	R	96	19	4.41*	16.52	
"	11	R	205	22	28.37***	9.69	
"	12	R	181	24	19.32***	11.71	
"	13	R	166	50	0.40	23.15	
"	14	R	191	23	23.18***	10.75	
"	15	R	166	46	1.23	21.70	
							11.6

/ I = immune R = resistant S = susceptible (see Table 4).

* x^2 significant at 0.05 level of probability.

** x^2 significant at 0.01 level of probability.

*** x^2 significant at 0.001 level of probability.

Three test cross plants with the N gene from Bombay, gave an aberrant segregation ratio (at the 0.05 level of significance) for rust reaction in their progeny, and two plants with the N^1 gene from Polk gave an apparently normal segregation ratio for rust reaction. Therefore, since the aberration effect could be removed from the N^1 gene and transferred to the N gene, it is assumed that the aberrant ratios are not caused by a property of these genes themselves. Furthermore, this recombination between genes N and N^1 and the postulated disturbance factor (or factors) occurred relatively frequently, indicating that the disturbance factor is not closely linked with these genes. However, too few progeny groups were tested to obtain a reliable estimate of the degree of recombination. Furthermore, it is possible that some aberrant segregation ratios differing only slightly from a 3:1 ratio were present, though undetected, because of the limited number of progeny tested in each family.

One feature of these results indicates that the process responsible for the aberrant ratios is not simply inherited. Among test cross plants producing aberrant segregation ratios, those with gene N from Bombay produced a much larger proportion of susceptible plants in their progeny (18.7%), than those with gene N^1 from Polk (11.6%)

($\chi^2_1 = 15.33$, $P < 0.001$).

Obviously, progeny from many more test cross plants need to be tested to obtain further information on the inheritance of these aberrant ratios, but the possible significance of the above results will be discussed in Section I. 6. ii.

e. Cytological observations

(1) Megagametogenesis in flax

It appears likely that the aberrant segregation ratios for rust reaction, observed in this study, were not due to differential viability of zygotes, but were restricted to the products of megagametogenesis. Therefore, knowledge of the details of megasporogenesis and embryo-sac formation in flax, was required to help decide the type of process causing these aberrant ratios.

Jönsson (1879) reported that young flax ovules possess many archespore cells, and frequently several of these function directly as megaspore mother cells, each dividing to give four daughter cells. He stated that all of the daughter cells degenerate except the innermost (chalazal) cell in one row, and this cell develops into an eight-nucleate embryo-sac.

Schürhoff (1924) also reported that several archespore cells occur in the flax ovule, but he considered that only

one of these functions as a megaspore mother cell.

Kappert (1935), in his study of abnormal segregations for flower colour, reported the presence of several archespore cells in the young ovule and he considered that more than one of these cells divides by meiosis to produce four daughter cells. He stated that according to the literature the innermost (chalazal) megaspore of a tetraspore row always develops into the embryo-sac, but he considered that a sister megaspore could develop instead. However, no direct evidence for this latter possibility was given.

Although the above workers did not agree on the number of mother cells dividing in each flax ovule, it was generally agreed that only one embryo-sac develops. Therefore, the aberrant segregation ratio which occurred in the female gametes from, for example, Bombay-Polk hybrids (Table 6) could have been due to, either (1) an abnormal event occurring during meiosis, which results in preferential segregation of the chromosome bearing the N^1 gene to the functional (chalazal) megaspore, or (2) preferential selection of those megaspores possessing the N^1 gene, for development into the embryo-sac.

These two processes can be distinguished relatively easily in those plant species in which a single mother cell divides to give a row of four megaspores, and the functional

megaspore normally occurs at either the chalazal or the micropylar end of the row, depending on the species. For example, Rhoades (1952) found that in maize plants heterozygous for two types of chromosome ten, 70% of the female gametes possessed the abnormal chromosome ten, instead of the 50% expected. The chalazal megaspore always developed into the embryo-sac in these hybrids and, therefore, he concluded that the aberrant ratio was due to preferential segregation of the abnormal chromosome ten to this megaspore, during meiosis.

The characteristics of the contrasting process of megaspore selection are shown in Renner's (1921) studies with Oenothera muricata, composed of the rigens and curvans chromosome complexes. Renner found that only the rigens complex was transmitted by the egg. In Oenothera species, one mother cell divides to give a row of four megaspores and normally the micropylar spore develops into the embryo-sac. However, Renner found that in Oe. muricata, either the micropylar or the chalazal megaspore could develop, indicating that the genotype of the megaspore, rather than its position, determined its future development. Since only the rigens complex was recovered in the egg, there must have been complete selection against megaspores with the curvans complex.

However, when several megaspore mother cells divide in each ovule it may be impossible to distinguish between these two processes. That is, if several rows of four megaspores are formed in plants which produce an aberrant segregation ratio among female gametes, and a chalazal cell regularly develops into the embryo-sac, this cannot be taken as proof that preferential segregation of a particular chromosome is occurring. It is possible that selection occurs among the several chalazal megaspores in an ovule, and that the particular chalazal spore with the favoured genotype is selected to develop into the embryo-sac.

As described above, Jönsson and Kappert reported that several megaspore mother cells divide in each ovule of flax. However, the writer considered that their observations did not demonstrate conclusively that these cells divide by meiosis, rather than mitosis. For example, Kappert (1935) reported that dyad and tetrad cell stages of meiosis occur in very young ovules, but, in the opinion of the writer, the particular cells shown in his figures could have resulted from mitotic divisions of archespore cells. Jönsson reported that several mother cells each divide to give four daughter cells, but it can only be inferred that these divide by meiosis, since his observations were made before the characteristics of meiosis

had been described. Furthermore, Schürhoff reported that only one mother cell divides in each flax ovule and this indicates that Kappert's and Jönsson's evidence for multiple mother cell divisions may have been based on mitotic, rather than meiotic divisions.

Consequently, megagametogenesis in a particular flax hybrid was studied to find, first, whether more than one mother cell divides in each ovule and, second, which one of the megaspores develops into the embryo-sac.

Flower buds of different ages were taken from a plant with pedigree Hoshangabad x (Polk x Bombay), and which produced an aberrant segregation ratio for rust reaction in its progeny derived from self-fertilization.

These buds were fixed in Randolph's (1935) modified Navashin's fluid, embedded in paraffin wax and serial sections (10μ thickness) were produced. The sections were stained with either crystal violet (Figure 7) or cotton red and the counter-stain, light green (Figures 5, 6, 8 and 9). The nucleoli and condensed chromosomes were stained intensely with crystal violet and cotton red, but the chromatin material in non-dividing, prophase and telophase nuclei was poorly stained. Light green stained the cytoplasm.

The stages in the development of the embryo-sac

observed in this study, are shown in Figures 5 to 9. The earliest stage observed, apparently before megaspore mother cells had begun to divide, is shown in Figure 5.

However, most observations were made at a later stage (Figures 6, 7 and 8), when the inner integuments had grown well beyond the nucellus and were closely apposed to produce a long narrow micropyle. The obturator had grown across the top of the ovule and the integumentary tapetum was clearly differentiated. The most conspicuous feature of the nucellar region at this stage is often a group of very long cells with tapered ends, which occur directly below a micropylar cap of cells (Figures 6 and 7). These elongated cells are considered to be megaspore mother cells and in fact their size, shape and arrangement, resembles megaspore mother cells described in Casuarina equisetifolia (Swamy, 1948). The presence of several of these cells, with at least two undergoing division, in the one ovule (Figures 6 and 7), is considered good evidence that more than one meiotic division may occur in each ovule of flax. It is conceivable that some of these cells represent megaspores developing into an embryo-sac, but the presence of a partly formed transverse cross-wall in one of the dividing cells (Figure 7) proves that this one, at least, is not a megaspore.

FIGURE 5.

Median longitudinal section of a young ovule of flax showing several non-dividing megaspore mother cells.

o, obturator; i.i., inner integument; o.i., outer integument; m.m.c., megaspore mother cell; no, nucleolus (stained); n., nucleus (unstained).

x 800

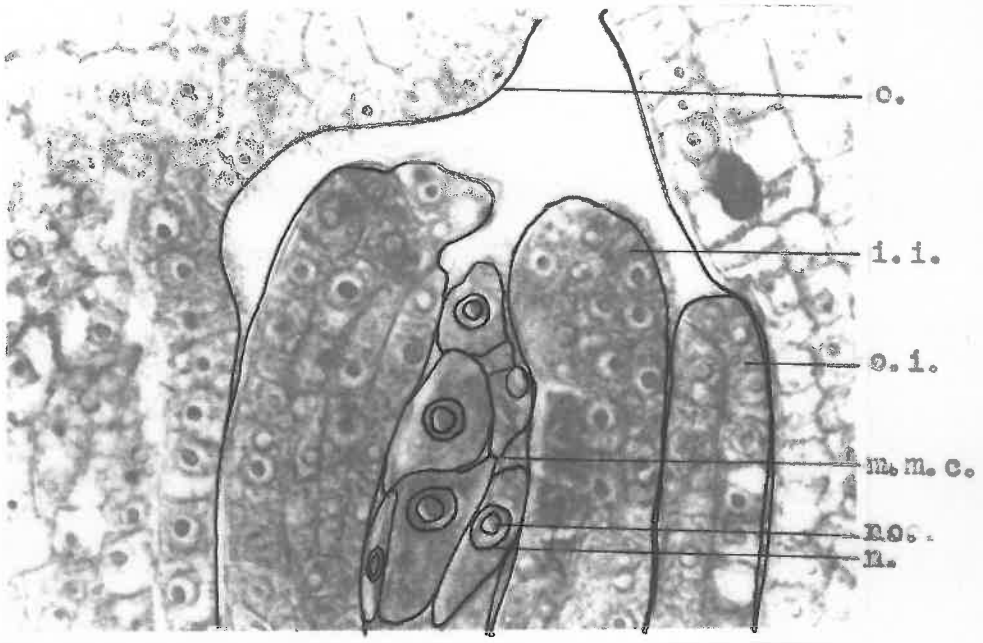

c.
i. i.
o. i.
m. m. c.
n. c.
n.

FIGURE 6.

Median longitudinal section of a flax ovule, at two magnifications, showing two dividing and two non-dividing cells in the sporogenous tissue.

cell (1) - The position of the single metaphase plate suggests that this cell is either a developing megaspore at the stage of second mitotic division, or a megaspore mother cell at the second division stage of meiosis. However, neither a second nucleus, nor a cross wall, was observed in either the preceding, or the following, serial section; therefore, this may be a megaspore mother cell, at metaphase I.

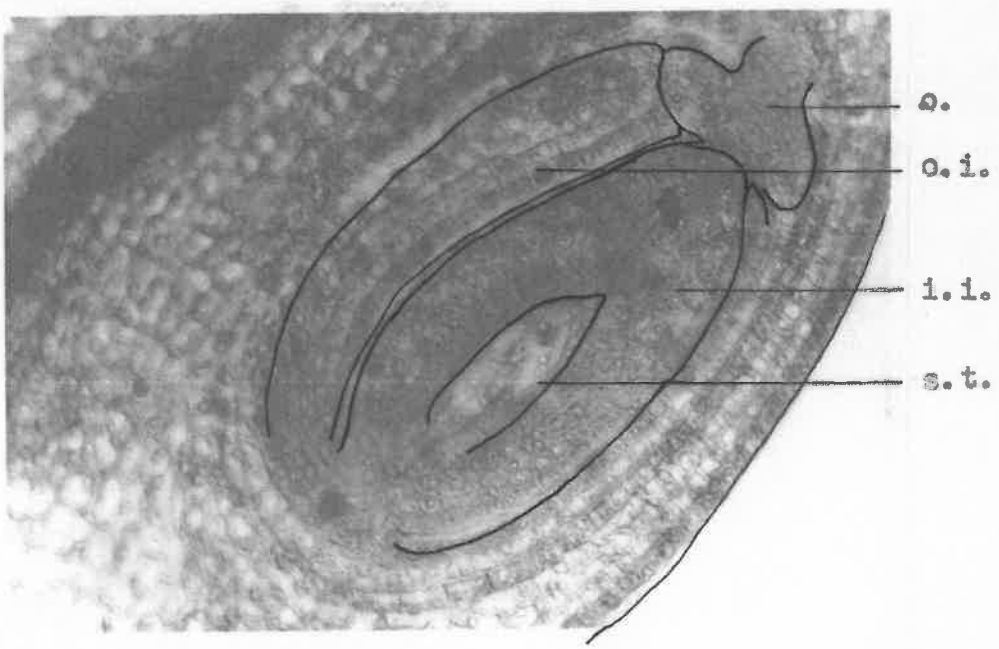
cell (2) - Megaspore mother cell at telophase I.

cells (3) and (4) - Non-dividing megaspore mother cells.

o., obturator; o.i., outer integument; i.i., inner integument; s.t., sporogenous tissue; i.t., integumentary tapetum; nu., nucellar tissue.

A - upper photograph x 210

B - lower photograph x 830


o.
o.i.
i.i.
s.t.

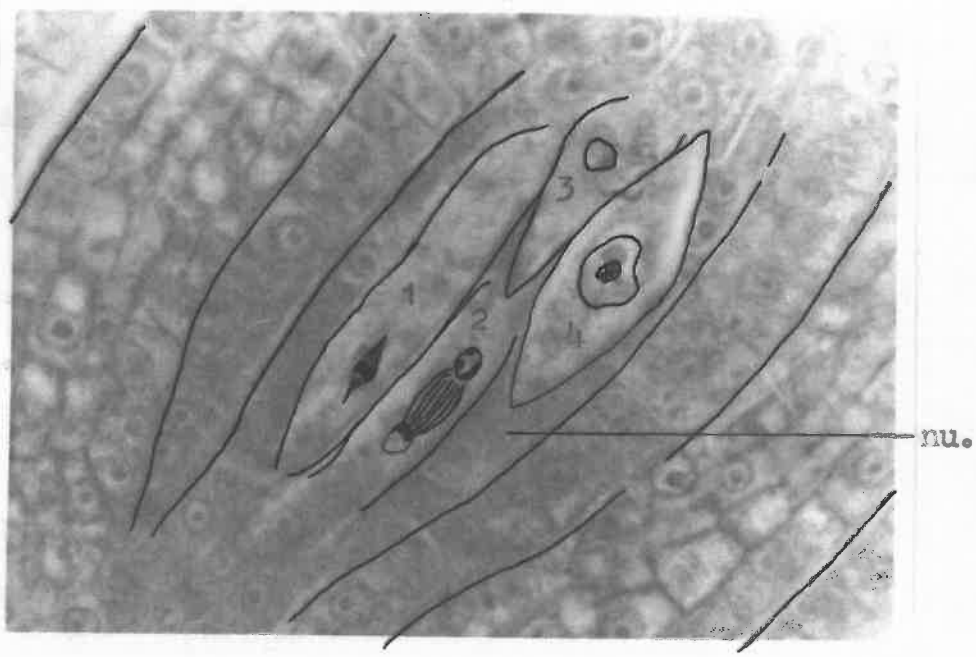

1
2
3
4
nu.

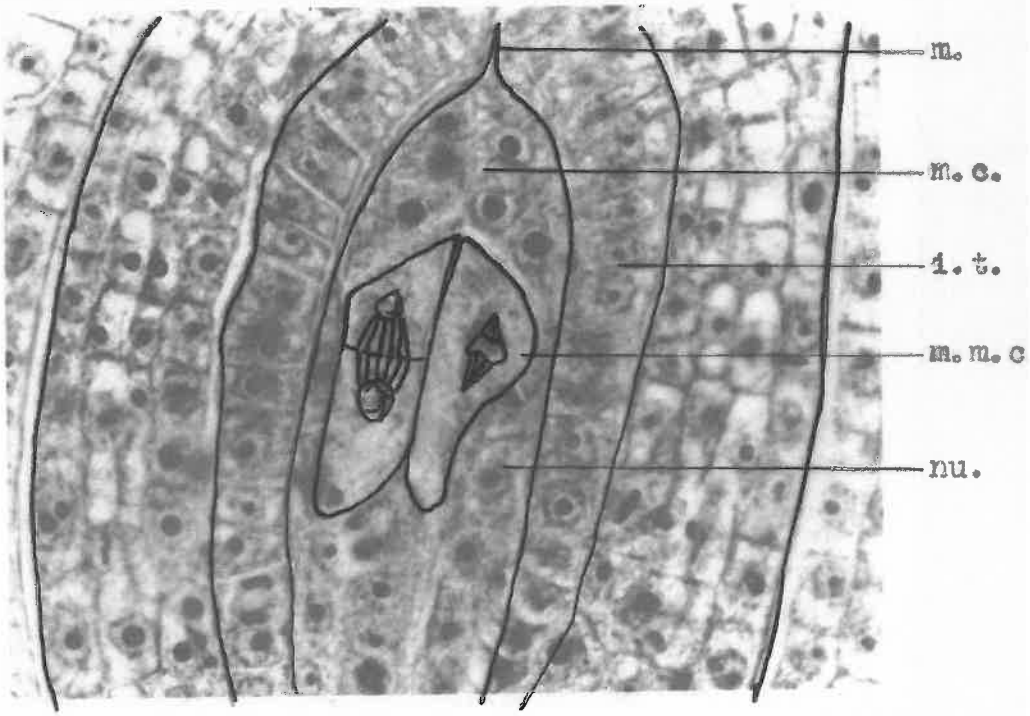
FIGURE 7.

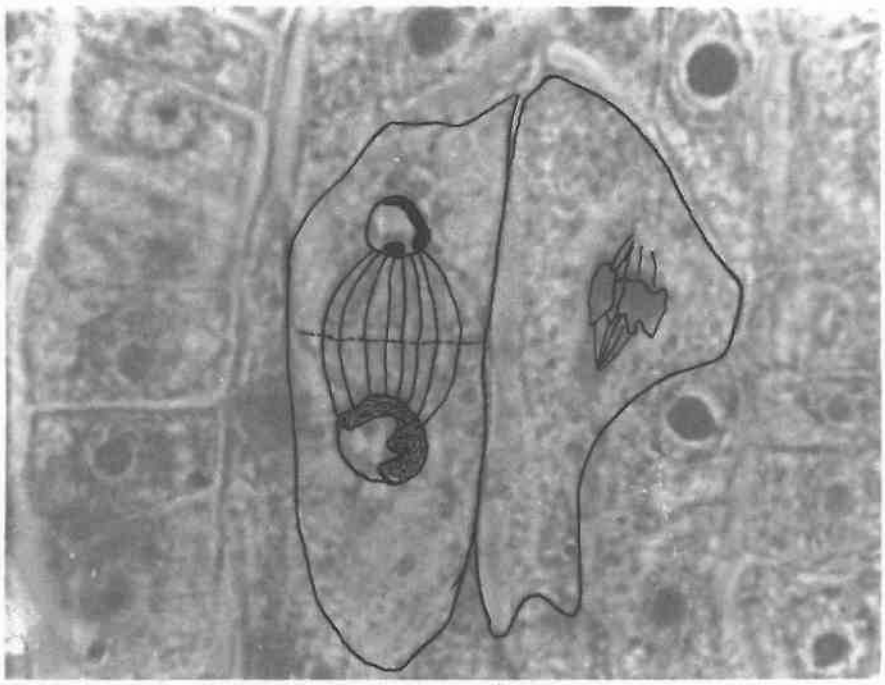
Median longitudinal section of a flax ovule, at two magnifications, showing two dividing megaspore mother cells; one at telophase I and the other at metaphase I. Note the partly formed cross-wall shown in the mother cell on the left.

m., micropyle; m.c., micropylar cap of cells; m.m.c., megaspore mother cell; nu., nucellar tissue.

A - upper photograph x 870

B - lower photograph x 1900


m.
m. c.
i. t.
m. m. c
nu.



Many ovules possess several of these elongated cells, but apparently only one such cell is present in the ovule shown in Figure 8. The division figure in this cell is probably metaphase I of meiosis, but bivalents cannot be distinguished with certainty.

Unfortunately, the stages in the development of the embryo-sac from a megaspore were not observed, but the large number, and crowded arrangement, of the presumed mother cells indicate that it will be difficult to decide which particular megaspore develops into the embryo-sac. Nevertheless, only one mature embryo-sac was observed in a given ovule (Figure 9).

It is concluded from this limited study that several mother cells occur, and divide, in flax ovules, as reported by Jönsson and Kappert. However, the interpretation of the stages in megasporogenesis differs from that of Kappert, since he postulated that the megaspore mother cells divide at least once, and often twice, before the integuments grow above the nucellus.

Furthermore, this study indicates that it will be difficult to obtain conclusive cytological evidence on whether the aberrant segregation ratios are due to either preferential segregation of a particular chromosome, or preferential selection of a particular megaspore for

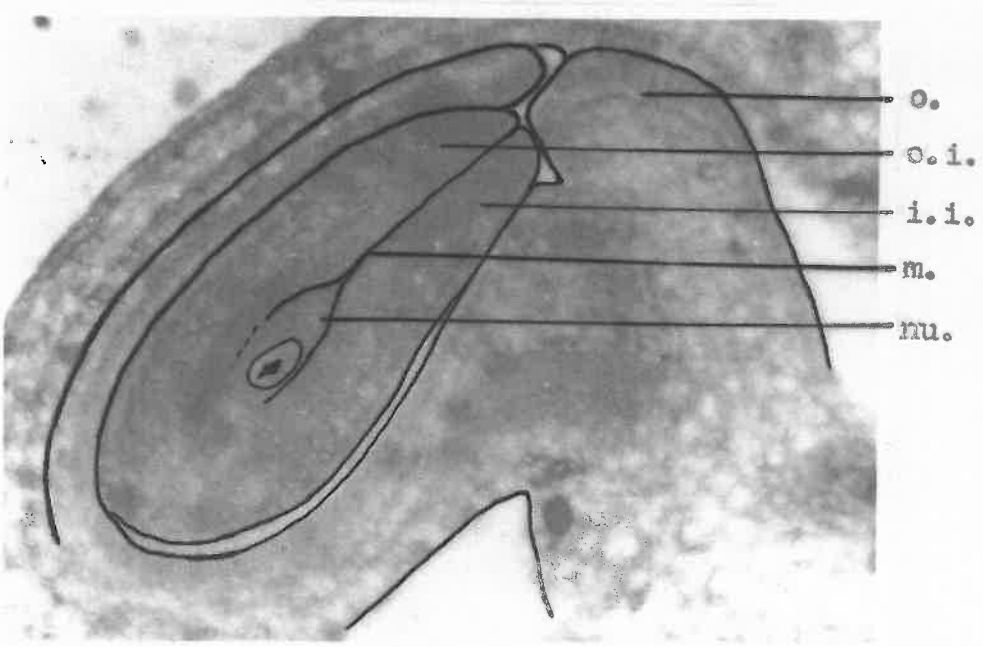
FIGURE 8.

Median longitudinal section of a flax ovule, at two magnifications, showing apparently only a single megaspore mother cell at metaphase I.

o., obturator; o.i., outer integument; i.i., inner integument; m., micropyle; nu., nucellus; i.t., integumentary tapetum; m.m.c., megaspore mother cell.

A - upper photograph x 210

B - lower photograph x 720


o.
o. i.
i. i.
m.
nu.

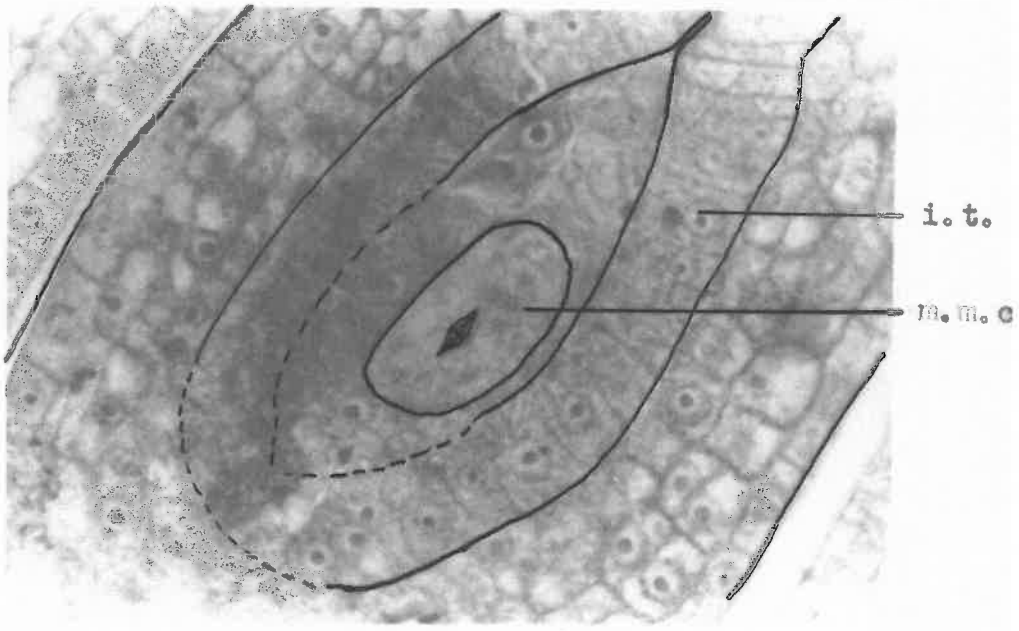
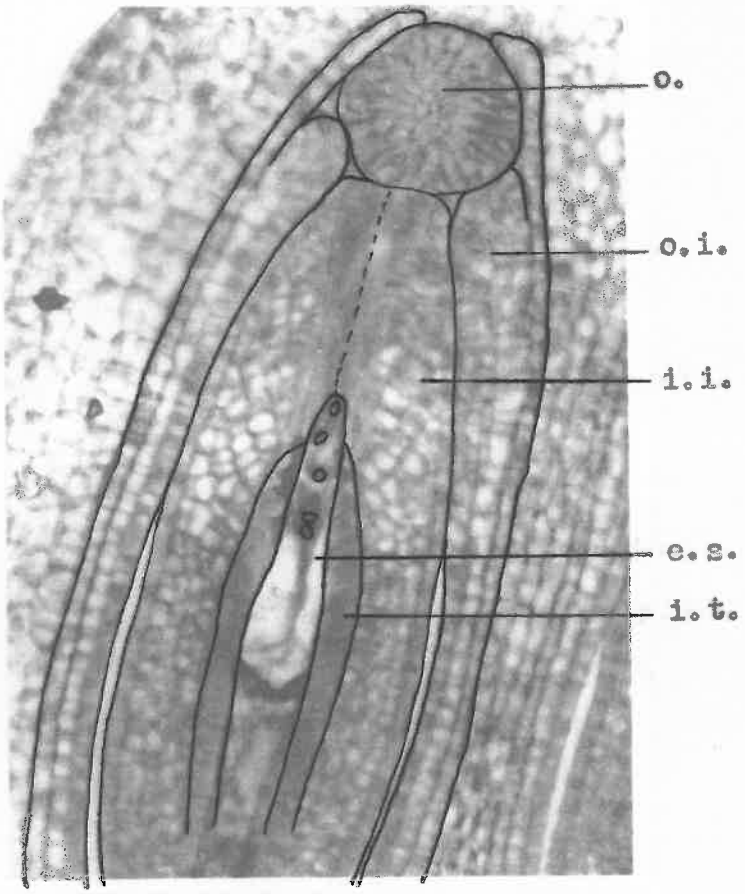

i. t.
M. m. c

FIGURE 9.

Median longitudinal section of a flax ovule showing a single mature embryo-sac.

o., obturator; o.i., outer integument, i.i., inner integument; e.s., embryo-sac; i.t., integumentary tapetum.

x 240


o.
o.i.
i.i.
e.s.
i.t.

development into the embryo-sac.

(ii) Microsporogenesis in flax hybrids

In studies of abnormal segregation ratios restricted to the female gametes, in other organisms, it has often been possible to show that the abnormal ratio is correlated with the presence of heteromorphic chromosome pairs.

For example, Rhoades (1952) showed, from a study of microsporogenesis, that the preferential segregation of chromosomes which occurred during megagametogenesis in certain maize hybrids, was correlated with the presence of both neocentromeres and one or more pairs of chromosomes heteromorphic for a heterochromatic knob. Furthermore, he constructed a model showing how neocentromeric activity could lead to preferential segregation of the knobbed member of a chromosome pair.

Also, in females of D. melanogaster with particular heteromorphic chromosome pairs, the physically shorter chromosome is usually included in the egg more often than its longer homologue. This phenomenon has occurred with chromosome pairs heteromorphic for deficiencies (Novitaki, 1951), translocations (Zimmering, 1955) and certain compound X chromosomes (Novitski and Sandler, 1956).

Thus, although the genetic effect of these chromosome aberrations was observed only among the female gametes, the particular aberration involved could be detected by a study

of meiosis in plant microsporocytes or animal spermatocytes.

Consequently, a study of microsporogenesis in Polk x Hoshangabad and Bombay x Polk hybrids was made, to find if the abnormal segregation ratios for rust reaction were also associated with a chromosomal aberration. Aceto-orcein squash preparations of microsporocytes were made, and first and second divisions of meiosis were observed.

There was no evidence of any abnormality in either chromosome structure or mode of division, in these hybrids. Fifteen bivalents were counted at metaphase I and these appeared to separate normally at anaphase I, just as observed by Ray (1944) in his studies of meiosis in flax. Similarly, the second division stages appeared to proceed normally. However, it is possible that small abnormalities in chromosome structure were present, but these were not detected, because of the very small size of flax chromosomes.

6. DISCUSSION

i. Tests for Close Linkage at L, M, N and P Loci

The main object of the present study was to search for recombination between genes postulated by Flor (1956a) to exhibit multiple allelism at the L, M, N and P loci in flax, and the experiments were designed to give sensitive tests for recombination between these genes.

The occurrence of a high frequency (greater than 1%, for example) of recombinants in these progeny groups would have provided good evidence that these genes were closely linked, rather than allelic. However, as shown in Table 17, recombinants occurred with a very low frequency and, since flax is not a suitable organism for investigating allelism per se, it is difficult to interpret the significance of these results. For example, we would like to know the mechanism, or mechanisms, responsible for producing the recombinants, and whether the genes involved in the crosses are functionally allelic or not.

As stated earlier (p.40), double-susceptible plants could have resulted from seed contamination, mutation (at a suppressor locus or at the resistance locus itself), and crossing over (equal or unequal) between the genes conferring resistance. The double-immune plants could have arisen from self-fertilization of the female parent, mutation and equal, or unequal, crossing over. Five of the six

TABLE 17

SUMMARY OF RESULTS FROM TESTS FOR RECOMBINATION BETWEEN GENES AT FLOR'S (1955) L, M, N AND P LOCI IN FLAX

F_1 HYBRID[ø]	GENES* CONTROLLING RUST REACTION IN PARENT LISTED: FIRST	SECOND	NUMBER OF TEST CROSS PROGENY ANALYSED	FREQUENCY OF RECOMBINANTS: NUMBER OBSERVED	%	97.5% CONFIDENCE LIMITS[≠] (%): LOWER	UPPER
Stewart-Birio	L^2	L^6	1,968	2	0.10	0.01	0.37
Dakota-Cass	M	M^3	2,300	2	0.09	0.01	0.31
Polk-Bombay	N^1	N	1,152	2	≠	≠	≠
Leona-Koto	P^3	P	439	0	0.00	0.00	1.39

ø Separation of host varieties with a hyphen indicates that reciprocal crosses were made.

* According to Flor (1955).

≠ Calculated from Table VIII, Fisher and Yates (1953).

≠ These values were not calculated because both recombinant classes could be detected in some of the progeny, and one class only, in others. Furthermore the occurrence of aberrant segregation ratios meant that the expectation of detectable recombinants was not necessarily p and p/2, in these respective tests.

recombinant plants survived to maturity and observations made on their progeny showed that the double-susceptible plants did not arise from seed contamination, or from a suppressor mutation, unless this occurred at a locus closely linked with the locus of the genes conferring resistance, and that the double-immune plants did not arise from self-fertilization of the female parent. However, marker genes closely linked with the genes conferring resistance are required, to help distinguish between the other alternatives.

The mechanism responsible for producing these recombinants could be decided from the following experiments, given suitable marker genes. If recombinant plants occurred in the progeny from plants homozygous for any of these genes conferring resistance, they must have arisen from either mutation or unequal crossing over. These two processes could be distinguished only if the parental plants were heterozygous for closely linked marker genes. The presence of non-parental combinations of marker genes in the recombinants would indicate that they arose from unequal crossing over, rather than from mutation.

On the other hand, if it was found that unequal crossing over did not occur in the homozygote, then the presence of non-parental marker genes in any recombinants derived from plants heterozygous for different genes conferring

resistance, would indicate that these recombinants arose from equal crossing over rather than mutation.

However, closely linked marker genes are not available in flax and, therefore, it is not possible to decide whether the recombinants arose from equal or unequal crossing over, or from mutation. Nevertheless, if all of the recombinants observed arose from spontaneous mutation of one or other of the genes conferring resistance in the F_1 plants, we must postulate a relatively high mutation rate for these genes. Furthermore, the particular rust reaction phenotypes of recombinants MR-1 and MR-2 (p. 43) is considered evidence that these, at least, did not arise from mutation.

Unequal crossing over between closely linked genes, or chromosome segments, has been reported to occur at, for example, the Bar locus (Sturtevant, 1925; Bridges, 1936) and the 'white' locus (Judd, 1961) in D. melanogaster, and the A (Laughnan, 1955) and R (Stadler and Emmerling, 1956) chromosome regions, controlling anthocyanin pigmentation in maize. The new phenotypes arising from unequal crossing over at the A and R chromosome regions occur with a frequency similar to that observed for recombinants in the present study. The occurrence of unequal crossing

over is considered evidence for duplication of loci (e.g. Laughnan, 1955) and, in particular, the presence of an adjacent serial duplication allowing asymmetric pairing between segments of homologous chromosomes. Therefore, demonstration that unequal crossing over occurs between genes controlling rust reaction would suggest that these genes may also have arisen by duplication of chromosome segments. However, the test of this hypothesis must await the production of suitable marker genes in flax, or the choice of a plant more suitable for genetical analysis.

The results obtained are also consistent with equal crossing over occurring between different closely linked genes and in some cases, at least, between heteroalleles of a single gene.

Although it is not possible to decide which type of process was responsible for producing the recombinants observed, we would like to decide whether the genes involved are either allelic or closely linked. The four double-susceptible recombinants are equally compatible with either possibility. The rust reaction phenotypes of the double-immune plants MR-1 and MR-2, with genes M and M^3 in coupling phase (*cis* arrangement), are apparently the same as the corresponding phenotypes of the F_1 plants, with these genes in repulsion phase (*trans* arrangement) (p.43). The signi-

ficance of this depends on the validity of the operational test for non-allelism between genes controlling qualitatively different phenotypes, described earlier (p. 13). This test is based partly on the assumption that non-allelic genes, each responsible for a recognizably different phenotype, would produce the same phenotype in coupling and repulsion arrangements. On the other hand, it is assumed that if these genes are allelic it would not always be possible to predict the *cis* phenotype from that of the *trans* arrangement. Therefore, on this assumption, if individuals with the *cis* arrangement of these genes were not detected among progeny with a large number of the other recombinant class, this could be taken as evidence for allelism.

Although it is an axiom of genetics that linked genes with different primary functions will produce the same phenotype in coupling and repulsion arrangement, there is very little specific evidence in the literature on whether mutant genes which are allelic, and produce qualitatively different phenotypes, will also produce the same phenotype in *trans* and *cis* arrangements. It is expected, assuming the Crick (1958) model of gene action, that the presence of two alterations in a gene product (e.g. polypeptide) would often produce an interaction, so that the properties of this doubly altered product would not be equivalent to the sum of the properties of the singly altered products.

A recent study, in which the properties of a particular protein produced by single and double mutants of E. coli were compared (Maling and Yanofsky, 1961), provides some direct information on this prediction. Maling and Yanofsky selected strains of E. coli which possessed different single deleterious mutations of the A gene concerned with the production of the enzyme tryptophane synthetase, and which produced a defective A protein. The mutations were considered to be allelic, since they were situated in the same cistron and affected the same protein. Some of the defective A proteins had qualitatively different properties; for example, some (e.g. A_{-23}) were more heat-labile than the wild type A protein, and others (e.g. A_{-11}) were precipitated by mild acid treatment, whereas the wild type A protein was not.

Strains of E. coli with two mutational alterations in the A gene were prepared, and the properties of the A protein produced by these double mutant strains were compared with those of the A protein produced by each of the parent single mutant strains. In most cases an interaction occurred between the two mutational sites, so that the properties of the doubly altered protein were different from those of either of the singly altered proteins. However, no such interaction occurred with a few combinations of mutants. For example, mutant A_{-11} produced

an A protein which was heat-labile but acid-stable, and mutant A_{-23} produced a heat-stable, but acid-precipitated A protein, and the double mutant A_{11-23} produced an A protein which combined the properties of acid-precipitability and heat-lability.

Therefore, although it was not possible to predict the double mutant phenotype from those of the single mutants in most examples, exceptions to this pattern did occur. Presumably, certain parts of the A protein have an autonomy of function so that alteration in one particular region of the molecule does not affect the properties determined by another part of the protein molecule.

Consequently, the observed rust reaction phenotypes of plants MR-1 and MR-2, possessing genes M and M^3 in coupling phase, do not necessarily mean that genes M and M^3 control different primary functions and are non-allelic. Although their phenotypes are consistent with that expected if these genes are non-allelic, it is possible to construct a model of gene action which will also explain these results, on the assumption that genes M and M^3 are heteroalleles. For example, if genes conferring rust resistance act by producing a specific antigen which interacts with a specific antibody in the rust, as has been suggested (Catcheside, 1951; Flor,

1956a; Flangas and Dickson, 1961), we may speculate that genes M and M^3 produce the same basic molecule (e.g. protein or polysaccharide), but differ by producing alterations (e.g. single amino-acid substitutions) in different parts of the molecule, which result in altered surface configurations of the molecule, providing antigenic specificity. Providing that these particular surfaces are non-overlapping, we might expect that a single molecule could possess both types of antigenic specificity.

Further information on the nature of genes M and M^3 can possibly be obtained from induced mutation studies with plants homozygous for both of these genes. If these genes are non-allelic they should mutate independently, but if allelic we would expect a high frequency of joint mutation to the inactive (susceptible) form. However, flax is not a suitable organism for such studies, because of the difficulty in producing a large number of progeny from controlled crosses.

Although it is not possible to decide the mechanism responsible for producing the recombinant plants observed, and whether the genes involved are allelic or not, we may conclude that these genes are very closely linked, and if not situated at different loci, they represent hetero-alleles rather than mutations at the same site in the

gene.

These results may be compared with other studies of recombination between genes conferring resistance to pathogens and, in particular, with the reports of allelism between genes in flax conferring resistance to rust. Since recombinants occurred with such a low frequency, it is not surprising that Flor (1947) did not observe any of these in the F_2 families he studied, where the expectation of detectable recombinants was $p^2/4$. However, recombination might have been expected to occur among the 584 F_3 families derived from crosses between the flax varieties Buda and J.W.S., and used to test for allelism between L genes (Flor, 1941), since the corresponding expectation in these families was approximately 2p. Recently, Flor (personal communication) produced 1,042 and 3,306 test cross progeny from Ottawa 770B x Stewart and Stewart x Birio hybrids, respectively. The rust reaction of these progeny was determined, to search for recombination between genes L and L^2, and L^2 and L^6, respectively, but no recombinant phenotypes were observed, although both classes of recombinants could have been detected in these families. The results obtained with the latter family may be contrasted with those found in the same family in the present study. Flor's results indicate that the true frequency of recombination between genes L^2 and L^6 is, most

likely, less than that observed in the present study (Table 17). For, if we assume a recombination frequency of 1/1,000, the probability that no recombinants will occur in a family of 2,206 test cross individuals is only 0.04.

Similarly, recombinants would not be expected in the F_2 families of flax plants studied by Mayo (1955) and Kerr (1960). Also, the test cross families analysed by Kerr were relatively small; the largest family in which the expectation of detectable recombinants was p/2, consisted of 83 individuals, and the largest family in which the corresponding expectation was p/4, consisted of 182 individuals. The non-occurrence of detectable recombinants in such test cross families is quite consistent with the results obtained in the present study.

There are several reports of multiple allelism, or close linkage, between host genes conferring resistance to other fungal pathogens. For example, Smith (1934), Cochran et al (1945) and Litzenberger (1949) considered that the 'Rainbow' and 'White Russian' genes in oats, conferring resistance to stem rust, were allelic. However, later Koo et al (1955) studied a variety of oats (LHMJA), which apparently possessed the 'Rainbow' and 'White Russian' genes in coupling phase, and showed that these genes can be recombined with a frequency of approximately 1.5%.

They concluded that these genes are either pseudo-alleles, which had been combined in variety LHMJA by equal crossing over, or alleles, which had been combined in this variety as a duplication arising from rare unequal crossing over. Unfortunately, they did not report any tests designed to distinguish between these hypotheses. However, on the criteria for non-allelism adopted in the present study, the 'Rainbow' and 'White Russian' genes conform to closely linked genes with different functions, rather than pseudo-alleles, since they can be recombined with a relatively high frequency and apparently function autonomously in coupling arrangement.

Several workers have reported that some of the genes in barley conferring resistance to powdery mildew are allelic, or closely linked (Schaller and Briggs, 1955; Luig et al, 1958; Moseman and Schaller, 1959). No recombination has been detected between at least six different genes postulated to occur at the Mla locus, and the most critical evidence for allelism between these genes, comes from the work of Schaller and Briggs (1955). Their studies exclude recombination percentages greater than 0.3%, with 95% certainty, between two genes at this locus. However, this limit is above the frequency of recombination observed in the present study and further studies may show that these genes at the Mla locus can also be recombined

with a very low frequency.

Hooker and Russell (1962) postulated that the six genes conferring resistance to rust, identified in maize, all occur at a single locus, the Rp locus. They studied F_2, F_3 and test cross progeny from hybrids heterozygous for different genes conferring resistance, and did not detect any plants which could be classified as recombinants with certainty.

However, in a total of 3,286 test cross seedlings derived from several different hybrids, and in which only the susceptible recombinant class could be detected, one susceptible plant occurred. This plant was detected among the progeny from the K148 x B38 hybrid (395*), but, since no susceptible plants occurred in the progeny from K148 x GG208R (487), K148 x Cuzco (390), B38 x GG208R (350) and B38 x Cuzco (401) hybrids, they concluded that this susceptible plant did not arise from crossing over. However, the non-occurrence of recombinants in these families cannot be considered evidence against the hypothesis that the susceptible plant arose from crossing over, since if recombination occurred with a low frequency, recombinants would not be expected to occur in these relatively small

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* Number of test cross progeny produced.

families. They suggested that this plant came from seed contamination, but apparently the test cross progeny did not possess diagnostic marker genes and, therefore, it is not possible to decide from their data whether seed contamination had occurred, or not.

If we assume that this plant originated from crossing over, then the frequency of recombination between genes at the Rp locus is of the same order as that observed in the present study, remembering that only one recombinant class could be detected in their studies.

Maize is relatively well adapted for studies of allelism, since large numbers of progeny can be obtained from a single cross and it possesses many marker genes. However, apparently there are no marker genes closely linked with the Rp locus (Flangas and Dickson, 1961). This locus has been located near the end of the short arm of chromosome ten (Rhoades, 1935) and the nearest marker is 'old gold stripe', 16 map units away (Rhoades, 1950). If marker genes can be induced closer to the Rp locus, or this locus can be translocated to a chromosome region with many marker genes, then it would be possible to make a detailed study of the relationship between the genes postulated to occur at the Rp locus. For example, if recombinants were detected it would be possible to decide whether they arose from mutation, or from equal or unequal crossing over. Furthermore, if

progeny plants analogous to plants MR-1 and MR-2 occurred, it would be possible to carry out mutation studies to find whether the genes involved were allelic, or not.

The above discussion emphasizes the limitation of our current knowledge of the 'fine structure' of genes concerned with disease resistance in plants, but special attention has been given to ways in which additional information on this may be obtained. This information should help us understand the mode of action, and evolution, of these genes conferring resistance to pathogens. The implications of the results from the present study on these questions will be discussed in Section III.

11. Nature of Aberrant Segregation Ratios

The present study, besides confirming some of Flor's reports of aberrant segregation ratios for genes located at the N and P loci in flax, shows some of the characteristics of these aberrations.

The aberrant segregation ratios occur among test cross progeny when the relevant F_1 hybrids are used as the female parent in crosses, but not when used as the male parent. Therefore, it is considered likely that they are not due to reduced viability of one class of zygotes, but occur at the gametic stage and, furthermore, only among

the female gametes.

This indicates that these aberrant ratios are due to either

(1) preferential segregation of a chromosome bearing a particular N or P allele, to the functional megaspore, during meiosis, or

(2) preferential selection of megaspores with a particular N or P allele, for development into the embryo-sac.

The limited observations of microsporogenesis and megagametogenesis in F_1 hybrids producing aberrant segregation ratios, made in this study, do not indicate which one of these processes is occurring. However, the study of megagametogenesis confirms previous reports that several mother cells divide in each flax ovule, and therefore, even with a more comprehensive cytological investigation, it would be difficult, and perhaps impossible, to determine whether preferential segregation or preferential selection is occurring.

It is not possible to decide the exact mode of inheritance of these aberrant ratios, from the present study. However, two particular observations indicate that more than one genetic factor (disturbance factor) is responsible for them.

First, if a single factor is responsible it would be expected that, with Hoshangabad x (Polk-Bombay) test cross plants producing aberrant segregant ratios in their progeny (Table 16), those test cross plants with gene N would produce the same proportion of susceptible plants among their progeny, as those with gene N^1. However, the plants with gene N gave a much higher proportion of susceptible plants than those with gene N^1. This result could have been caused by the presence of two or more disturbance factors linked with the N^1 gene, in the Polk parent. Given these two (or more) disturbance factors, recombination in Polk-Bombay hybrids would occur more frequently between the N gene (from Bombay) and the disturbance factor (assumed to be from Polk) more distant from the N locus; that is, the disturbance factor having least effect on segregation at the N locus by virtue of its distant position. However, progeny from many more test cross progeny must be tested to obtain further information on this suggestion.

Secondly, the survey of literature showed that aberrant segregation ratios do not occur in all cases expected, assuming that flax varieties exist in two hypothetical groups, A and B, with the properties described previously (p.58). If several genetic factors are

responsible for the aberrant ratios, only crosses between parental varieties possessing a widely different number of disturbance factors would be expected to produce detectable aberrant ratios in a limited number of F_2 progeny. Nevertheless, the results obtained in the present study from F_2 and/or test cross progeny from crosses between Polk, Leona, Hoshangabad, Koto and Bombay in all combinations (Table 10, 12, and 13 and p. 65) showed that Polk and Leona exist in one hypothetical group (A), and Koto, Bombay and Hoshangabad occur in the other group (B).

The hypothesis that all host varieties exist in one or other of these two groups could be tested by producing large F_2 families from crosses between Bombay (A group) and a series of host varieties, and from crosses between Polk (B group) and the same varieties, and observing the segregation ratio of genes at the N locus in the progeny. The hypothesis predicts that if an aberrant ratio occurs in the progeny derived from the Bombay parent, a normal ratio would occur in the progeny from Polk, and vice versa. These crosses have been made and F_2 progeny will be produced to test this hypothesis.

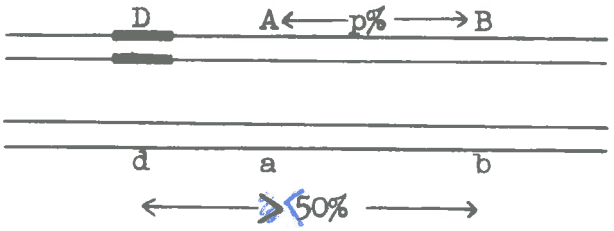
Assuming several disturbance factors, it may be postulated, as the simplest hypothesis, that the same disturbance factors are responsible for the aberrant

segregation ratios occurring at both the N and P loci. If correct, it follows that some of these factors must be linked with both the N and P loci. Otherwise, the factors would occur equally frequently with the contrasting alleles present at these loci in hybrids, and normal segregation ratios would be expected in the female gametes.

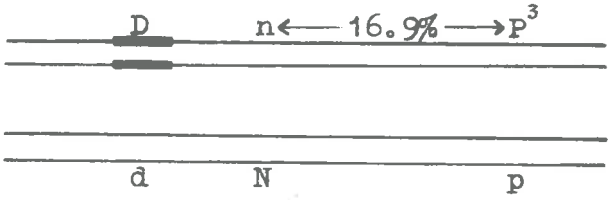
If the disturbance factors are located on the N-P chromosome in the order disturbance factors - N locus - P locus, it should be possible, in suitable hybrids, to predict the expected gametic segregation ratio of genes at the P locus, from knowledge of the gametic segregation ratio occurring at the N locus and the amount of recombination occurring between these loci. This is shown in the following general model.

Assume a disturbance factor D, representing the central position of the postulated disturbance genes, linked with two loci, A and B. The suggested arrangement of these genes in a hybrid is shown in Figure 10. Given that recombination equal to p% occurs between the A and B loci, that genes A and a segregate in the ratio x:1 among female gametes, that there is no chromosomal interference over the interval D to B and that the process responsible

I. GENERAL MODEL



II. LEONA-BOMBAY HYBRIDS



III. POLK-KOTO HYBRIDS

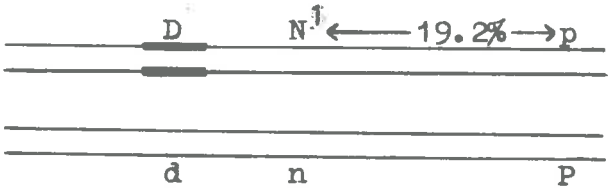


Figure 10 - Models, representing the four strand stage of meiosis in each of three hybrids, showing the proposed location of a disturbance region, D, causing aberrant segregation ratios at two linked loci.

for aberrant ratios does not depend on crossing over in the interval A to B, it can be shown that the expected segregation ratio of genes B:b will be (100 - p)x + p:100 - p + xp. Furthermore, the expected ratio of the reciprocal recombinant products (Ab:aB) will be x:1.

The observed results obtained from (Leona-Bombay) x Hoshangabad and (Polk-Koto) x Hoshangabad test cross progeny (Table 8) were examined to find if they are consistent with this model.

The suggested arrangement of the relevant genes in the Bombay-Leona hybrids is shown in Figure 19. The estimate of p in this family was 16.9% and the observed segregation ratio of genes n and N was 208:82, that is, 2.54:1. The predicted segregation ratio of genes P:p, where x = 2.54 and p = 16.9, is equal to 1.81:1 and the observed results of 144:66 agree with this (x_1^2 = 1.58, P = 0.2-0.3). The observed ratio of the reciprocal recombinant types np to NP was 23 to 12, which agrees well with the ratio of 2.54 to 1, also expected from the model (x_1^2 = 0.63, P = 0.8-0.9).

The above type of analysis could not be applied directly to the data from (Polk-Koto) x Hoshangabad test cross progeny, because it was not possible to detect the segregation ratio of genes N^1: n in this family.

However, we may infer that the N^1 gene occurred more often than the n gene, among female gametes of the Polk-Koto hybrids, since these genes were linked in repulsion phase with genes at the P locus in these hybrids, and the segregation ratio of genes p:P was observed to be 2:1. It is assumed, as a working hypothesis, that the segregation ratio of gene N^1 to its allele was of the same order as that occurring in Polk-Bombay and Polk-Hoshangabad hybrids. The most reliable estimate of this ratio is 4.37:1, obtained from the (Polk-Bombay) x Hoshangabad test cross progeny (Table 6).

The suggested arrangement of the relevant genes in the Polk-Koto hybrids is shown in Figure 10. Given that the segregation ratio of genes N^1:n equals 4.37:1, then it follows from the general model that the expected ratio of the recombinant types N^1P:np will be 4.37:1. That is, the frequency of double-susceptible recombinants provides an estimate of p/5.37, where p is the recombination percentage between the N and P loci. Nine out of 273 test cross plants from (Polk-Koto) x Hoshangabad crosses, were susceptible to rust stock P-i, giving an estimate of p:

$$p = \frac{5.37 \times 9 \times 100}{273} = 17.7\%$$

This value of p is similar to that obtained from the reciprocal test cross progeny (19.2%), where it was assumed that the frequency of susceptible plants represents p/2 (p.66). This model, therefore, reconciles the results obtained from the reciprocal test crosses. Furthermore, the expected segregation ratio of genes p:P, given x = 4.37 and p = 19.2%, is equal to 0.44:1 and the observed result (87:186) agrees with this (x_1^2 = 0.138, P = 0.7 - 0.8).

Thus, all of the results obtained with these two families of test cross progeny are consistent with the proposed location of the disturbance factors, and the model provides a basis for understanding the segregations observed. However, many more progeny need to be tested, and, furthermore, rust stocks must be obtained capable of detecting the segregation of genes N^1 and n in the Polk-Koto hybrids, to provide a more sensitive test for this model.

It is of some interest to consider the possible relationship between the aberrant segregation ratios observed for flower colour and those observed for rust reaction. In both examples the aberrant ratios are restricted to the female gametes of certain hybrids, and these two occurrences of this rather unusual phenomenon,

in the one organism, suggested that the aberrations have a common cause. However, the analysis of the F_2 progeny from Ottawa 770B x Bombay hybrids showed that an aberrant segregation ratio for flower colour could occur jointly with a normal segregation ratio for the N gene controlling rust reaction. Therefore, these two aberrations must be caused by separate events.

Nevertheless, it is possible that these separate events are caused by the same type of abnormal process. This possibility is illustrated by Rhoades' (1952) study of preferential segregation occurring during megasporogenesis in maize. Rhoades found that, in maize hybrids possessing abnormal chromosome ten, and chromosome pairs heteromorphic for heterochromatic knobs, preferential segregation of the knobbed member of each chromosome pair occurred. He postulated that in each case preferential segregation resulted from the formation of neo-centromeres on the knobs. That is, abnormal segregation did not occur with a given chromosome pair unless it was heteromorphic for the knob, but when it did occur, the same type of process, namely neo-centromere formation, was responsible for each example.

Kappert (1935) concluded from cytological studies that

the aberrant segregation ratios for flower colour are caused by megaspore selection. However, the author considered that his cytological observations, like those made in the present study with a plant showing aberrant segregations for rust reaction, did not distinguish between the occurrence of either preferential segregation of a particular chromosome, or the alternative process of preferential selection of megaspores for development into the embryo-sac (see p. 81). Therefore, it is not possible to decide from these cytological studies whether the two examples of aberrant ratios are caused by similar processes.

The investigations of the mode of inheritance of these aberrations indicate that they may be controlled by different types of processes. According to Kappert (1935), several disturbance genes, segregating independently from the B_1 locus, interact in some way with the b_1 gene, to produce the aberrant segregations for flower colour. In contrast, the present study showed that the aberrant ratios for rust reaction are not caused by a property of the genes at the N and P loci, but it is suggested that they are due to two or more disturbance genes which are linked with both the N and P loci.

Kappert (1935) reported that there was a correlation between the average number of seeds per capsule on

heterozygous plants producing aberrant ratios for flower colour, and the proportion of white-flowered plants occurring among their progeny. However, the corresponding comparison was not made in the present study, with the aberrant ratios for rust reaction. If a similar correlation occurred, this would suggest that the same kind of process is responsible for the two examples of aberrant segregation ratios.

Conclusive evidence on the nature of the process causing each of these aberrant segregation ratios could be obtained only from cytological studies, but, as indicated previously, this would be difficult to obtain with flax, since this organism is not well adapted for studies of either megagametogenesis or microsporogenesis.

iii. Recombination Between the N and P Loci

A feature of the present study of recombination between the N and P loci is the occurrence of widely different recombination values. These values ranged from 9.76% (5.63% to 15.60%)*, with Bombay-Koto hybrids, to 16.91% (12.07% to 22.72%) with Leona-Bombay hybrids used as the male parent in test crosses, and 21.2% (17.69% to 25.08%) with the same hybrids used as the

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* 95% confidence interval.

female parent. However, the recombination percentage obtained with Polk-Koto hybrids used as the female parent in test crosses, namely 19.19% (11.76% to 29.16%), was similar to that obtained with the Leona-Bombay hybrids.

Only one recombinant class, that of plants susceptible to all tester rust stocks, could be detected among progeny from Polk-Koto and Koto-Bombay hybrids. The former hybrids, when used as the female parent in test crosses, produced a significantly lower proportion of these susceptible plants than when used as the male parent. As indicated previously, this difference was probably a consequence of the aberrant segregation ratio, which occurred among the female gametes but not among the male gametes, of these hybrids (see p.109).

However, the Koto-Bombay hybrids produced approximately the same proportion of susceptible plants among the progeny from reciprocal test crosses, and since these hybrids also produced normal segregation ratios in both groups of progeny, the total percentage of susceptible plants most likely represents p/2, where p is the recombination percentage between genes N and P. Therefore, the relatively low value of 9.76%, given above, is considered to be a valid estimate of p.

The Leona-Bombay and Polk-Koto hybrids, giving

relatively high recombination percentages between the N and P loci, both produced aberrant segregation ratios among their female gametes. In contrast, the Bombay-Koto hybrids gave normal segregation ratios. It may be significant that the low recombination value obtained with Bombay-Koto hybrids is similar to that obtained by Kerr (1960) with several hybrids which also produced normal segregation ratios. Additional hybrids, heterozygous for genes at the N and P loci, need to be tested to find if this association also occurs in other examples.

However, even if the observed association is real, the increased recombination which occurred in hybrids producing aberrant segregation ratios, could not have been caused by the aberrant ratio per se, since with Leona-Bombay hybrids the recombination percentage observed in the male gametes, showing a normal segregation ratio, was greater than that observed in the female gametes, showing an aberrant ratio.

It is possible that the observed differences in recombination value were caused by the influence of different background genotypes on crossing over in the N-P chromosome interval, independent of the occurrence of aberrant segregation ratios.

II. STUDY OF PATHOGEN GENES CONTROLLING PATHOGENICITY

1. INTRODUCTION

After Craigie's (1927a, 1927b) discovery of heterothallism and the function of the pycnidia in rusts, it became possible to carry out inheritance studies with these fungi. Melampsora lini exhibits physiological specialization (Flor, 1935) and, since it is heterothallic (Allen, 1934), it was possible to determine the genetical control of these patterns of pathogenicity on host varieties.

Flor (1942) described methods for breeding flax rust and making pathogenicity determinations. He made crosses between six different 'races' , four of which had been collected in North America and two in South America, and determined the pathogenicity of parents and F_1 progeny on the eleven differential varieties of flax then in use. He found that avirulence was dominant to virulence on each host variety, except that avirulence was incompletely dominant on Buda.

Flor's use of the term 'race' is synonymous with the term 'strain' adopted for use in the present study (see p. 22).

He also produced ninety-eight F_2 progeny from the cross 'race' 6 x 'race' 24 and these progeny segregated for pathogenicity on three host varieties. Pathogenicity was controlled by a single gene on each of Bombay and Akmolinsk, varieties possessing a single gene controlling rust reaction, and by two genes on Buda, a variety with two genes controlling reaction.

Subsequently, Flor (1946) produced 133 F_2 progeny from crosses between 'races' 22 and 24, and tested these progeny on sixteen host varieties. Avirulence was dominant to virulence, with the possible exception that virulence was dominant on Williston Brown. Segregation for pathogenicity occurred on fourteen of these varieties: monohybrid segregation ratios occurred on each of nine host varieties possessing a single gene conferring resistance, dihybrid ratios occurred on each of two host varieties possessing two genes conferring resistance, and a trihybrid ratio occurred on a host variety with three genes conferring resistance to the avirulent parent 'race'.

The segregation on two host varieties, Williston Brown and Williston Golden, possessing the same single gene controlling rust reaction, did not agree with either a monohybrid or a dihybrid ratio. However, Flor concluded that this result represented a disturbed monohybrid ratio, with virulence dominant to avirulence.

Flor (1946) produced ninety-eight F_2 progeny from crosses between 'races' 6 and 22, and tested these on the sixteen differential varieties. Later, he produced an additional sixty-seven F_2 progeny from these parents and these progeny were tested on thirty-two host varieties (Flor, 1955). In these latter tests, segregation for pathogenicity occurred on twenty-four of the host varieties and in all examples, except on Ottawa 770B, this agreed with a monohybrid ratio. According to Flor, each of these varieties, with the possible exception of Ottawa 770B, possesses a single gene conferring resistance to parent 'race' 6.

Flor (1942, 1946, 1955) concluded from the combined results of host and pathogen studies that for each gene in the host controlling reaction to rust, there is a specific and complementary gene in M. lini controlling pathogenicity on the host. Flor's results show that specificity of interaction, previously envisaged as between host variety and pathogen 'race', is more specifically related to genes in the host controlling reaction and genes in the pathogen controlling pathogenicity.

Flor also determined the linkage relationships between the genes controlling pathogenicity which segregated in these F_2 progeny. Fifteen different genes were identified in the F_2 progeny from the cross 'race' 22 x 'race' 24

and nineteen in the progeny from crosses between 'races' 6 and 22, and these genes occurred in nine linkage groups, in both examples.

In several cases, pathogenicity segregated as a unit on groups of two or more host varieties (see p.139 and Table 21). Flor (1955) accounted for unit segregation on some of these varieties, for example on Clay and Bowman, by assuming that the varieties within a group each possess a common gene which interacts with a single gene in the pathogen. On the other hand, since rust strains occur having contrasting pathogenicity on host varieties within the other groups, for example on Pale Blue Crimped and Kenya, Flor (1946, 1955) assumed that these varieties possess different genes controlling rust reaction. He concluded that the pathogen also possesses different genes controlling pathogenicity, and that these are so closely linked that they did not recombine in the F_2 progeny analysed.

Flor (1947) commented on an apparent parallelism of linkage between genes controlling reaction in the host and genes controlling pathogenicity in the pathogen. He reported that the genes controlling pathogenicity on Pale Blue Crimped and Kenya, on Abyssinian, Akmolinsk and Leona, and on Billings and Newland were linked, and that

genes controlling reaction in these varietal groups occurred at the L, P and M loci, respectively. Later, Flor (1955) found that the genes controlling pathogenicity on host varieties Polk and Marshall (both possessing genes controlling rust reaction at the N locus) were also closely linked. However, as an exception to the above pattern, he observed that one of the genes controlling pathogenicity on Buda (with genes controlling reaction at the L and M loci) was closely linked with the gene controlling pathogenicity on Akmolinsk (with a gene at the P locus).

These studies of Flor were made with a restricted number of North and South American strains of flax rust. It is possible that other strains, particularly those occurring in a region isolated from the above continents, possess different or additional genes controlling pathogenicity on some flax varieties. The present study was made to check this possibility with two strains of M. lini collected in New Zealand.

Flor (1946, 1955) suggested that some of the genes controlling pathogenicity, which segregated independently in his studies, may be loosely linked, and this linkage was not detected because of the small number of F_2 progeny tested. This appears likely, since Shepherd (1957) showed,

from a study of mitosis in basidiospores, that M. lini possesses a haploid chromosome number of only 5 or 6.

In Flor's studies most of the genes controlling pathogenicity occurred in coupling phase in the F_1 hybrids and although F_2 data are efficient for detecting rare recombination between genes closely linked in coupling phase, they are inefficient for detecting loose linkage between these genes (Mather, 1936). Test cross data are efficient for detecting both. Flor (1946) had planned to back cross some of his F_2 hybrids, but was unable to synchronize the germination of F_2 and recurrent parent teleutospores. Subsequently, Clark (1955) developed a method for inducing teleutospore germination, making it practicable to carry out back crosses and test crosses with M. lini.

Consequently, a test cross breeding programme was carried out in the present study, with two New Zealand strains of M. lini differing widely in pathogenicity, to determine the number of genes controlling pathogenicity on host varieties, and to provide data suitable for determining the linkage relationships between these genes. Also, it was expected that this programme would provide rust stocks suitable for analysing some of the host gene segregations (Section I).

2. BIOLOGY OF MELAMPSORA LINI

Flor (1956a) has given a general account of the biology of M. lini. The features relevant for inheritance studies with this organism are described below.

M. lini is an autoecious long-cycle rust. The dikaryotic mycelium growing in susceptible flax plants produces dikaryotic uredospores and these are the repeating stage of the life cycle. Eventually the dikaryotic mycelium produces teleutospores instead of uredospores. The thick-walled teleutospores are resistant to adverse environmental conditions and represent an overwintering stage. At or just prior to germination, the two nuclei in the teleutospore fuse to give a transient diploid phase. This diploid nucleus divides meiotically and the four haploid nuclei migrate, one to each of the four basidiospores produced on a promycelium. The haploid basidiospores re-infect susceptible flax plants and produce a monokaryotic mycelium. Eight to ten days after basidiospore infection, the mycelium resulting from a single basidiospore produces pycnidia of either (+) or (-) mating type. The pycnidia produce pycnidiospores in a liquid exudate (nectar). Transfer of pycnidiospores from one pycnidium to another, of opposite mating type, initiates the production of dikaryotic aeciospores. These spores re-infect susceptible flax plants and after nine to ten days, uredospores are produced.

Flor (1956a) has pointed out that the dikaryotic aeciospores, resulting from pycnidiospore transfer between haploid pycnidia of opposite mating type, and the subsequent uredospores, are genetically equivalent to the diploid progeny resulting from union of haploid gametes in animals and higher plants. However, a useful characteristic of the rusts is that uredospores, representing a particular parental or progeny genotype, can be maintained indefinitely as stable clonal lines by periodic passage through the host, so that a single pathogen genotype can be used in repeated tests.

3. MATERIALS AND METHODS

Just as the segregation of genes in L. usitatissimum conferring resistance to rust can be detected by testing host progeny with a standard array of tester rust stocks, so the segregation of genes in M. lini controlling pathogenicity on the host can be detected by testing progeny stocks on a standard array of host varieties.

In the present study the test cross rust progeny were tested on the differentials listed in Table 2. Seeds of the other varieties used by Flor (1955) were not available.

Rust stocks P-1 and P-2 were selected as the parents in this breeding programme, since they differ widely in their range of pathogenicity (Table 2). Furthermore, since stock P-2 possesses a wide range of virulence, it was used as the 'homozygous recessive' parent in the test cross.

1. Breeding and Testing Rust Progeny

In outline, the procedure adopted for breeding M. lini consisted of producing teleutospores of the relevant rust stocks, germinating these to obtain basidiospores, and obtaining single basidiospore infections on host plants. Crosses were made by transferring nectar between the pycnidia originating from these single basidiospore infections. The aeciospores produced from successful crosses were inoculated onto susceptible host plants and

the resulting uredospores were collected, and inoculated on the host differential varieties, to determine the pathogenicity phenotype of the progeny stocks.

All stocks of rust grown to produce teleutospores were initiated from single uredospore inoculations and the infected plants were grown in isolation from other rust-infected plants. When the teleutospores were collected, the uredospores on the same plant were also taken and tested on the differential varieties, to check that the stock had remained homogeneous.

After collection the teleutospores were induced to germinate by subjecting them to a regime of temperature and moisture treatments developed by Clark (1955). This consisted of moistening the stem segments bearing the spores and incubating them at 13°C for two days, followed by drying and incubation at -5°C for a further two days. These treatments were continued for a total of twenty days. These treated spores were then dried, placed in stoppered glass phials and stored at a temperature of 4°C.

When these treated teleutospores were incubated at 13°C, either immediately after treatment or even after six months storage, they usually germinated profusely within four to five days. However, the best results were usually obtained with teleutospores which had been left on the plant for at least three weeks after their formation.

With this procedure it is relatively easy to synchronize the germination of teleutospores derived from different rust stocks, making it possible to carry out a test cross breeding programme.

The methods of Flor (1942) were used to inoculate flax plants with basidiospores, and to make crosses between the resulting pycnidia.

Equipment, similar to that described by Flor, was used to suspend the germinating teleutospores above flax plants in humidity chambers, so that the basidiospores were shed directly onto the upper leaves of the plant. These plants were inoculated for ten to twenty-four hours, at a temperature of 10 - 14°C, and were then removed to the plant growth cabinet. All inoculations were made on Hoshangabad plants, since this host variety is susceptible to the parental rust stocks and, furthermore, supports vigorous growth of the monokaryon.

Nine days after inoculation, pycnidia began to erupt through the leaf epidermis, and sometimes the stem epidermis also, of infected plants. All leaves with more than a single focus of infection were removed from the plant, and the other leaves, apparently infected with a single basidiospore, were marked with coloured cotton and the developing pycnidia were used in crosses. As suggested by Flor (1942), some of these apparently single

infections may have arisen from two or more basidiospores developing in close association in the leaf. However, if these basidiospores were of opposite mating types, as would be expected in one half of such double infections, these infections would produce aeciospores. As a check for this, pycnidia were not used in crosses until at least four days after their eruption through the leaf. Very few of the pycnidia developed aeciospores during this time and, from this, it is considered that most, if not all of them, resulted from infection with a single basidiospore.

The nectar produced by pycnidia attracted insects and, consequently, infected plants were placed under wire frames covered with colourless 'cellophane', to prevent insects gaining access to, and spreading, this nectar.

Crosses were made by transferring nectar, with a sterile platinum wire loop, from one pycnidium to another. The recipient pycnidia were subsequently inspected each day to check for the development of aeciospores. With a successful cross, aeciospores began to erupt through the lower, and sometimes the upper epidermis of the leaf, four days after crossing.

In crossing, care was taken that a pycnidium was used in only one successful cross, thus ensuring that the crosses were genetically equivalent to the random union

of gametes in higher organisms.

The aeciospores produced from a successful cross were collected on a glass microscope slide and inoculated onto young Hoshangabad plants. The uredospores produced from these inoculations were collected, and tested on the differential host varieties, to determine the pathogenicity of the progeny stock. The methods of culturing the differentials, inoculating them with uredospores and scoring rust reaction phenotypes, were identical with those described in Section I.

All aeciospore progeny were named according to their pedigree and sequence of occurrence, as described earlier (p.23). However, uredospores were not obtained from aeciospores of many of the progeny which had been named, because very few aeciospores were produced from these particular crosses.

ii. Rust Progeny Material

The above procedure for breeding the pathogen was used to produce:

a. First generation progeny from crosses between stocks P-1 and P-2.

b. Test cross progeny derived from crosses between four (F-a, F-i, F-h, F-k) of the first generation progeny and stock P-2.

c. Selfed progeny from stock P-2.

Pycnidia of stock P-2 were used as the recipient for nectar from P-1 pycnidia (donor parent) in crosses between P-1 and P-2, so that self fertilization could be detected if it occurred.

The first generation progeny with the narrowest range of virulence, that is, virulent on the least number of differential varieties and presumably heterozygous at the greatest number of loci controlling pathogenicity, were used in crosses with P-2 to produce the test cross progeny. Stock F-a was originally used, but it was lost soon after isolation and was replaced with stock F-i.

Five groups of F-i teleutospores were used in the test crosses with P-2. Each group was derived from a different single uredospore (F-i) inoculation of Hoshangabad plants and the five groups of infected plants were grown in isolation until the teleutospores were collected.

Seventy-five of the ninety-eight progeny were derived from using P-2 pycnidia as the donor parent, and twenty-three from using F-i pycnidia as this parent, in test crosses with pycnidia of F-i and P-2, respectively.

First generation stocks F-h and F-k were also crossed with P-2 to produce test cross progeny: though their overall host virulence range exceeded that of F-i, both were avirulent on Abyssinian and Leona and hence could be

used to provide information on the genetical control of pathogenicity on these host varieties. The pycnidia of these stocks were used as the donor parent in crosses with P-2.

Parental stock P-2 was selfed and the progeny were tested for segregants as a check for homozygosity: parent P-1 was lost before a similar test could be carried out.

4. OBSERVATIONS AND THEIR ANALYSIS

The interaction phenotypes, which resulted from testing the parental and progeny rust stocks on the differential host varieties, are given in the Appendix. These phenotypes have been described in terms of degree of host resistance, to be consistent with the description of the reactions of the host to the parental and some of the progeny stocks, given previously (Section I). However, it is more convenient to describe these phenotypes in terms of degree of rust virulence when studying the segregation of genes controlling pathogenicity. Therefore, the reactions of the rust progeny on each host variety, upon which segregation occurred, were classified into two categories corresponding to the characteristic less virulent (resistant or immune host reaction) reaction of parental stock P-1, and the more virulent (susceptible or semi-resistant host reaction) reaction of parental stock P-2, on each of these varieties. These data, taken from the Appendix, are summarized in Table 18.

The data obtained from reciprocal test crosses between stocks F-1 and P-2 were pooled, since both crosses gave similar segregation ratios for pathogenicity. Furthermore, the segregation ratios obtained from the five (three after grouping) separate teleutospore collections of F-1 were homogeneous, and the pooled results are given

TABLE 18

NUMBER OF PROGENY STOCKS IN EACH GROUP WITH INDICATED PATHOGENICITY ON SELECTED* HOST DIFFERENTIAL VARIETIES

CROSS	PROGENY GROUP	PROGENY STOCKS			HOST DIFFERENTIAL VARIETY								
		TOTAL NUMBER	PATHOGENICITY✓		ABYSSINIAN LEONA	BIRIO WILDEN	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
P-2 selfed	Self fertilization	10	LV										
	S-series		MV		10	10	10	10	10	10	10	10	10
P-2 x P-1	First generation	13	LV		7	4	13	6	13	8	7	9	13
	F-series		MV		6	9		7		5	6	4	
F-1 x P-2	Test cross	98	LV			43	55	38	54	49	56	51	58
P-2 x F-1	Ti-series		MV		98	55	43	60	44	49	42	47	40
				Het. x_2^2		0.39	1.58	1.38	0.65	0.07	2.89	0.78	0.51
				Dev. $x_1^2(1:1)$		1.47	1.47	4.94*	1.02	0.00	2.00	0.16	3.31
F-a x P-2	Test cross	16	LV			10	7	8	7	9	8	8	5
	Ta-series		MV		16	6	9	8	9	7	8	8	11
F-h x P-2	Test cross	12	LV		8	≠	4	≠	4		4	≠	7
	Th-series		MV		4		8		8	12	8		5
F-k x P-2	Test cross	17	LV		11		8		9	9	12	7	8
	Tk-series		MV		6	17	9	17	8	8	5	10	9

* Where tested all progeny were virulent on Akmolinsk, Bison and Williston Brown and avirulent on Koto, Polk and Ottawa 770B and therefore these varieties are not included here.

✓ LV = less virulent and corresponds to reaction of P-1 on the particular differential variety.
MV = more virulent and corresponds to reaction of P-2 on the particular differential variety.

≠ Both F-h and P-2 were virulent on these varieties and no segregation was expected in the progeny, therefore these tests were not made.

* x^2 significant at 0.05 level of probability.

in Table 18. The segregation ratios obtained from the Ta and Ti test cross progeny groups were not homogeneous for all host varieties and, consequently, these data have been listed separately in Table 18.

Assuming that a single gene controlled pathogenicity of parental stock P-2 on each differential host variety, we may conclude, with 95% certainty, that this stock was homozygous for these genes, since on selfing no segregation for pathogenicity occurred among ten progeny. However, parental stock P-1 must be heterozygous for genes controlling pathogenicity on at least nine of the differential varieties, since the first generation progeny segregated on these varieties.

The test cross progeny did not segregate for pathogenicity on Akmolinsk, Bison, Williston Brown, Koto, Polk and Ottawa 770B: the parental rust stocks were wach virulent on the former three, and avirulent on the latter three varieties. However, segregation occurred on each of the other host varieties in at least one test cross family. As shown in Table 18, the segregations for pathogenicity in each test cross progeny group, except Ti progeny tested on Clay and Bowman, are consistent with monohybrid segregation ratios. That is, with the possible exception noted, these results are consistent

with the assumption that the first generation stocks possess a single gene controlling less virulence on each of the relevant host varieties.

The reactions of the Ti progeny on Clay and Bowman could be classified into two discrete classes without difficulty, hence it is unlikely that the significant deviation observed among this group was due to misclassification. Since the F and Ta progeny both gave a 1:1 segregation ratio for pathogenicity on these varieties, it is assumed that stock F-1 also possesses a single gene controlling less virulence on these varieties, and the observed deviation was due to chance.

There was a complete association between the reactions of rust progeny on each of the host variety pairs Abyssinian and Leona, Clay and Bowman and Birio and Wilden. Flor (1946, 1955) reported a similar association between the reactions of F_2 progeny on Abyssinian and Leona, and on Bowman and Clay. Although the joint segregation of pathogenicity on Birio and Wilden could not be observed in these progeny, his mutation experiments (Flor, 1956b, 1958) showed that the reactions on these varieties are also intimately associated. He suggested that closely linked genes control pathogenicity on Abyssinian and Leona, and on Birio and Wilden, and possibly the same gene controls pathogenicity on both Bowman and Clay.

In summary, the combined test cross and first generation progeny results are consistent with the hypothesis that stock P-1 possesses a single gene controlling less virulence on host varieties Bombay, Cass, Dakota, Kenya, Stewart and Victory A, and also a single gene, or closely linked genes, controlling less virulence on each of the pairs of varieties Leona and Abyssinian, Clay and Bowman, and Birio and Wilden.

The results of an analysis of the Ti progeny data, designed to detect linkage between the segregating genes identified, are given in Table 19. This analysis was based on the assumption that, with pairs of independently segregating genes, parental and non-parental combinations of pathogenicity will occur with equal frequency. This expectation will be valid providing that at least one of the two segregations compared, agrees with the 1:1 ratio expected for a single gene segregation in test cross progeny. Therefore, from the results shown in Table 18, it was appropriate to use this analysis on the Ti progeny data included in the Appendix.

The reactions of each Ti progeny stock, on pairs of host varieties, were classified as either parental or non-parental. The classifications obtained from the five separate teleutospore collections (three after grouping)

TABLE 19

SUMMARY OF ANALYSIS OF DATA OBTAINED FROM T1 TEST CROSS PROGENY JOINTLY CLASSIFIED FOR PATHOGENICITY ON PAIRS OF SINGLE HOST VARIETIES OR VARIETY GROUPS

HOST DIFFERENTIAL VARIETY OR VARIETY GROUPS	Type of analysis[/]	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
Birio	i	1.19	0.75	2.37	1.02	0.51	0.65	1.94
Wilden	ii	50:48	56:42	55:43	48:50	41:57	54:44	51:47
	iii	0.04	2.00	1.47	0.04	2.61	1.02	0.16
	i		0.15	8.62*	5.99*	2.01	1.79	0.26
Bombay	ii		43:45	53:45	56:42	54:44	50:48	52:46
	iii		0.04	0.65	2.00	1.02	0.04	0.37
	i			0.57	0.31	0.91	6.44*	0.34
Clay	ii			45:53	51:47	44:54	49:49	44:54
Bowman	iii			0.65	0.16	1.02	0.00	1.02
	i				1.32	0.86	0.55	0.31
Cass	ii				50:48	51:47	55:43	47:51
	iii				0.04	0.16	1.47	0.16
	i					0.68	0.13	1.55
Dakota	ii					48:50	48:50	51:47
	iii					0.04	0.04	0.16
	i						0.78	0.30
Kenya	ii						47:51	54:44
	iii						0.16	1.02
	i							0.69
Stewart	ii							52:46
	iii							0.37

[/] i Heterogeneity x^2 with 2 d.f. Obtained by comparing the numbers of parental and non-parental combinations of pathogenicity in Ti progeny derived from each of the five collections (three after grouping) of teleutospores.

ii Ratio of parental to non-parental combinations of pathogenicity among Ti progeny.

iii x_1^2 on expectation of a 1:1 ratio for parental : non-parental combinations.

* x^2 significant at 0.05 level of probability.

were homogeneous on all variety pair combinations, except three (Table 19). It was assumed that these exceptions represented chance deviations, since with twenty-eight independent comparisons, the probability of observing three or more significant deviations from expected (at the 0.05 level of significance), due to chance alone, is equal to 0.16. Consequently, the data obtained from separate teleutospore collections were pooled (Table 19).

There is no indication that any of the eight segregating single genes (or gene pairs) identified among T1 progeny are linked, since a significant deficiency of recombinant phenotypes did not occur among any of the twenty-eight comparisons listed in Table 19.

Linkage between any two loci will be detected among ninety-eight test cross progeny (at the 0.05 level of significance, using a one-tailed test) only if forty or fewer recombinant phenotypes are observed. Given that the recombination percentage between two loci is 33%, there is 95% probability that forty or fewer recombinants will be observed among ninety-eight progeny. Therefore, the data given in Table 19 are sufficient to exclude only recombination percentages less than 33%, with 95% certainty.

5. DISCUSSION

This study of the inheritance of pathogenicity, with two stocks of *M. lini* collected in New Zealand, gave results which were similar to those obtained by Flor in his studies with North and South American stocks of rust.

The present study, with mainly test cross progeny, did not provide direct information on the dominance relationship between genes controlling less and more virulence in the pathogen, except the first generation progeny results showed that the genes controlling avirulence on Bombay and Cass were dominant over their respective alleles controlling virulence on these varieties. However, in those test cross progeny groups where segregation for pathogenicity occurred on other host varieties, the results were consistent with Flor's (1946) postulate that the genes controlling less virulence are dominant.

Apparently a single gene controls the pathogenicity of stock P-1 on each of the differential varieties Bombay, Cass, Dakota, Kenya, Stewart and Victory A and either a single gene or closely linked genes control pathogenicity on each of the variety pairs Abyssinian and Leona, Wilden and Birio, and Bowman and Clay. Each of these host varieties possesses a single gene conferring resistance (or immunity) to stock P-1 (Table 5). Therefore, the

combined results from host and pathogen studies are consistent with Flor's (1942) gene-for-gene hypothesis.

Linkage was not detected between any of the eight segregating single genes or presumed closely linked gene pairs. Although there are probably only five or six linkage groups in M. lini, the observed joint segregational data were quite consistent with this, especially since we could expect, with 95% certainty, to detect linkage values only less than 33%, in ninety-eight test cross progeny.

Genes controlling pathogenicity on Stewart, Victory A and Wilden did not segregate in Flor's (1946, 1955) F_2 progeny, but he reported that the genes controlling avirulence on the other five single varieties or variety pairs, listed in Table 19, segregated independently among F_2 progeny. The gene controlling pathogenicity on Victory A segregated independently from the genes controlling pathogenicity on Dakota and Akmolinsk, Abyssinian and Leona, in the F_2 progeny of 'race' 1 (Flor, 1960b).

Flor's (1955) data indicate that there are at least ten independent or loosely linked loci concerned with pathogenicity in M. lini. If there are only five or six linkage groups in this organism, we might expect that at

least one of these loci is linked with the locus determining mating type. However, the occurrence of this type of linkage in flax rust has not been reported in the literature.

Rust stocks which are heterozygous for a gene controlling pathogenicity linked with the mating type locus, would produce an excess of individuals heterozygous for this particular gene, in their F_2 progeny. That is, the linkage would result in the occurrence of more avirulent F_2 progeny than expected. For example, if there is 20% recombination between a gene controlling pathogenicity and the mating type locus, we would expect a 5.25:1 ratio of avirulent to virulent F_2 progeny, rather than the 3:1 ratio expected for a single gene segregation. However, it would not be possible to detect this linkage among test cross progeny.

Flor (1946, 1955) noted that among the F_2 progeny derived from crosses between rust 'races' 6 and 22, the ratio of progeny avirulent on Ottawa 770B to those virulent (145:20) approximated 7:1, instead of the 3:1 expected. This result could have been due to linkage between the locus controlling pathogenicity on Ottawa 770B and the mating type locus. However, if this is correct, the gene in 'race' 6 controlling avirulence on

this variety must be situated at a different locus from the gene in 'race' 24, since crosses between 'races' 22 and 24 gave a normal 3:1 segregation ratio for pathogenicity on Ottawa 770B in F_2 progeny (Flor, 1946).

On the other hand, the observed approximate 7:1 ratio could have been due to differential viability of progeny or the occurrence of two linked genes in 'race' 6 controlling pathogenicity on Ottawa 770B. Further experiments are required to distinguish between these three possibilities.

A striking feature of Flor's results, and those obtained in the present study, is the frequent occurrence of a complete association between the reactions of segregating rust progeny on two or more host varieties. Reactions of rust stocks have been found to segregate (Flor, 1946, 1955) and/or mutate (Flor, 1956b, 1958, 1960a, 1960b) as a unit on each of the following groups of host varieties:

a. Akmolinsk, Abyssinian, Leona, Koto, Ward and Wells.

b. Pale Blue Crimped, Bolley Golden selection, Kenya and Norman.

c. Bowman, Clay, Grant and Minnesota selection.

d. Argentine selection, Cortland and Lino 6899M.A.

e. Wilden, Birio and Barnes.

These results could be explained most simply by assuming that each host variety within a group possesses a common gene conferring resistance, which interacts with a single gene controlling avirulence in the relevant rust stocks. Flor (1955) recognized this possibility and, in the absence of evidence to the contrary, suggested that Wells and Leona, Norman and Bolley Golden selection, and the varieties in each of groups c and d above, may possess a common gene controlling rust reaction.

However, Flor (1946, 1955) considered that since rust stocks occur, which have contrasting pathogenicity on the host varieties Akmolinsk, Abyssinian, Leona and Koto, these varieties must possess different genes conferring resistance or immunity (named P^1, P^2, P^3 and P_s respectively). He then suggested that the complete association between the reactions of F_2 rust progeny on these varieties was due to close linkage between different single genes in the pathogen (named Ap^1, Ap^2, Ap^3 and Ap, respectively), which interacted with the different host genes.

Later, Flor showed that at least three different linked genes (or three parts of a single gene) control avirulence on these host varieties. He found that genes controlling avirulence on Akmolinsk and Koto occurred in

repulsion phase in 'race' 1 and he did not detect recombination between these genes in eight test cross progeny (Flor, 1958) and twenty-six F_2 progeny (Flor, 1960a) derived from this 'race'. Furthermore, in his mutation studies (Flor, 1960b), X-ray induced mutation to virulence on Koto was always accompanied by mutation to virulence on Abyssinian and Leona as well, showing that the genes (or gene) controlling avirulence on Abyssinian and Leona must be either closely linked or allelic with the gene controlling avirulence on Koto.

If four different closely linked genes in the rust each control pathogenicity on one of four host varieties, it would be possible, theoretically, to obtain the sixteen rust stocks representing all combinations of avirulent and virulent phenotypes on these varieties (Table 20). However, apparently nine of these stocks have not yet been found in nature or produced in breeding experiments. Table 20 shows that whereas only four of the eight possible combinations of pathogenicity patterns on Akmolinsk, Abyssini and Leona have been reported, all combinations of pathogenicity on Koto occur within this group of four, with one exception. Also, among the combinations which have been reported, all rust stocks which attack Leona, also attack

TABLE 20

THE SIXTEEN COMBINATIONS OF PATHOGENICITY OF *M. LINI* ON HOST VARIETIES AKMOLINSK, ABYSSINIAN, LEONA AND KOTO AND THE REGIONS OF OCCURRENCE OF STOCKS WITH THE INDICATED PATHOGENICITY

M. LINI STOCK WITH INDICATED PATHOGENICITY TYPE	PATHOGENICITY* ON				REGION OF OCCURRENCE†
	AKMOLINSK	ABYSSINIAN	LEONA	KOTO	
i	A	A	A	A	N.A., Aust., E.
ii	A	A	A	V	N.A.
iii	V	A	A	A	N.A., Aust., N.Z.
iv	V	A	A	V	None reported
v	V	V	A	A	N.A., Aust., N.Z., E.
vi	V	V	A	V	N.Z.
vii	V	V	V	A	Aust., N.Z.
viii	V	V	V	V	N.Z., S.A.
ix	A	A	V	A	None reported
x	A	A	V	V	" "
xi	A	V	A	A	" "
xii	A	V	A	V	" "
xiii	A	V	V	A	" "
xiv	A	V	V	V	" "
xv	V	A	V	A	" "
xvi	V	A	V	V	" "

* A = avirulent V = virulent (see Table 4).

† N.A. = North America S.A. = South America Aust. = Australia
N.Z. = New Zealand E = Europe.

Compiled from Flor (1954), Kerr (1954), Cruickshank (1956) and s'Jacob (1955).

Abyssinian and Akmolinsk, and all stocks which attack Abyssinian, but not necessarily Leona, also attack Akmolinsk. Although the non-occurrence of particular combinations may be due to close linkage between the different genes controlling pathogenicity and the lack of opportunity for certain types of recombination to occur, it is possible that there is another explanation for this.

For example, Person (1959) noting the pattern of pathogenicity on Akmolinsk, Abyssinian and Leona, suggested that these varieties possess genes A, AB and ABC conferring rust resistance, respectively. On this hypothesis, rust stocks which attack Leona must also attack Abyssinian and Leona, and so on. If these three loci do exist in the host, it is likely that they are linked; otherwise we would sometimes expect to detect di- or tri-hybrid segregation ratios in progeny from Abyssinian and Leona parents, respectively, and these varieties gave monohybrid segregation ratios only, in Flor's (1947) study and in the present investigation.

The type of evidence required to decide whether these host varieties each possess a different gene controlling reaction (Flor, 1946) or different combinations of the same genes (Person, 1959), will be described later (see p.165)

As described above, Flor has shown that at least three different and closely associated genetic units control pathogenicity of M. lini on Akmolinsk, Abyssinian, Leona and Koto. Irrespective of whether these genetic units interact with either different combinations of the same gene or different single genes, in these host varieties, it is of some interest to decide whether the units occur in M. lini as different genes at closely linked loci or as different parts of a single gene. Mutational studies would be expected to provide information on this.

Flor (1958) has shown that with a rust stock heterozygous for genes (or a gene) controlling pathogenicity on Abyssinian, Leona and Koto, selection for X-ray induced mutation to virulence on either Leona or Koto, gives rust stocks which are virulent on all three varieties. Flor (1960b) found that some of the mutant stocks gave disturbed segregation ratios in their progeny, and he inferred from this, that the X-ray treatment had caused mutation by deleting a portion of the chromosome. Therefore, the results from these studies are consistent with the existence of either closely linked genes or a single gene, controlling pathogenicity on these four host varieties.

More decisive evidence would be expected from a study of spontaneous mutation of these genes, since it is more

likely that this mutation would result from a 'point' change rather than deletion of portion of a chromosome.

Flor did not observe spontaneous mutation to virulence on any of the above four varieties, but he reported (Flor, 1958, 1960a) seven separate occurrences of apparent spontaneous mutation of pathogenicity on Birio, with 'race' 22 x 'race' 1 hybrids. In all cases, a change from avirulence to virulence on Birio was accompanied by a similar change of pathogenicity on Wilden, and when tested, on Barnes also.

Furthermore, in studies of mutation induced by ultraviolet irradiation (Flor, 1956b) and X-rays (Flor, 1958), mutation from avirulence to virulence selected on any one of the three varieties Birio, Wilden and Barnes, always resulted in a joint change of pathogenicity on all three varieties.

These results could be explained most simply by assuming that the rust hybrids used were heterozygous for a single gene controlling avirulence, which interacted with a common gene conferring resistance, present in each of the varieties Birio, Wilden and Barnes.

However, Flor (1956b) reported that rust stocks are known which will attack (i) Birio, but not Wilden and Barnes, (ii) Wilden, but not Birio and Barnes and (iii) Barnes, but not Wilden and Birio, and therefore he con-

cluded that these varieties must possess different genes controlling rust reaction. Furthermore, he suggested that different genes in M. lini control pathogenicity on each of these three varieties and they are so closely linked that, in mutation studies, a single 'hit' could alter all three genes.

Critical tests have not yet been reported to find whether the three different genes identified in these host varieties by the selective rust stocks, were also effective against the hybrid rust stocks used in the mutation studies. If they were then the mutational data, especially those from spontaneous mutation (assuming that they did represent spontaneous mutation, and not contamination), would provide good evidence that a single locus, with alleles able to exhibit at least three separate specificities, controls pathogenicity on Birio, Wilden and Barnes. That is, if three closely linked loci in M. lini are each concerned with pathogenicity on a single host variety, we would not expect spontaneous mutation to affect all three loci simultaneously, since this type of mutation would most likely result from a point change, rather than deletion of a portion of the chromosome. On the other hand, if a single locus is involved, we might expect that point mutation would frequently lead to complete inactivation of the presumed single gene product and, assuming that

this product was necessary to produce the avirulent phenotype (Catcheside, 1951), the inactivation would be expressed as a change to virulence, occurring jointly on all three host varieties.

In the present study a complete association was observed between the reactions of rust progeny on Clay and Bowman, on Abyssinian and Leona and on Birio and Wilden. This result could have been due, in each example, to a single pathogen gene interacting with a common host gene present in each variety of a pair. Abyssinian (resistant) and Leona (immune) reacted differently to stock P-1, as also did Wilden (near immune) and Birio (immune), suggesting that each variety in a pair possesses a different gene controlling reaction to this stock. However, the observed differences could, instead, have been due to the presence of different modifier genes in these varieties.

Even if these host varieties do possess different genes controlling rust reaction, the observed results do not help in deciding whether either closely linked or allelic genes control pathogenicity on each of these two variety pairs, since no recombinants occurred among the rust progeny.

Bowman and Clay reacted identically to stock P-1 and they may possess a common gene conferring resistance, as suggested by Flor (1955).

Just as with genes controlling rust reaction in the host, it is important to decide whether genes controlling pathogenicity in the pathogen are closely linked or allelic, because these two possibilities have different implications for the mode of action and evolution of these genes. Further mutational studies with genes controlling pathogenicity should provide additional information on the 'fine structure' of these particular genes. Critical evidence is less likely to come from segregational data, because of the difficulties involved in producing the large number of rust progeny required for such an analysis.

III. GENERAL DISCUSSION

One aspect of the present study, concerned with obtaining more critical data on Flor's (1955) L, M, N and P allelic series in flax, showed that pairs of genes representative of each of the L, M and N series, could be recombined with the low frequency of approximately 0.1%. However, on the definition adopted for allelism, it is not possible to decide whether the genes in each pair are allelic or closely linked. Nevertheless, we may conclude that these genes, if not hetero-alleles at a single locus, must occur at very closely linked loci.

There are three ways in which this type of gene arrangement could have arisen. First, it is possible that different mutations occurred at a single locus in the host, giving rise to alleles which produce qualitatively different phenotypes for rust reaction.

Second, it is possible that some of these genes arose from unequal crossing over. That is, beginning with a single host locus controlling rust reaction, rare unequal crossing over may have produced a duplication for this particular locus, and if these originally identical loci diverged in function, they would appear as closely linked loci with different, but related, functions. For example, Laughman (1955) postulated that the α and β

components of the A locus, controlling anthocyanin pigmentation in maize, arose in this way.

Finally, we must consider the possibility that beginning with genes controlling rust reaction located at separate loci, there has been an evolution of close linkage between these loci. It is widely accepted that under certain conditions, selection can modify the degree of recombination occurring between two loci. For example, Sheppard (1953, 1955) commented on the surprisingly frequent association between polymorphism, linkage and strong selective forces with colour patterns of some insects, snails and fishes, and with some examples of blood groups in birds and mammals. He suggested that these features may be causally related, and that there has been an evolution of close linkage. Kimura (1956) developed a mathematical model appropriate for analysis of a particular polymorphic population, where the coupling arrangement of two pairs of genes has a selective advantage over the repulsion arrangement. This model shows that if genes at one of the loci are kept in balanced polymorphism by heterozygote superiority in fitness, contrasting genes at the other locus would be retained in the population and consequently would exhibit a polymorphism also, providing that linkage between these loci is sufficiently close. He inferred from this, that there would be selection for

closer linkage in these examples.

Schaller and Briggs (1955) noted that the genes controlling reaction to disease, in several examples in plants, were distributed non-randomly among the chromosome complement. Later, Mode (1958) constructed a mathematical model, using Flor's (1956a) data, which shows that host and pathogen genotypes will both exhibit a stable polymorphism, providing that there is no recombination between pairs of host genes conferring resistance. He inferred from this, that there would be an evolution of close linkage between these loci in the host, to the point of no recombination.

It would be difficult to distinguish between these three possibilities experimentally. However, if some host genes controlling rust reaction had arisen by unequal crossing over, these genes themselves would be expected to exhibit unequal crossing over. The original cross over event, producing duplication of a chromosome segment, would be expected to occur very infrequently. This duplication, if in tandem rather than reverse arrangement, would provide opportunity for asymmetric pairing between homologous parts of the chromosome and, therefore, unequal crossing over would be expected to occur much more frequently between the duplicated genes than with the original single locus.

If evolution of close linkage between host genes controlling rust reaction had occurred, the genes concerned

would have controlled separate primary functions initially, and presumably would have retained them on becoming closely linked. On the other hand, if these genes arose from mutation at a single locus in the host, the different genes would be expected to control the same primary function.

Therefore, if it can be shown that these genes are closely linked, rather than allelic, and unequal crossing over does not occur, this could be taken as suggestive evidence that they arose from evolution of close linkage, rather than from unequal crossing over or mutation occurring at a single locus. However, the present study did not provide the type of evidence required to distinguish between the possible origins of these genes.

Any theory proposed to explain their origin must take account of the large number of qualitatively different genes reported to exist at a single "locus" in the host. For example, Flor (1955) reported that at least eleven different genes occur at the L locus in flax. But the linkage relationship between most of these genes has not been critically tested. However, since the four pairs of host varieties used as parents in the present study were selected only because of their particular reactions with available rust stocks, and since the genes in each of these pairs of varieties were found to be allelic or closely

linked, it is reasonable to assume that most, if not all, of the other genes at Flor's L, M, N and P loci are also closely linked or allelic.

Assuming that these eleven genes at the L locus are all closely associated, it is conceivable that evolution of close linkage could have caused this large number of genes to become associated. However, this close association could, perhaps, be more simply explained by postulating that separate mutations had occurred at the L locus, giving at least eleven qualitatively different forms of a single gene product. For, it has been widely shown that a single gene may contain many different mutational sites (e.g. Benzer, 1957), and that mutation at these sites may lead to gene products which are qualitatively different (e.g. Maling and Yanofsky, 1961). For example, at least five different forms of abnormal haemoglobin, with altered β polypeptide chains, are known in humans and it has been inferred from chemical evidence (Ingram, 1959) that these forms arose from different mutations occurring at a single locus.

Furthermore, repeated mutation occurring at a single locus in the host would fulfil, at the time of mutation, Mode's (1958) condition for a stable polymorphism in both host and pathogen populations, namely, no recombination between host genes conferring resistance.

Turning to genes in the pathogen, Flor (1942) postulated that for each gene controlling reaction to rust in the host there is a complementary gene controlling pathogenicity in the pathogen, and a list of the genes identified in the pathogen, corresponding to those identified in the host, is given in Table 21. This table shows three general features of the relationship between genes in the pathogen and those in the host.

First, host genes which are allelic or closely linked, in many cases, interact with genes in the pathogen which are loosely linked or segregate independently. For example, in the present study, whereas genes conferring immunity to rust in Stewart and Biri0, namely L^2 and L^6, respectively, and in Cass and Dakota, namely M^3 and M, respectively, were found to be either closely linked or allelic (Table 6), the genes in M. lini controlling avirulence on each of these varieties segregated independently (Table 19).

If it could be shown that the genes at Flor's L, M, N and P loci are closely linked, rather than allelic, the above feature would raise the question of whether evolution of close linkage had occurred with genes controlling rust reaction in the host, but not to the same extent, if at all, with genes controlling pathogenicity in the pathogen.

On the other hand, if the eleven genes at Flor's L

TABLE 21

GENES IN L. USITATISSIMUM CONTROLLING REACTION TO M. LINI AND GENES IN M. LINI CONTROLLING PATHOGENICITY ON L. USITATISSIMUM, AND THEIR SEGREGATIONAL RELATIONSHIPS[‡]

HOST			PATHOGEN			
VARIETY	GENES CONTROLLING REACTION TO RUST		GENES CONTROLLING PATHOGENICITY ON INDICATED HOST VARIETIES			
	NOMENCLATURE[≠]	ALLELIC, OR CLOSELY LINKED, GROUP	NOMENCLATURE[≠]	SEGREGATE AND/OR MUTATE AS		
				SINGLE UNIT		SEPARATE UNITS[ø]
				COMMON GENE	DIFFERENT GENES	
Clay	K	K	A_K	*		
Bowman	≠		≠	*		
Grant	≠		≠	*		
Minnesota seln	≠		≠	*		
Ottawa 770B	L	L	A_L			*
Burke	L^1		A_L1			*
Stewart	L^2		A_L2			*
Pale Blue Crimped	L^3		A_L3		*	
Kenya	L^4		A_L4		*	
Bolley Golden seln	L^{10}		A_L10	*	*	
Norman	≠		≠	*		
Wilden	L^5		A_L5		*	
Birio	L^6		A_L6		*	
Barnes	L^7		A_L7		*	
Towner	L^8		A_L8			*
Bison	L^9		≠			
Dakota	M	M	A_M			*
Williston Brown	M^1		V_M1			*
Ward	M^2		A_M2			*
Cass	M^3		A_M3			*
Victory A	M^4		A_M4			*
Cortland	M^5		A_M5	*		
Lino 6899M. A.	≠		≠	*		
Argentine seln	≠		≠	*		
Bombay	N	N	A_N			*
Polk	N^1		A_N1			*
Marshall	N^2		A_N2			*
Koto	P	P	A_P		*	
Akmolinsk	P^1		A_P1		*	
Abyssinian	P^2		A_P2		*	
Leona	P^3		A_P3	*	*	
Wells	P^3		A_P3	*		

≠ Flor's (1955) notation for genes in host controlling immunity or resistance, and for genes in the pathogen controlling avirulence or moderate virulence.

ø The genes listed in this column segregated independently in those combinations tested, except according to Flor (1955):
(1) A_N1 is closely linked with A_N2, and loosely linked with A_M3
(2) A_M2 is closely linked with A_P, A_P1, A_P2 and A_P3
(3) A_L8 is loosely linked with A_L10, A_L4 and A_L3.

≠ Gene not identified

‡ Compiled from Flor (1942, 1946, 1955, 1956b) and Table 19.

locus, for example, arose by repeated mutation occurring at a single locus and are consequently allelic, this feature would have different implications. For, if it is assumed further that restriction of pathogen growth in the host results from an interaction between host and pathogen substances, we would then have to postulate that a single host product is able to exist in at least eleven different forms and that each of these provides specificity for interaction with one of the products from at least six different pathogen genes. The pathogen genes must be different because they are situated at six separate loci (see Table 21). If this speculation is extended to Flor's K, M, N and P loci, we would have to postulate further that there is a restriction on the number of different host substances (at least six, that is, one for each host locus), which possess the properties required to interact with pathogen products, but that there is no such restriction on the number of different pathogen products (at least seventeen, that is, one for each pathogen locus), with which these host products can interact.

Considering the second general feature, Table 21 shows that where segregation, or mutation, of pathogenicity occurs as a unit on two or more host varieties with different genes controlling rust reaction, these host genes, in

each example, are closely linked or allelic. Flor (1955) reported that pathogenicity segregated as a unit on host gene M^2 as well as host genes P, P^1, P^2 and P^3, representing a possible exception to the above feature. However, in earlier studies infrequent recombination was observed between pathogenicity on host genes M^2 and P^1 (Flor, 1942), and on host gene M^2 and genes P^2 and P^3 (Flor, 1946).

As suggested previously, segregation of pathogenicity as a unit could be due to either a single gene for pathogenicity interacting with a common gene present in each host variety within a group, or alternatively, to the presence of closely linked (or allelic) genes in the pathogen interacting with different host genes. The experiments required to distinguish between these possibilities with, for example, the genes controlling pathogenicity on Wilden and Birio, are set out in Table 22 and described below.

Rust stocks corresponding to types (1), (2), (3) and (4) of Table 22, have been reported by Flor (1956b), but only types (3) and (4) were available in the present study. Experiment I of Table 22, involves testing a large number of test cross progeny with stocks (1) and (2) to find whether the different genes identified in Wilden and Birio, by these stocks, are closely linked or allelic. That is, we would search for recombinant plants either

TABLE 22

SUGGESTED PROCEDURE FOR DETERMINING THE LINKAGE RELATIONSHIP BETWEEN GENES CONTROLLING IMMUNITY IN BIRIO AND WILDEN AND PATHOGEN GENES CONTROLLING AVIRULENCE ON THESE VARIETIES

EXPERIMENT NUMBER	PEDIGREE	RUST STOCK	REACTION[/]				
			PARENTS			PROGENY	
			WILDEN	BIRIO	SUSCEPT.	JOINT SEGREGATION	
	HOST						
I	(Wilden x Birio) x suscept.	(1)	S	I	S	S I I S	
		(2)	I	S	S	I S I S	
		(3)	I	I	S	I I I S	
						parental	non-parental
II	(Wilden x suscept.) F_2	(2)	I		S	I S I S	
		(3)	I		S	I S S I	
						parental	non-parental
III	(Birio x suscept.) F_2	(1)		I	S	I S I S	
		(3)		I	S	I S S I	
						parental	non-parental
	PATHOGEN	HOST VARIETY	(1)	(2)	(4)		
IV	(Stock (1) x Stock (2)) x Stock 4	Wilden	S	I	S	S I I S	
		Birio	I	S	S	I S I S	
						parental	non-parental

/ I = immune S = susceptible (see Table 4).

immune to both stocks or susceptible to both.

If double-susceptible recombinants occurred and they were also susceptible to stock (3), this would indicate that stock (3) did not interact with a common gene present in both Wilden and Birio, but most likely interacted with the same host genes as did stocks (1) and (2).

This latter possibility could be checked by testing a large number of F_2 progeny, derived from each of Birio and Wilden crossed with a susceptible host variety, with the pairs of stocks (2) and (3), and (1) and (3), respectively. (Experiments II and III of Table 22, respectively). If stock (3) interacted with host genes different from those interacting with stocks (1) and (2), it would be possible to recover recombinants in these F_2 progeny, providing a large number of progeny plants were tested.

Experiment IV, involving test crosses between rust stocks, would indicate whether the different genes in stocks (1) and (2) controlling pathogenicity on Wilden and Birio, are closely associated: that is, are either closely linked or allelic.

Furthermore, if it was found in Experiment I that stock (3) did not interact with a gene common to both Wilden and Birio, then studies of spontaneous mutation in stock (3) x stock (4) hybrids, should indicate whether the genes for

pathogenicity on these varieties are allelic or not. That is, the occurrence of a high frequency of joint mutation of pathogenicity on both host varieties, rather than independent mutation for pathogenicity on each host variety, would suggest that these pathogen genes are allelic, rather than closely linked.

Flor's (1958, 1960a) observations of spontaneous mutation of pathogenicity on Wilden and Birio with 'race' 1 hybrids, suggested that these genes were allelic, but critical experiments were not reported to prove that 'race' 1 interacted with different genes in these host varieties.

If these genes in the pathogen are allelic, we would then have to account for the fact that some of the host genes at, for example, Flor's L locus interact with allelic genes, whereas others interact with non-allelic genes, in the pathogen. Could this mean that some of the genes at the L locus are allelic, whereas others are non-allelic (closely linked), reflecting a parallel relationship with genes for pathogenicity in the pathogen?

Finally, Flor's system of naming host and pathogen genes, shown in Table 21, indicates the third general feature of the relationship between these genes; namely, an apparent one-to-one relationship between genes controlling rust reaction in the host and genes controlling pathogenicity in the pathogen. The occurrence of mutual monohybrid,

dihybrid or trihybrid segregation ratios in studies of both host gene segregations and pathogen gene segregations, led Flor (1942, 1946, 1947, 1955) to postulate his gene-for-gene (or complementary gene) hypothesis, namely, that for each gene controlling reaction to rust in the host there is a specific and complementary gene controlling pathogenicity in the rust.

Gene-for-gene relationships have been reported in other parasitic systems, for example with powdery mildew infection of barley (Moseman, 1959) and wheat (Powers and Sando, 1960), and it has been suggested (Flor, 1956a; Person, 1959) that such a relationship would be expected to occur as a consequence of the co-evolution of obligate parasites with their hosts.

Person (1959) developed a method for detecting gene-for-gene relationships by analysing the pattern of pathogenicity of pathogen strains on host varieties. This method depends on the analysis of, ideally, all possible combinations of pathogenicity on host varieties or, at least, a random sample of possible pathogen genotypes. Person used this method to analyse the published data on pathogenicity of Phytophthora infestans on potato varieties, and M. lini on flax varieties, and concluded that both

groups of data showed those features expected if a gene-for-gene relationship existed in both systems. However, it is considered that this method of analysis was not appropriate for the latter data, since most of the strains of M. lini analysed were derived from Flor's breeding experiments and represented a highly biassed sample of pathogen genotypes.

Catchesides (1951) suggested that the resistant reaction of the host could be due to the interaction of a specific host substance with a specific pathogen substance. If this is correct, a gene-for-gene relationship would be expected to exist whenever these interacting substances represent the products of single genes in both host and pathogen. However, as suggested by Person (1959) exceptions to this one-for-one relationship would be expected if gene interaction occurred within either host or pathogen: that is, on Catchesides's hypothesis, if a single host or pathogen substance was controlled by the interaction of two, or more, genes.

Flor did not report the occurrence of modified di-hybrid segregation ratios, indicative of gene interactions, in either his host or pathogen studies. However, re-analysis of some of his data indicates that two genes may be interacting in M. lini to control pathogenicity on the host variety Williston Brown. Flor (1946) crossed 'races'

22 and 24 of *M. lini* and used the F_1 progeny to produce 133 F_2 progeny. Parental 'races' 22 and 24, the nine and fifteen progeny derived from self fertilization of these respective races, and the seven F_1 progeny, were all virulent on Williston Brown. Segregation for pathogenicity occurred in the F_2 and 17 of these progeny were avirulent, and 116 were virulent, on Williston Brown. Although this latter result did not agree with a 1:3 ratio ($\chi^2_1 = 10.6$, $P < 0.01$), Flor concluded that a single gene controlled pathogenicity on this host variety, and that the gene for virulence was dominant to the gene for avirulence, representing an exception to the rule.

If Flor's hypothesis is accepted, at least one of the parental 'races' must have been heterozygous for the recessive gene controlling avirulence on Williston Brown. However, none of the fifteen progeny (Flor, 1946) and thirteen progeny (Flor, 1946, 1960a) derived from self-fertilization of 'races' 24 and 22, respectively, were avirulent on Williston Brown. Therefore, it is difficult to reconcile the observed results with the hypothesis that a single dominant gene controls virulence on this variety.

As an alternative explanation, we may postulate that a dominant inhibitor gene (I) is present in one of the parents, and this interacts with a dominant gene (A)

normally controlling avirulence on Williston Brown, to give a virulent pathogen phenotype. If we assume that 'races' 22 and 24 have genotypes IIAA and iiaa, respectively, the parents and the F_1 progeny would be virulent on Williston Brown and we would expect a 13:3 ratio of virulent to avirulent F_2 progeny. In fact, Flor's F_2 segregation ratio agreed with this expectation ($\chi^2_1 = 3.11$, $P = 0.05 - 0.1$). Furthermore, on this hypothesis, we would expect all progeny derived from self fertilization of the parents to be virulent on Williston Brown, and this was observed.

Although confirmatory experiments are required to test this hypothesis, it appears likely that gene interaction may occur in M. lini, resulting in an exception to a one-to-one relationship between host and pathogen genes.

Also, the results from the present study of host gene segregations suggest how an apparent exception to the one-to-one relationship between host and pathogen genes, could occur. That is, plant MR-1 possessed genes M and M^3 closely linked in coupling phase (or, if allelic, in cis arrangement) and these genes segregated as a unit in the progeny from this plant (Table 7). Therefore, without prior knowledge, we would consider that plant MR-1 possessed a single gene conferring immunity to stocks Ti-15 and Ti-58,

and presumably F-i, also. However, if this plant had been used as one of the host differentials in testing the pathogenicity of Ti rust progeny, we would have expected to observe a dihybrid segregation ratio among these progeny, since genes A_M3 (conferring avirulence on Cass) and A_M (conferring avirulence on Dakota) in stock F-i, segregated independently among the Ti progeny (Table 19).

If similar recombination (or mutation) had occurred in nature during the evolution of flax genes controlling rust reaction, an apparent one-to-two relationship between host and pathogen genes might have been expected to occur in Flor's studies and in the present investigation.

The absence of any such report may have special significance, perhaps indicating that all host differential varieties possess only one gene at Flor's L, M, N and P loci. On the other hand, it could merely be a consequence of the limited range of rust strains used in breeding experiments; for, both genes conferring avirulence on the postulated two closely linked (or allelic) host genes must be present in the pathogen, before this type of exception could be detected.

The above description of the genetics of host-pathogen interactions is appropriate, if the interacting host and pathogen genetic units represent different single genes.

However, as indicated previously, it is possible that some of these interacting units are allelic. Therefore it would be more appropriate to describe the observed one-to-one relationship between host and pathogen genetic units as a unit-for-unit, rather than a gene-for-gene relationship, since the former term does not specify whether the interacting units represent different single genes, or different parts of a single gene. If several alleles do occur at a single locus in the host and in the pathogen, we would like to know whether there is any relationship between these loci; for example, whether alleles at one locus in the host interact, without exception, with alleles at a single locus in the pathogen.

Another consequence of the demonstration that two genes at Flor's M locus in flax can be combined in a single plant is that some host varieties classified as 'single-gene differentials' could each have a gene (or genes) in common. For example, Person (1959) postulated that Akmolinsk, Abyssinian and Leona have gene A and gene combinations AB and ABC, respectively, to account for the observed pattern of pathogenicity of M. lini on these varieties (Table 20). It was suggested in the present study that, if these genes exist, they are closely linked. (p. 142). Furthermore, Flor (1960b) has shown that the

different units in M. lini for pathogenicity on Akmolinsk and on Abyssinian and Leona, at least, are either closely linked or allelic.

The following model may indicate the significance of the observed pathogenicity patterns on the above three host varieties. According to Flor (1955), host varieties Wilden, Birio and Barnes possess genes L^5, L^6 and L^7, respectively, and Flor (1956b) also postulated that the genes for pathogenicity on these varieties are closely linked or allelic. Suppose that recombination or mutation had occurred in the past with these varieties, giving plants with gene combinations L^5L^6 and $L^5L^6L^7$. If only plants with gene L^5 and gene combinations L^5L^6 and $L^5L^6L^7$ had survived, these would be classified as different 'single-gene differentials'. Furthermore, the same closely linked or allelic genes in M. lini controlling pathogenicity on the original varieties, would also be effective against the new varieties. Therefore, pathogenicity on these three hypothetical varieties would be expected to exhibit features identical to those observed with pathogenicity on Akmolinsk, Abyssinian and Leona.

This model raises the question of whether the observed pathogenicity patterns on the latter three varieties are due to the occurrence of common host genes, as suggested by Person, which arose from events similar to those described

in the model.

Person's hypothesis could be tested by crossing Leona (and also Abyssinian) to a susceptible host variety and raising a large number of F_2 progeny. If Leona possesses the closely linked genes ABC, recombination would be expected to occur in the hybrids, giving F_2 progeny plants with all combinations, theoretically, of these three genes, either singly or in pairs. The recombinant plants with, for example, gene A only, could be detected by testing the F_2 progeny with two rust stocks corresponding to types i and iii of Table 20.

Detection of these recombinant plants would prove that 'compound' host genes may occur at the P locus and, furthermore, would support Person's postulation that common genes occur in these varieties.

Person's hypothesis predicts that it would not be possible to obtain stocks of M. lini with pathogenicity corresponding to that of stocks ix to xvi of Table 20. However, if closely linked genes occur in the pathogen and these interact with different single genes in Akmolinsk, Abyssinian, Leona and Koto, as suggested by Flor (1946), it would be possible to obtain all of the rust stock types shown in Table 20. Therefore, the occurrence of any rust stocks corresponding to stocks ix to xvi, in breeding experiments or mutation studies, would support Flor's

hypothesis.

This discussion emphasizes the need for further investigations on the 'fine structure' of both genes controlling reaction to rust in flax, and genes controlling pathogenicity in M. lini. An attempt has been made to describe the types of experiments which would be expected to provide this information.

ADDENDUM

At a late stage in the preparation of this thesis an abstract was noticed in which Flor also reported recombination between genes M and M^3, in Dakota and Cass, respectively (Flor, H.H. (1962) - Linkage of genes conditioning resistance to rust in flax. Phytopathology 52, 732). He produced 16,188 test cross plants and observed eleven double-susceptible and sixteen double-immune recombinant plants among these. This result does not alter the arguments developed in this thesis, but it shows that these recombinants were most likely produced from reciprocal cross-over events. However, it does not help us decide whether they arose from equal or unequal crossing over.

APPENDIX

REACTION OF DIFFERENTIAL HOST VARIETIES TO PARENTAL AND PROGENY RUST STOCKS

PEDIGREE	NOMENCLATURE OF RUST STOCKS	NUMBER OF STOCKS WITH INDICATED PATHOGENICITY	REACTION[1] OF INDICATED DIFFERENTIAL VARIETY											
			AKMOLINSK BISON WILLISTON BROWN	KOTO POLK OTTAWA 770B	ABYSSINIAN	LEONA	BIRIO WILDEN	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
Parents	P-1*	1	S	I	R	I	I	I	R	I	I	IR	I	R
	P-2*	1	S	I	S	SR	S	S	S	S	S	SR	S	SR
P-2 selfed	S(P-2)-1 to 10	10	S	I	S	SR or S	S	S	S	S	S	SR or S	S	SR or S
First generation progeny	F-a,i*	2	S	I	S	SR	I	I	R	I	I	R	I	R
	b	1	S	I	S	SR	S	I	S	I	S	SR	I	R
P-2 x P-1	c,j	2	S	I	R	-	S	I	R	I	I	SR	I	R
	d	1	S	I	R	I	S	I	R	I	I	I	I	R
	e	1	S	I	S	SR	I	I	S	I	S	S	S	R
	f	1	S	I	R	I	S	I	S	I	S	I	I	R
	g	1	S	I	R	I	S	I	S	I	S	I	S	R
	h	1	S	I	R	I	S	I	S	I	S	SR	S	R
	k*	1	S	I	R	I	S	I	S	I	I	R	I	R
	l	1	S	I	S	SR	I	I	S	I	I	R	S	R
	m	1	S	I	S	SR	S	I	R	I	I	SR	I	R
Test cross progeny	Ta-1*	1	S	I	S	S	I	I	R	I	I	I	S	SR
	2	1	S	I	S	SR	S	S	R	S	I	R	I	R
F-a x P-2	5,10	2	S	I	S	SR	I	S	S	S	S	SR	I	S
	6	1	S	I	S	S	I	S	S	I	S	SR	S	S
	7	1	S	I	S	SR	I	S	R	I	I	R	I	R
	11	1	S	I	S	SR	S	I	R	I	I	R	I	S
	12	1	S	I	S	R	S	S	R	I	S	I	I	SR
	13	1	S	I	S	-	S	I	S	S	S	R	S	R
	14	1	S	I	S	-	I	S	R	S	S	SR	I	S
	15	1	S	I	S	-	I	S	R	S	I	I	I	S

APPENDIX - continued

PEDIGREE	NOMENCLATURE OF RUST STOCKS	NUMBER OF STOCKS WITH INDICATED PATHOGENICITY	REACTION[1] OF INDICATED DIFFERENTIAL VARIETY											
			AKMOLINSK BISON WILLISTON BROWN	KOTO POLK OTTAWA 770B	ABYSSINIAN	LEONA	BIRIO WILDEN	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
Test cross progeny F-a x P-2	Ta-16	1	S	I	S	SR	I	S	R	I	S	S	S	S
	17,18	2	S	I	S	SR	I	I	S	S	I	S	S	R
	19	1	S	I	S	S	S	I	S	I	I	R	S	S
	20	1	S	I	S	S	S	S	S	S	I	SR	S	S
Test cross progeny (F-i x P-2 (P-2 x F-i	Ti-2	1	S	I	S	SR	I	I	R	I	S	I	I	R
	3,6	2	S	I	S	S	S	I	S	I	S	R	I	S
	4,140	2	S	I	S	SR	S	I	R	I	I	I	I	R
	8	1	S	I	S	S	I	I	S	I	I	I	S	R
	10	1	S	I	S	SR	S	I	S	S	S	R	S	R
	11	1	S	I	S	SR	S	S	S	S	S	SR	S	R
	12,71	2	S	I	S	SR	S	S	S	I	I	I	I	R
	15*,85	2	S	I	S	SR	S	I	R	I	S	SR	I	R
	16	1	S	I	S	S	I	I	S	I	I	S	S	R
	17	1	S	I	S	SR	I	I	S	S	I	I	S	R
	20	1	S	I	S	SR	I	I	R	S	S	R	I	S
	21,49	2	S	I	S	SR	S	S	S	I	S	I	S	R
	22,83	2	S	I	S	SR	S	I	S	I	S	I	S	S
	24	1	S	I	S	SR	I	I	S	I	I	SR	S	S
	32	1	S	I	S	SR	S	S	S	I	I	S	S	S
	33	1	S	I	S	SR	S	S	S	S	I	I	S	R
	35	1	S	I	S	SR	S	I	R	I	S	R	S	S
	36	1	S	I	S	SR	I	I	R	S	I	SR	I	R
	37	1	S	I	S	S	S	S	S	I	I	SR	I	SR
	39	1	S	I	S	SR	S	S	S	S	I	SR	S	R
	44	1	S	I	S	S	S	S	S	S	S	I	S	SR
	51	1	S	I	S	SR	I	S	S	I	S	SR	I	R

APPENDIX - continued

PEDIGREE	NOMENCLATURE OR RUST STOCKS	NUMBER OF STOCKS WITH INDICATED PATHOGENICITY	REACTION[1] OF INDICATED DIFFERENTIAL VARIETY											
			AKMOLINSK BISON WILLISTON BROWN	KOTO POLK OTTAWA 770B	ABYSSINIAN	LEONA	RIRIO WILDEN	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
Test cross progeny (F-i x P-2 (P-2 x F-i	Ti-52	1	S	I	S	SR	S	I	S	S	S	SR	I	R
	55	1	S	I	S	SR	I	S	R	S	I	I	I	S
	56	1	S	I	S	SR	S	I	R	S	S	SR	I	R
	57,136	2	S	I	S	SR	S	I	S	I	I	I	S	S
	58*	1	S	I	S	SR	S	I	R	S	I	I	I	R
	60,64,84	3	S	I	S	SR	I	I	S	I	S	I	I	R
	61	1	S	I	S	SR	S	S	S	I	S	I	S	R
	62	1	S	I	S	SR	I	S	R	S	S	SR	S	R
	63	1	S	I	S	SR	I	I	R	I	I	R	I	SR
	65	1	S	I	S	SR	S	S	S	S	S	R	I	SR
	68	1	S	I	S	SR	I	I	R	I	I	SR	S	SR
	69	1	S	I	S	SR	I	I	S	S	I	SR	S	R
	70	1	S	I	S	SR	I	S	S	S	S	I	S	R
	72,139	2	S	I	S	SR	I	S	S	I	S	SR	I	S
	75	1	S	I	S	SR	S	S	R	I	I	I	S	R
	76	1	S	I	S	S	S	S	R	S	I	R	S	R
	77	1	S	I	S	SR	S	I	S	S	I	I	S	S
	81	1	S	I	S	SR	I	S	R	I	I	SR	I	SR
	91	1	S	I	S	SR	I	I	S	S	S	S	I	R
	93	1	S	I	S	SR	I	S	S	I	S	I	I	R
	94	1	S	I	S	SR	I	S	R	I	I	SR	I	R
	95	1	S	I	S	SR	S	I	R	S	S	I	S	R
	96	1	S	I	S	SR	S	I	R	S	I	R	S	R
	98	1	S	I	S	SR	S	I	S	S	I	I	I	SR
	99	1	S	I	S	SR	S	I	S	S	S	SR	S	SR
	100	1	S	I	S	SR	I	S	R	S	S	S	S	S
	101,148	2	S	I	S	SR	I	I	R	S	S	R	S	R

APPENDIX - continued

PEDIGREE	NOMENCLATURE OF RUST STOCKS	NUMBER OF STOCKS WITH INDICATED PATHOGENICITY	REACTION OF INDICATED DIFFERENTIAL VARIETY											
			AKMOLINSK BISON WILLISTON BROWN	KOTO POLK OTTAWA 770B	ABYSSINIAN	LEONA	RIRIO WILDEN	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
Test cross progeny (F-1 x P-2 (P-2 x F-1	T1-103	1	S	I	S	SR	I	I	R	S	I	S	S	S
	104	1	S	I	S	SR	I	S	S	S	I	I	I	SR
	105,127	2	S	I	S	SR	S	I	S	S	I	SR	I	R
	108	1	S	I	S	SR	S	I	S	S	I	I	I	R
	109	1	S	I	S	SR	S	I	S	S	I	S	S	R
	110	1	S	I	S	SR	S	S	S	S	S	I	S	R
	111	1	S	I	S	SR	S	S	S	I	S	R	I	R
	113	1	S	I	S	SR	I	I	S	I	S	S	I	SR
	115	1	S	I	S	SR	S	I	R	I	I	I	I	R
	116	1	S	I	S	SR	I	I	S	I	I	I	I	R
	118,138	2	S	I	S	SR	I	I	R	I	I	S	S	R
	119	1	S	I	S	SR	S	S	S	I	S	SR	I	SR
	125	1	S	I	S	SR	S	I	R	S	S	SR	I	SR
	126	1	S	I	S	SR	I	I	S	I	I	SR	S	R
	129	1	S	I	S	SR	I	I	S	I	I	I	I	S
	131	1	S	I	S	SR	S	S	S	I	S	SR	S	R
	132	1	S	I	S	SR	S	S	R	I	S	S	I	R
	133	1	S	I	S	SR	I	S	S	I	S	R	I	SR
	141	1	S	I	S	SR	I	S	R	I	S	I	S	S
	142	1	S	I	S	SR	S	S	S	S	I	I	S	R
	143	1	S	I	S	SR	I	I	R	I	I	I	S	SR
	144	1	S	I	S	SR	I	S	S	I	S	SR	S	R
	145	1	S	I	S	SR	I	I	R	I	I	SR	I	R
	146	1	S	I	S	SR	I	S	R	S	S	SR	I	SR
	147	1	S	I	S	SR	I	S	R	S	S	R	I	R
	149	1	S	I	S	SR	S	S	R	S	S	S	S	S
	152	1	S	I	S	S	S	S	S	S	I	I	I	R

APPENDIX - continued

PEDIGREE	NOMENCLATURE OF RUST STOCKS	NUMBER OF STOCKS WITH INDICATED PATHOGENICITY	REACTION[1] OF INDICATED DIFFERENTIAL VARIETY											
			AKMOLINSK BISON WILLISTON BROWN	KOTO POLK OTTAWA 770B	ABYSSINIAN	LEONA	BIRIO WILDEN	BOMBAY	CLAY BOWMAN	CASS	DAKOTA	KENYA	STEWART	VICTORY A
Test cross progeny (F-i x P-2 (P-2 x F-i	Ti-153	1	S	I	S	SR	S	S	S	S	I	I	S	SR
	154	1	S	I	S	S	S	S	S	I	I	SR	S	SR
	155	1	S	I	S	SR	I	S	S	I	I	SR	I	R
	156	1	S	I	S	SR	I	S	S	S	S	I	I	R
	157	1	S	I	S	SR	S	I	R	I	I	I	S	S
	160	1	S	I	S	SR	S	S	R	S	S	R	S	SR
	161	1	S	I	S	SR	S	S	R	S	I	S	I	SR
	162	1	S	I	S	SR	S	I	S	I	S	I	I	R
	163	1	S	I	S	SR	S	I	S	S	I	SR	I	SR
Test cross progeny F-k x P-2	Tk-31,45	2	S	I	R	I	S	I	S	S	I	I	I	R
	32	1	S	I	R	I	S	S	S	I	S	I	S	SR
	33	1	S	I	R	I	S	I	S	I	I	SR	S	SR
	34	1	S	I	R	I	S	S	S	S	I	R	S	R
	35	1	S	I	R	I	S	S	S	S	I	I	I	R
	36	1	S	I	S	SR	S	I	S	I	I	SR	I	SR
	37	1	S	I	S	SR	S	S	S	I	S	SR	S	R
	38,40	2	S	I	S	SR	S	S	S	I	S	I	S	SR
	39	1	S	I	R	I	S	I	S	S	I	R	S	SR
	41*	1	S	I	R	I	S	S	S	I	I	SR	I	R
	42	1	S	I	R	I	S	I	S	I	I	I	S	R
	43	1	S	I	R	I	S	I	S	S	S	SR	I	R
	44	1	S	I	S	S	S	S	S	S	S	I	S	SR
	46	1	S	I	S	SR	S	I	S	I	S	R	I	SR
	47*	1	S	I	R	I	S	S	S	S	S	I	S	SR

APPENDIX - continued

PEDIGREE	NOMENCLATURE OF RUST STOCKS	NUMBER OF STOCKS WITH INDICATED PATHOGENICITY	REACTION OF INDICATED DIFFERENTIAL VARIETY							
			AKMOLINSK BISON WILLISTON BROWN	KOTO POLK OTTAWA 770B	ABYSSINIAN LEONA	BIRIO WILDEN	BOMBAY CLAY BOWMAN	CASS	DAKOTA	KENYA STEWART	VICTORY A
Test cross progeny	Th-1	1	S	SR	I	I	S	SR		R
F-h x P-2	2	1	R	I	S	S	S	R		R
	3	1	R	I	I	I	S	SR		R
	4,6	2	S	SR	S	S	S	R		R
	5,7	2	R	I	S	S	S	SR		S
	13	1	R	I	S	S	S	SR		R
	14	1	S	SR	S	S	S	R		R
	15	1	R	I	I	S	S	SR		R
	16	1	R	I	S	S	S	SR		S
	17	1	R	I	I	I	S	SR		S
	18	1	S	SR	S	I	S	R		SR

/ As for Table 4, I = immune, IR = immune to resistant, SR = semi-resistant, S = susceptible

* Rust stocks used in study of host gene segregations

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