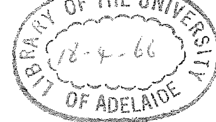
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"GENETICAL STUDIES IN TETRAPLOIDS"

Submitted for the degree of Doctor of Philosophy
in the University of Adelaide by

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SUMMARY

Following an account of genetical studies in induced and naturally occurring tetraploids, the need for some revision of terminology for such studies is considered. The 'duplex segregation frequency' is suggested as a useful parameter for such studies, and this parameter is compared with those used by other authors for similar purposes. The term 'di-tetrasomic inheritance' is introduced to describe a form of inheritance intermediate between disomic and tetrasomic inheritance which has been demonstrated on a number of occasions in newly synthesized allotetraploids, but not, apparently, in naturally occurring tetraploids.

Efficient procedures for detecting and estimating duplex segregation, double reduction and linkage are considered. The general superiority of the coupling genotypes for the detection and estimation of linkage is demonstrated, and breeding procedures for producing such genotypes are outlined. Relationships between the recombination frequency and the number of crossovers have also been derived, and it is shown that the upper limit of recombination is $\frac{3}{4}$. In general the recombination

frequency for a particular region in tetraploids may exceed the corresponding diploid frequency even when the mean frequency of crossing-over is the same in the two cases, and an upper bound is given for the amount of such an excess which may be expected in practice. A discussion is included of the interpretation of data from experiments in which the diploid and tetraploid recombination frequencies are compared.

Genetical studies in the locally occurring tetraploid *Oxalis pes-caprae*, L. have been directed at elucidating the inheritance of tristyly and a number of leaf and calyx pigments. Two linked loci are postulated to control the inheritance of tristyly, one controlling the mid:long difference, and another which is epistatic to the latter controlling the short:non-short difference. Results from some crosses point to this linkage being tight, and possible explanations for such linkage in terms of adjacent origin or evolution of close linkage are considered. Results from a number of crosses cannot be explained in terms of the simple two-locus model given above, and the possibility of a more complex interaction between some of the genes postulated is

discussed.

Results from studies with the leaf pigments are generally in agreement with the hypothesis that the inheritance of these pigments is controlled by an allelic series. Critical tests for allelism are outlined, although no results are as yet available. Similarly results indicate that the inheritance of a number of calyx pigments may be controlled by another series of closely linked or allelic genes.

The majority of single gene segregations have given results in agreement with tetrasomic expectations, but several results have strongly suggested di-tetrasomic inheritance. In particular one plant has given for three possibly independent markers duplex segregation frequencies of $\frac{1}{69}$, $\frac{0}{69}$ and $\frac{0}{78}$ respectively. It appears that the variability in duplex segregation frequencies may extend to loci throughout the genome.

To explain these results a detailed examination has been made of the evolution which may be expected in newly synthesized allotetraploids. A model of pairing in which pairing initiation takes place independently at two sites is considered and tested against several

published results. On the basis of this model it is shown that segregation and recombination of chromosomal regions may cause the preferential pairing of a newly created allotetraploid to be lost within comparatively few generations. The manner in which this evolution towards tetrasomic inheritance may be opposed by selection is discussed, although it is argued that such selection would need to be quite severe to successfully oppose the loss of preferential pairing.

The results in O. pes-caprae may be explained if it is assumed that this species originated as an allotetraploid showing di-tetrasomic genetical behaviour, and is in the process of evolving tetrasomic genetical behaviour. The evolution in this case could be somewhat retarded since reproduction in O. pes-caprae appears to be principally asexual.

DECLARATION

I 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.

J. A. Sved.

PREFACE

The first genetical study of a tetraploid was made by Gregory (1914) some ten years or so after the rediscovery of Mendelism. Since that time there have been numerous studies in both naturally occurring and artificially produced tetraploids, the majority of which have been reviewed by Little (1945, 1958) and Burnham (1962). An attempt has been made in Part I to list most of the genetical studies, giving prominence to four or five studies which appear to be of most importance. Theories of segregation in autotetraploids are reviewed, and the need for a more general nomenclature and a theory of segregation for tetraploids in general is discussed.

Procedures for detecting and estimating linkage, testing for allelism, etc. are relatively straightforward in diploids. In tetraploids however most of these procedures are considerably more complex, and there are much larger numbers of genotypes which can possibly be used in such tests. On both of these counts there is need for an evaluation of the efficiencies of various breeding procedures in tetraploids. This has been attempted in Part II.

The locally occurring tetraploid *Oxalis pes-caprae*, L. was found to have considerable variation in leaf, calyx and flower pigments, as well as a well-developed system of tristyly, and therefore appeared very suitable for genetical studies. Preliminary studies, especially in tristyly, had been in progress for two or three years before being taken over by the present author, and appropriate acknowledgements are given in the text of Part III.

The genetical findings in *O. pes-caprae* have been of considerable interest and have led to a broader consideration of the type of inheritance to be expected in all classes of tetraploids than has previously been given. While these considerations are largely independent of the studies in *O. pes-caprae* it has been thought preferable to report the majority of this work in Part IV after the experimental studies, since many of the results are somewhat speculative and the need for such speculation is best seen in conjunction with the *O. pes-caprae* studies.

Part V is a largely independent theoretical study concerned with the derivation of the relationship between the recombination frequency and the amount of crossing over in tetraploids. Essentially the same results as reported

in this section have previously been published in 'Heredity' Vol. 19, pp.585-596 (1964).

This study was carried out under the supervision of Professor J.H. Bennett. I am grateful for his critical advice throughout the course of the study. In addition I am indebted to a number of colleagues who have helped in various ways, particularly to Dr.O.R. Byrne and Mr. I.R. Franklin for their advice and assistance in the early stages of the work on O. pes-caprae, and to Mr. A.J. Pryor for his assistance with the cytological studies. Professor J. Venkateswarlu and Dr. R.N. Oram have kindly given me permission to quote unpublished results from their Ph.D. theses. Finally I should like to express my appreciation to Miss T.Siekmann for her help in the preparation of the thesis, and to C.S.I.R.O. and the University of Adelaide for financial support.

I. INTRODUCTION

1. THE THEORY OF TETRASOMIC INHERITANCE

i Single locus

The possibility of meiotic pairing in a tetraploid between two chromosomes derived from the same gamete was first pointed out by Muller (1914). He considered the case where the four chromosomes of a set were equally attracted to each other in meiosis, leading to a form of inheritance which has become known as tetrasomic inheritance. Muller derived the expectations for a single locus assuming that chromosomes associated in pairs, i.e. in bivalents. The most important finding was the gametic output from the parental genotype AAaa, viz. 1 AA : 4 Aa : 1 aa, as compared to the 1 AA : 2 Aa : 1 aa ratio expected for duplicate disomic inheritance.

Later Haldane (1930) derived expectations for a single tetrasomic locus considering the case where chromatids, rather than chromosomes, paired and assorted at random. The two types of segregation were labelled chromosome and chromatid segregation respectively.

Mather (1935, 1936a) first indicated that these two were limiting segregation types, and that in general the expectations should lie somewhere between the two. He introduced

a parameter, α , defined in cytological terms, which specified the segregation at any single tetrasomic locus. However he mistakenly supposed that the simplex (Aaaa) and duplex (AAaa) segregations must be specified by different parameters. This was later corrected (Fisher and Mather, 1943), when it was shown that the gametic expectations could be given independently of cytological considerations. The segregation from all classes of parental genotypes was given (Table 1) in terms of the frequency of double reduction (Darlington, 1929), i.e. the frequency with which gametes are formed containing at a particular locus two genes derived

ZYGOTE		GAMETES			DIVISOR
		AA	Aa	aa	
Nulliplex	a_4	.	.	1	1
Simplex	Aa_3	α	$2(1-\alpha)$	$2+\alpha$	4
Duplex	A_2a_2	$1+2\alpha$	$4(4-\alpha)$	$1+2\alpha$	6
Triplex	A_3a	$2+\alpha$	$2(1-\alpha)$	α	4
Quadruplex	A_4	1	.	.	1

Table 1. Expected gametic frequencies in tetrasomic inheritance (From Fisher and Mather, 1943)

from sister chromatids. The parameter α was redefined by Fisher and Mather as the frequency of double reduction, making

it equal to half Mather's (1936a) value. Unfortunately some confusion is still caused by workers adhering to the original definition of α, e.g. Little (1958), Moens (1964).

In reviewing experimental studies in tetraploids, Little (1945, 1958) has pointed out that although the work of Mather (1936a) is well known, the practice of testing results for agreement with the chromosome or chromatid theories still persists to a large extent. Chromosome segregation represents one extreme of the range of possible values for tetrasomic segregation (i.e. $\alpha = 0$), and since these values are expected under the simple model of bivalent formation, there appears to be some justification for testing for deviations from chromosome segregation. However if dominance is complete the test for deviations from chromosome segregation may be made with much more precision by back-crossing a triplex parental genotype. The appearance of even a single double recessive gamete from such a genotype is sufficient to invalidate the hypothesis that $\alpha = 0$.

On the other hand there appears to be no similar justification for the test for deviations from chromatid segregation, since this segregation neither represents the upper extreme of the range of possible segregation ratios, nor is to be expected under any realistic models of chromosome pairing and disjunction. This segregation, corresponding to

a value of α of $\frac{1}{7}$, is to be expected in the limit with an infinite number of partner exchanges and crossovers between the locus and the centromere. However, as argued by Burnham (1962), $\frac{1}{7}$ is evidently not an upper bound for α, since for instance one partner exchange, plus one crossover between the centromere and the locus, together with random disjunction of chromosomes will give a value of α of $\frac{1}{6}$. Burnham argues that $\frac{1}{6}$ should be regarded as an upper bound for α, but this also seems a little unsatisfactory, since an excess of adjacent disjunction of chromosomes could cause the value of $\frac{1}{6}$ to be exceeded. In addition the possibility of non-random orientation of chromatids at the second division of meiosis cannot be ignored (Whitehouse and Haldane, 1946), and its occurrence could increase the value of α still further. There seems to be no simple answer to the problem of giving a realistic upper bound for α.

The above discussion has assumed regular disjunction of chromosomes in meiosis, producing a gamete disomic at each locus. However numerical non-disjunction is known to occur, leading to both monosomic and trisomic gametes which are frequently viable. Catcheside (1956, 1959) has considered the effect on the phenotypic expectations of numerical non-disjunction, showing how this may superficially resemble the effect of double reduction, and has shown how

the joint estimation of the frequencies of double reduction and numerical non-disjunction may be carried out.

In the absence of such disturbances as viability differences a knowledge of the value of α is sufficient to specify the expectations of all disomic gametes at a single tetrasomic locus. However it is of interest to investigate the possible effect on α of variation in such factors as the frequency of quadrivalent formation and the type of chromosome disjunction. These questions have been considered by Mather (1936a) and Catcheside (1956). However it appears from the work of Moens (1964) that the simple multiplicative relationships suggested by Mather and Catcheside may have to be considerably modified in some cases to take account of the fact that the probability that the chromosomes are associated in a quadrivalent at metaphase is not independent of the possibility of crossing-over between the locus and the centromere. The values predicted by Mather and Catcheside must from this point of view be regarded as lower limits for the true value.

A further contribution, of mathematical rather than genetical interest, is that of Moran (1962), who derived the value of α to be expected under any specified sequence of

re-pairing and crossing-over, but assuming random chromosome disjunction. Some infinite sequences of partner exchanges and crossing over are shown to lead to a value of α of $\frac{1}{7}$, although this is not so for all such sequences.

ii Two or more loci

Based on the assumptions of chromosome and chromatid segregation respectively, de Winton and Haldane (1931) and Sansome (1933) gave approximate methods for estimating the recombination frequency between two linked tetrasomic loci. The first of these methods was subsequently improved by Mather (1936a) to take account of double reduction.

The problem of the exact treatment of linkage was solved by Fisher (1947), when he introduced the important notion of a mode of gamete formation. For a single tetrasomic locus for instance he delineated two modes of gamete formation, viz. formation through double reduction, and non-double reduction. In terms of a single tetrasomic parental genotype $A_1A_2A_3A_4$, the gametes arising by the two modes of formation would be respectively A_1A_1, A_2A_2 ..... etc. and A_1A_2, A_1A_3 ..... etc. For two loci Fisher (1947, 1950) showed there were eleven modes of gamete formation, and in

general for l (linked) loci $\frac{1}{48}(16^l + 16.4^l + 16)$.

The gametic expectations for any parental genotype for an arbitrary number of loci and level of ploidy could then be given in terms of the frequencies of the appropriate modes of gamete formation. The procedure for deriving these expectations was summarised in the generalization of Mendel's law that "The gametic frequencies are invariant in respect of any gene substitution applied systematically to the genic content of an organism and of the gametes it produces". Fisher himself gave the expectations for all classes of parental genotypes with two tetrasomic loci. Bennett (1953) and Griffing (1956) later used the same principles in deriving the gametic expectation for two hexasomic and trisomic loci respectively. For the analysis of tetraploid tetrad data (Sved, 1963) the same principles have again been applied.

It was shown by Fisher that the frequency of recombination between two loci could be given as a linear combination of the mode frequencies. This could be estimated for tetrasomic loci by back-crossing any except one of the nineteen doubly digenic genotypes possible with two loci, followed by a second backcross to identify the genotype of the gamete produced in the first backcross. A first

backcross of any or all of the nineteen genotypes was however shown to give insufficient information to allow the recombination frequency to be estimated.

In spite of the fact that Fisher's (1947) treatment constitutes the generalised analysis for an arbitrary number of loci and level of ploidy it does not appear to have received wide recognition or use. However undoubtedly one of the main reasons for the scarcity of studies applying these principles is the amount of labour involved in a second backcross programme. In view of these difficulties it is important to know the efficiencies of the different possible backcrosses. From the point of view of estimating the recombination frequency, these efficiencies have been considered in Section II 5.

2. REVIEW OF EXPERIMENTAL GENETICAL STUDIES IN TETRAPLOIDS

i Newly arisen tetraploids

a. Autotetraploids

The work of Blakeslee, Belling and Farnham (1923) on *Datura* may be considered in this category, the tetraploids in this case having arisen spontaneously from a few of several thousand diploids grown in the field. Two loci were studied, large numbers of plants being obtained from crosses involving nearly all possible combinations of parental genotypes. Perhaps the most interesting result was the finding for both loci of recessive plants from triplex back-crosses, the first recorded occurrence of double reduction. Blakeslee et al. realized the possibility of recessive gametes arising as a result of reductional separation at the first division of meiosis, but did not indicate how such reductional separation could occur. Retrospectively it may be seen (Burnham, 1962) that this might have been taken as an indication that crossing-over takes place at the four strand stage, some time before the demonstration by Anderson (1925) using attached-X in *Drosophila*.

The data of Blakeslee et al. have been further analysed

by Little (1945) to show that they are in agreement with neither chromosome nor chromatid segregation. However they agree well with expectation under the more general theory of Mather (1936a).

A further large experiment in autotetraploid segregation was carried out by Welch (1962), who studied the segregation of four chromosome 2 markers in maize. He showed that the frequency of double reduction is approximately proportional to the map distance between the locus and centromere. On the other hand Moens (1964) working with markers in the long arm of chromosome 2 in autotetraploid tomatos failed to find any relationship between the genetic output for a single locus and the map distance between the locus and centromere. The choice of relatively closely linked markers distal to the centromere, a low frequency of quadrivalent formation (approximately 30%), and the use of duplex selfings, a relatively inefficient estimator (see Section III 2) evidently contribute to this. However Moens has shown that the specialized structure of chromosome 2 where no chiasmata appear to occur in the short arm, would tend to lead to a distribution of chiasmata in the long arm which would mask the usual relationship between α and the frequency of crossing-over between gene and centromere. As pointed out

in the previous section, a similar argument to that used by Moens may be used to show that in general the multiplicative relationships for α given by Mather (1936a) and Catcheside (1956) will tend to underestimate its true value.

Several other studies in artificially induced autotetraploids have given information on single gene segregations ratios. Those of Sansome (1933) in tomato, and Little (1945) in antirrhinum may be mentioned in this category.

Several experiments have been carried out to compare the recombination frequencies in similar segments of diploids and autotetraploids. Studies relevant to this question include those of Sansome (1933) in tomato, Oram (1959) and Welch (1962) in maize, and de Winton and Haldane (1931) in _Primula sinensis_. In all these studies except those of Oram the recombination frequency in the tetraploids was determined by approximate methods from first backcross data, which methods Mather (1936a) has suggested cannot be trusted to give accurate results over fifteen map units. The results of Oram suggested that for one of two pairs of loci considered the recombination frequency in the tetraploid exceeded that in the diploid. An interesting question arises in this

connection of whether the same recombination frequencies are necessarily to be expected in the two cases. This point is considered further in Part V.

Many basic problems exist in genetics, e.g. mechanism of crossing-over, mechanism of dominance, etc., in which a comparison of properties of diploid and tetraploid forms might give useful information. The best approach to such problems is usually through doubling the chromosome complement of a genetically known diploid. Perhaps the best known such study is that of the comparison of the action of self-sterility alleles in diploids and autotetraploids (Lewis, 1943; Brewbaker, 1954, 1955). The first accurate study of dominance in tetraploids has recently been made by Nelson and Douglas (1963), who investigated some relationships in yeast between gene dosage and enzyme activity. Finally in the field of quantitative genetics, where studies in tetraploids could be expected to yield much useful information, the only study appears to have been a relatively small one of Lindstrom (1935).

b. Allotetraploids

An outstanding study of segregation in an allotetraploid was made by Collins and Longley (1935) using a tetraploid plant arising from the pollination of an

apparently unreduced gamete of diploid _Zea mays_ with pollen from tetraploid teosinte (_Euchlena perennis_, but see Reeves and Mangelsdorf (1959) and Shaver (1962) for arguments that maize and teosinte are co-generic). These authors used the maize gene waxy, which enabled scoring to be carried out at the pollen grain stage, and extremely large numbers of gametes to be classified (approximately five hundred per plant).

The original plant, 56 F_2 plants, and 453 F_3 plants from nine families were grown. The table embodying the principal results has been reproduced in Section IV 2 iii where some aspects of the data are considered in more detail. However some salient features of the results may be described here. The frequency of recessive (waxy) pollen grains from the original plant was 0.046 ± 0.009. Deviations from the value one-sixth expected for tetrasomic inheritance were thus clearly established. Similarly the majority of duplex F_2 and F_3 plants gave a frequency of waxy pollen grains intermediate between disomic and tetrasomic expectation, while the simplex plants gave good agreement with a 1 non-waxy : 1 waxy ratio. Furthermore the variance of the frequency of recessives observed from duplex plants was far higher than expected by chance, whereas the variance from simplex plants was close to

chance expectation. This demonstrated that the variation in genetic behaviour shown by WxWxwxwx plants was not due to differential survival of the waxy pollen grains, but could more reasonably be ascribed to variation in the frequency of pairing between chromosomes of different genomes. A few backcrosses were made with the segregating plant as female parent, and no evidence was found to suggest that the results from male and female gametogenesis were in any way different. Overall a slight, but non-significant increase in the frequency of recessives was found from F_1 to F_2 to F_3.

A further experiment in maize-teosinte allotetraploids has been reported by Shaver (1962). A number of different loci were studied in several allotetraploid stocks. All showed segregation frequencies less than expected with tetrasomic inheritance, and there was significant variation between markers.

Several other small studies of segregation in allotetraploids have yielded diverse results. A hybrid tetraploid in the genus Rubus produced by Crane and Darlington (1927) segregated 471:19 for one character, but did not segregate for another. Lindstrom (1936) studied the segregation from several markers in hybrid tetraploid

tomato and found the results for five genes to be in agreement with expectations for tetrasomic inheritance. Swaminathan (1956) studied the segregation in a synthesized allotetraploid of the genus *Solanum*. One character segregated 73:19, i.e. in agreement with tetrasomic expectations, while two other characters failed to segregate.

Within recent years a large series of experiments on segregation of allopolyploids within the genus *Gossypium* has been carried out (summarised in Gerstel and Phillips, 1958). This has been followed by a series in the genus *Nicotiana* (Gerstel, 1960, 1963). Large numbers of species have been studied in both genera, and a close relationship has been demonstrated between the taxonomic relationships of species and the genetic behaviour of their hybrid tetraploids. For example the tetraploid *G. arboreum X thurberi*, a hybrid of Old World and North American species gave only one recessive amongst over one hundred plants classified for three loci. On the other hand the tetraploid from the closely related *G. arboreum* and *G. herbaceum* gave ratios in agreement with tetrasomic expectations. Other tetraploids gave segregation ratios somewhere intermediate between these two. One difficulty in interpreting these results has been the inability to test for disturbing influences such as viability differences and misclassification.

Many of these difficulties would be removed if it were possible to backcross the simplex genotype, as practised by Collins and Longley (1935), since any deviation from a 1:1 ratio would be evidence of such disturbing factors.

A feature of the results in Gossypium was the similarity, with few exceptions, of segregation ratios given by unlinked genes within various tetraploids. However the studies in Nicotiana have shown much larger differences between the ratios for different loci, so that in view also of the results of Swaminathan and Crane and Darlington given previously the similarity is evidently not a general phenomenon.

In addition to the genetical studies, cytological studies of the metaphase configurations of most of the Nicotiana allopolyploids have been made (Sarvella, 1958; Phillips, 1964). A high correlation between the frequency of recessive segregants, and the frequency of multivalent formation was found. There were however some deviants from a linear relationship between the two variables, and Phillips has proposed an explanation for these. This explanation, together with a slightly different one proposed by the present author, will be given in Sec. IV 2 ii.

ii Naturally occurring tetraploids

The type of problem faced in the study of naturally occurring tetraploids is usually quite different from that of induced tetraploids. Usually little is known about the genetics of the plant except perhaps by inference in comparison with a closely related diploid. Studies to elucidate the inheritance of particular characters must be carried out in conjunction with tests to determine the type of segregation at the postulated loci.

Studies on two species, *Lythrum salicaria* and *Medicago sativa*, are especially worthy of mention. The studies in *L. salicaria* have been directed towards elucidating in considerable detail the inheritance of two characters, tristyly and pink or rosy flower colour. Two loci have been shown to control the inheritance of style length (Fisher and Mather, 1943), one controlling the inheritance of the mid:long difference, and another which is epistatic to this locus controlling the short:non-short difference. A single gene has been shown to control the inheritance of flower colour. This gene is linked to the short gene, and estimates of the strength of linkage have been obtained by Fisher (1949a) using second backcross data. The results at all three loci individually have been shown to be in

agreement with tetrasomic expectations (Fisher and Mather, 1943; Fisher and Martin, 1947; Fyfe, 1953). The critical approach adopted by Fisher and Mather for eliminating the hypothesis of duplicate disomic inheritance for the mid locus serves as a model which has unfortunately been followed on few occasions by other workers in genetical studies in tetraploids.

Probably more genetical studies have been made in the tetraploid Medicago sativa (alfalfa or lucerne) than in any other tetraploid. Studies prior to 1950 were reviewed by Atwood and Grun (1952) who reported that most studies had been explained by investigators on the basis of disomic inheritance. In virtually all cases however it is clear that the possibility of tetrasomic inheritance has not been critically excluded (Stanford and Clement, 1958). This scarcity of clear-cut results has now been remedied, starting with the work of Stanford (1950) (see Stanford and Clement, 1958 for references, and also Goplen and Stanford, 1960). Some nine or ten chracters have been investigated where single gene control has been demonstrated, and all results have been explicable on the basis of tetrasomic inheritance. Most of the tests have not been based on large numbers, and have been made using selfing rather than backcrossing, but the use of F_3 families is sufficient to

critically exclude the hypothesis of disomic inheritance.

Since M. sativa contains only eight chromosome sets, it seems likely that the genetical results now available include loci on a large proportion of these chromosome sets. A method for systematically investigating the segregation of genes on all chromosome sets using plants deficient for single chromosomes was outlined by Stanford (1959), although results for only one chromosome set were then available. The possibility of homology throughout the genome was indicated by the cytological behaviour of chromosomes in a reduced (i.e. 2n = 16) plant studied by Stanford and Clement (1958). Less than five percent of chromosomes were found as univalents.

Several reports of disomic inheritance in M. sativa have been made within recent years (Twamley, 1955; Oldemeyer, 1956; Dudley and Wilsie, 1957, 1958; Childers and McLennan, 1960). In all of these cases interactions between two or more disomically or tetrasomically inherited genes have been postulated to explain genetical results. In no case has the possibility of completely tetrasomic inheritance been critically excluded.

A third species which has been extensively studied is the cultivated potato, Solanum tuberosum. As was the case

with the alfalfa studies, early studies reported disomic inheritance, but later studies, principally those of Lunden (1937, 1950) and Cadman (1942) have interpreted results on a tetrasomic basis. Critical tests to distinguish between disomic and tetrasomic inheritance have not been made, although Cadman was aware of such tests but had not at the time of writing applied them. The evidence for tetrasomic inheritance rests largely on the finding of ratios in agreement with 5 dominant:1 recessive (rather than 3 dominant:1 recessive expected for duplicate disomic inheritance) from backcrossing commercial varieties segregating for anthocyanin pigments and disease resistance. For some four or five loci the evidence can be considered as strongly favouring the hypothesis of tetrasomic inheritance. No conclusive finding of disomic inheritance appears to have been obtained.

The genetics and cytology of the cultivated garden plant Dahlia variabilis have been studied by Lawrence (1929, 1931), Lawrence and Scott-Moncrieff (1935). The chromosome number of D. variabilis has been shown to be sixty-four, which by comparison with related species shows that it is an octoploid. Lawrence has shown that the pigments produced by this species include those produced by two distinct tetraploid species and proposes that D. variabilis has been

produced by hybridization between two such tetraploids. Although critical tests have not been made, backcrosses of many plants have given typical tetrasomic ratios, and tetrasomic inheritance has been claimed for four loci controlling the production of different pigments. This claim is somewhat at variance with cytological observations which indicate that hexavalents and perhaps even octovalents are formed with appreciable frequency. The genetical expectations for octosomic inheritance are not very different from those of tetrasomic inheritance, and the possibility of octosomic inheritance or perhaps inheritance intermediate between tetrasomic and octosomic inheritance would be very difficult to exclude.

Another species in which tetrasomic inheritance has been reported is *Lotus corniculatus*, a plant showing almost complete bivalent formation at metaphase (Dawson, 1941). Dawson reported tetrasomic inheritance of cyanogenic activity, although the alternative hypothesis of duplicate disomic inheritance has not been completely excluded (see Sec. II 1). In a recent short communication, Bubar and Miri (1965) have reported without presenting data that several characters have been found to be tetrasomically inherited. However the data for the inheritance of incompatibility are said to differ from tetrasomic expectations, but to be explicable on

a duplicate disomic basis.

One further study which may be mentioned here is that of Norderskjöld (1953) in hexaploid Phleum pratense, although the strain studied by this author was of recent origin. In fact it does not even seem certain that P. pratense actually occurs as a wild species (Clausen, Keck and Hiesey, 1945). Nordenskjöld has claimed to have demonstrated hexasomic inheritance, a claim which is evidently at variance with the conclusions of Clausen et al. based on biosystematic considerations. The conclusions of Nordenskjöld must be regarded as somewhat speculative, since the possibilities of disomic and tetrasomic inheritance combined with incomplete penetrance or viability differences have not been critically excluded. More critical tests are evidently needed to clarify the mode of segregation in this species.

The only studies mentioned so far are those on tetraploids known or suspected to have a large amount of homology between all chromosomes of a set. However a class of tetraploids exists, labelled as typical allopolyploids by Stebbins (1950), in which inter-genomal (i.e. heterogenetic) pairing does not occur or occurs only very rarely. Whilst rare segregants from duplex genotypes might be expected in

species of this nature, no attempt has been made to cover the genetical literature for such species, and no reported cases of departure from disomic inheritance are known by the author.

In summarizing the results from the studies in naturally occurring tetraploids two points should be noted. First, with the possible exception of the studies of Bubar and Miri (1965) in *Lotus corniculatus* where no data have been presented, there appears to be no bona fide example of disomic and tetrasomic inheritance occurring in the same species. Secondly no reports have been found of inheritance intermediate between disomic and tetrasomic inheritance such as described in Sec. I 2 ib.

3. REVIEW OF TERMINOLOGY

i Genetical parameters

In view of the demonstration that a form of inheritance intermediate between disomic and tetrasomic inheritance is possible, in newly produced allotetraploids at least, it might be argued that the existing terminology is inadequate for describing inheritance in naturally occurring tetraploids. For if the possibility of an intermediate type of inheritance is to be taken into account, then no matter how extensive the genetical data available it can never be said that either disomic or tetrasomic inheritance has been 'demonstrated'. Clearly both the terms disomic inheritance and tetrasomic inheritance may only be defined conceptually and not operationally.

However in considering the need for a revision of terminology it is relevant to note that an intermediate type of inheritance has not been found in any of the studies on naturally occurring tetraploids described in the previous section. This would at least indicate that such a type of inheritance does not occur frequently. Theoretical arguments will be put forward in Part IV which could account for the scarcity of such cases, since it will be argued that an intermediate type of inheritance is unstable and will

evolve rapidly towards either disomic or tetrasomic inheritance. In view of these considerations therefore it is felt that while a revision of terminology could be justified on the grounds of rigour, it would not be of general utility and would thus introduce unnecessary complications. By the same token, although it might be possible to give a comprehensive theory of the intermediate type of inheritance along the same lines as Fisher's (1947) theory of tetrasomic inheritance, it does not seem that such a theory would be of much utility. It will however be necessary to introduce briefly one or two terms in anticipation of their use in connection with the genetical studies in <u>Oxalis pes-caprae</u>.

Consider a tetraploid species with a qualitative character under study. For simplicity this may be supposed to be presence or absence of a particular pigment. A pigmented plant when crossed to unpigmented gives approximately 1 pigmented:1 unpigmented progeny; selfing the pigmented gives approximately 3 pigmented:1 unpigmented; and selfing the unpigmented gives all unpigmented progeny. A single dominant gene 'A' is thus postulated to control the presence of pigment.

The mode of segregation at the A locus is now investigated by backcrossing to unpigmented plants progeny having

two A genes from the pigmented selfing. If disomic inheritance is operative all the progeny from such a cross would have one A gene. The appearance of individuals containing no A genes, i.e. unpigmented, will be said to demonstrate duplex segregation. The frequency with which such individuals occur is then described as the duplex segregation frequency. (A strong case could perhaps be made for defining this term as the frequency of backcross progeny with either two or no A genes, since the appearance of progeny with two A genes also indicates segregation not expected under the hypothesis of disomic inheritance. However since the phenomenon of dominance is widespread, the former definition is adopted for the sake of utility.)

It will be convenient to introduce a term to describe the type of inheritance intermediate between disomic and tetrasomic inheritance, viz. 'intermediate di-tetrasomic inheritance', henceforth abbreviated to 'di-tetrasomic inheritance'. (This should be distinguished from the term 'tetra-disomic inheritance', used by Nordenskjöld to describe a form of inheritance possible in an autoallohexaploid.) A character showing duplex segregation, with a frequency significantly less than one-sixth, is said to be di-tetrasomically inherited. This definition assumes that possible explanations for the deficiency from one-sixth such as viability and

misclassification have been ruled out by comparison with the simplex segregation ratios.

ii <u>Parameters of pairing</u>

The idea of a parameter such as the duplex segregation frequency is not really new. The coefficients of allosyndesis of Collins and Longley (1935) and Burnham (1962, Ch. 8) are simple functions of this parameter. (The term allosyndesis is used by these authors in the same sense as heterogenetic pairing will be used throughout this thesis, viz. to describe pairing between chromosomes or chromosome segments derived originally from different diploid ancestors; see Waddington (1939) and Stebbins (1947) for a clarification of these terms.) However it seems unnecessary and perhaps a little misleading to consider parameters of pairing unless these may be estimated by direct cytological observation, as for example by Menzel (1964). Clearly one parameter cannot describe the overall pairing of a chromosome set, since more than two types of pairing are possible; viz. bivalents with homogenetic pairing, bivalents with heterogenetic pairing, and an infinite number of possible quadrivalent pairings. Neither however can such a parameter be legitimately used to describe pairing in the region of the locus, or perhaps in the region of the centromere as advocated by Burnham

(1962, P. 215). The estimation of this parameter involves assumptions regarding the relative frequency of bivalent and quadrivalent pairings and the type of disjunction from quadrivalents. While it seems reasonable to suppose that the duplex segregation frequency is roughly proportional to the amount of heterogenetic pairing, to attempt to give a specific formula for this relationship may be misleading.

To consider this point in more detail a model chromosome set may be constructed (Fig. 1) in which two homeologous pairs of chromosomes of a newly hybridized tetraploid have one segment in which heterogenetic pairing can occur freely and another segment in which no heterogenetic pairing can occur. The gene A is contributed only by parent I. Parent II may have contributed the 'a' allele on the homeologous chromosome, or on another part of the genome. Alternatively the locus may have been lost altogether in parent II. The model requires that combinations such as three chromosomes of type I and one of type II, or other numerically balanced combinations other than two of both types, be viable.

At meiosis two types of pairing configurations are possible (Fig. 2 a, b) such that strands are paired at all points. A third possible type of configuration, two

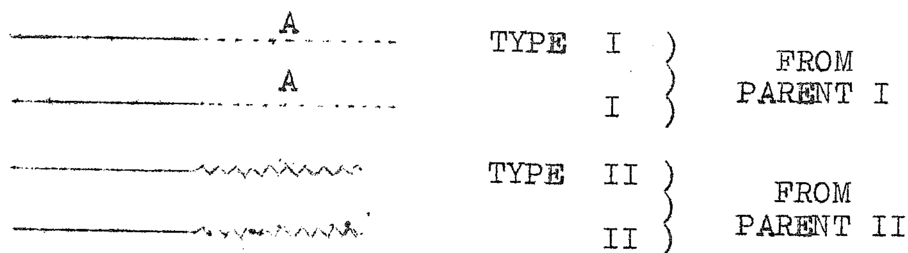


Figure 1. Model chromosome set of newly synthesized tetraploid

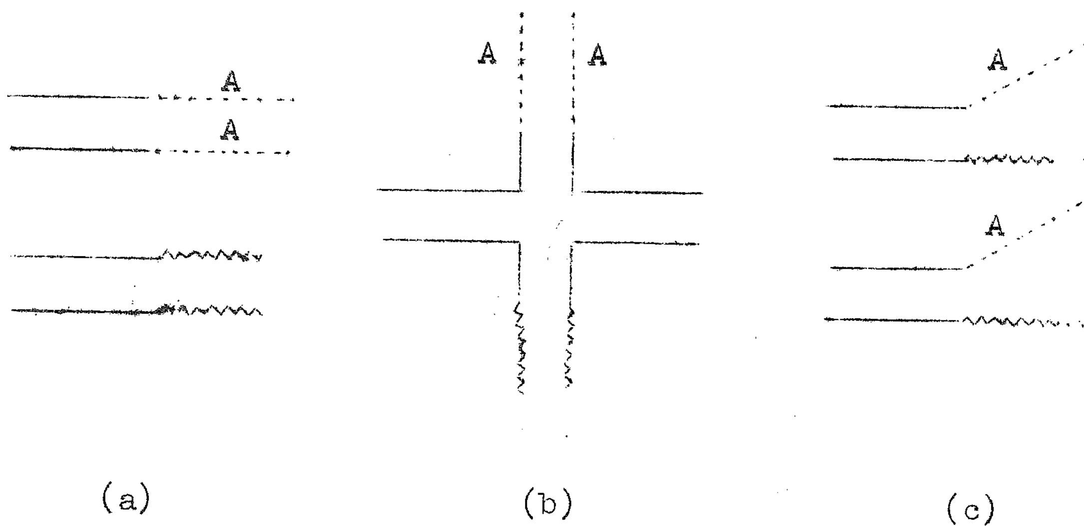


Figure 2. Possible configurations at meiosis
(Double nature of strands not shown)

bivalents with incomplete pairing is depicted in Fig. 2 c, while other more complex configurations are presumably possible. Provided that pairing is initiated some or all of the time in the first segment, configuration such as (b) and (c) are expected, which will lead to the formation of gametes containing two or no A alleles. Thus duplex segregation, and perhaps even close agreement with tetrasomic inheritance expectations, is expected for a locus in a region where no heterogenetic pairing can occur and where possibly only two of the four chromosomes of the set contain the locus.

The above example shows that only restricted inferences concerning chromosome homology may validly be made from the observation of typical tetrasomic ratios. It appears that duplex segregation is a propoerty of genes along the entire chromosome, rather than genes on portion of the chromosome. The segregational properties of these genes are determined only by the regions in which pairing occurs, which may not include the entire chromosome, and in particular by the regions in which pairing is initiated. Duplex segregation at any particular locus may be merely a reflection of pairing homology in a distant region of the chromosome.

II BREEDING PROCEDURES IN TETRAPLOIDS

1 TESTS OF THE MODE OF SEGREGATION

The most important indicator of the mode of segregation in a tetraploid is the duplex segregation frequency. The failure to demonstrate duplex segregation, and the finding of a duplex segregation frequency not significantly less than one-sixth, may be taken as indications of disomic and tetrasomic inheritance respectively. The finding of a frequency intermediate between zero and one-sixth demonstrates di-tetrasomic inheritance (with the proviso mentioned in Section I 3 i). The estimation of the duplex segregation frequency strictly requires that an individual containing two genes, say A genes, known to be identical by descent be produced for backcrossing. The requirement that the genes be identical by descent, thereby ensuring that duplicate disomic inheritance is not mistakenly classed as tetrasomic inheritance, appears to have been first explicitly stated by Cadman (1942) and Fisher and Mather (1943).

It is apparent that in order to obtain such a duplex individual it is necessary to start with an individual simplex for the A gene, i.e. one giving a 1:1 ratio on backcrossing. Backcross progeny containing the A gene may then be intercrossed or alternatively backcrossed to the simplex

parent, giving in either case an approximate 3:1 ratio regardless of the mode of segregation at the A locus. The desired duplex individual may be selected from these progeny, although in general it may only be distinguished from the simplex by its backcross ratio. The appearance of one or more nulliplex individuals amongst the backcross progeny of the duplex establishes duplex segregation.

The above remarks refer specifically to an outbreeding organism. In an organism capable of self-fertilization the duplex individual may be obtained in one generation, thus allowing the programme to be carried through in one generation less than for outbreeding organisms. Such a procedure has been used by Stanford (1950 et seq.). It is seen however (see Sec. II2) that intercrossing gives a less efficient estimate of the duplex segregation frequency than given by backcrossing.

In outbreeding organisms an alternative demonstration may in some circumstances be possible in two generations. The backcross of a simplex individual is expected to produce a proportion $\frac{\alpha}{4}$ of duplex individuals by double reduction. If dominance is not complete, as often seems to be the case with pigment markers in tetraploids, phenotypic expression may be used as a guide in selecting the duplex individuals.

Such a demonstration establishes at the same time duplex segregation and double reduction. However it should be noted that if double reduction is not occurring this programme demonstrates nothing. Thus it would be wasteful to attempt such a programme if for instance cytological observation had indicated the absence of quadrivalent formation.

Two other types of less direct evidence may also be used to argue against the possibility of disomic inheritance. The finding of an approximate 5:1 ratio from backcrossing a tetraploid individual of unknown ancestry is often taken as an indication that tetrasomic rather than disomic inheritance is operative. Such an argument is strengthened considerably if simplex backcross ratios are also available and agree with a 1:1 ratio. Thus for instance Dawson (1941) classifies plants on the basis of their backcross ratio into two classes. The results from backcrosses of the first class are homogeneous and total 696A:689a. Those from the second class are homogeneous and total 512A:89a. Taking into account the agreement of the first class with a 1:1 ratio, the demonstration that the results in the second class differ considerably from the 3:1 ratio expected for duplicate disomic genes may be considered as reasonably conclusive refutation of the disomic inheritance hypothesis.

It should be noted that if the duplex backcross results are obtained from one parent, which is not the case with the results of Dawson, the 5:1 ratio could possibly be explained by loosely linked disomic genes.

The second type of evidence concerns the absence of non-segregating dominant genotypes. All plants giving an excess of dominants on backcrossing, say in the range 3-5 dominant:1 recessive, must be classified as $A_1a_1A_2a_2$ on the hypothesis of duplicate disomic inheritance. However the occurrence of a number of plants classified as doubly heterozygous demands that the frequency of non-segregating genotypes such as $A_1A_1a_2a_2$ etc. should also be high. For example in a random mating population the relative frequency of non-segregating genotypes to double heterozygotes is

$$\frac{p_1^2 + p_2^2 - p_1^2p_2^2}{4p_1p_2(1 - p_1)(1 - p_2)}$$

where p_1 is the frequency of A_1, p_2 is the frequency of A_2. This can never be less than one-half, the value occurring when the frequency of the A phenotype is low. If either p_1 or p_2 is high, the failure to find a high frequency of non-segregating genotypes constitutes strong evidence against the hypothesis of duplicate disomic inheritance.

2 ESTIMATION OF THE FREQUENCY OF DOUBLE REDUCTION

It can be seen from Table 1 that, where dominance is present, positive recognition of double reduction can only be achieved through backcrossing or intercrossing the triplex genotype. Thus the most satisfactory procedure for estimating α is evidently through backcrossing the triplex genotype. However in view of the fact that this genotype may not always be available it seems useful to investigate the efficiency of other procedures for estimating α, and the possible effect that such disturbances as inviability and misclassification may have on these estimates.

The amount of information per observation, i.e. the inverse of the variance per single observation, may be calculated using binomial formulae. The calculation for the simplex intercross is given as an example. The expectation for the 'a' class (assuming dominance) is for this case $(\frac{2+\alpha}{4})^2$, and for the 'A' class $1 - (\frac{2+\alpha}{4})^2$.

Then

$$i_{(\frac{2+\alpha}{4})^2} = \frac{1}{\left(\frac{2+\alpha}{4}\right)^2 \left[1 - \left(\frac{2+\alpha}{4}\right)^2\right]}$$

$$\therefore \quad i = \frac{\left[\frac{\partial}{\partial \alpha} \quad (\frac{2+\alpha}{4})^2\right]^2}{\left[\frac{2+\alpha}{4}\right]^2 \left[1 - (\frac{2+\alpha}{4})^2\right]}$$

$$= \frac{\left[\frac{1}{8}(2+\alpha)\right]^2}{\left[\frac{2+\alpha}{4}\right]^2 \left[\frac{12 - 4\alpha - \alpha^2}{16}\right]}$$

$$= \frac{4}{(2-\alpha)(6+\alpha)}$$

The results for several possible estimation procedures are summarized in Table 2.

ESTIMATION PROCEDURE	i = AMT. OF INF. PER OBS.	i FOR PARTICULAR VALUES OF α $\alpha=0$	.05	.10	.15	.20
SIMPLEX BACKCROSS	$\frac{1}{(2-\alpha)(2+\alpha)}$	.250	.250	.251	.251	.253
" INTERCROSS	$\frac{4}{(2-\alpha)(6+\alpha)}$	.333	.339	.345	.352	.358
DUPLEX BACKCROSS	$\frac{4}{(1-2\alpha)(5+2\alpha)}$	.800	.742	.694	.655	.621
" INTERCROSS	$\frac{16}{(5-2\alpha)(7+2\alpha)}$	.457	.460	.463	.466	.470
SIMPLEX X DUPLEX	$\frac{(5+4\alpha)^2}{(1+2\alpha)(2+\alpha)(22-5\alpha-2\alpha^2)}$	.568	.551	.539	.529	.522
TRIPLEX BACKCROSS	$\frac{1}{(4-\alpha)}$	∞	5.063	2.564	1.732	1.316
" INTERCROSS	$\frac{4}{(4-\alpha)(4+\alpha)}$	.250	.250	.250	.250	.251

Table 2. Efficiencies of various procedures for estimating α.

With the exception of the triplex and to a lesser extent the duplex backcross, the values are largely unchanged over the range of values considered for α, the order of magnitude being constant throughout.

The effect of viability disturbances may be assessed as follows. For the simplex backcross for example the expected frequency of the 'a' phenotype will be

$$\frac{\left(\frac{2+\alpha}{4}\right)(1-v)}{1-v\left(\frac{2+\alpha}{4}\right)},$$

where $1 - v$ is the selective value of the 'a' phenotype. Estimating α in the usual manner we get

$$\hat{\alpha} = 4\left\{\frac{\left(\frac{2+\alpha}{4}\right)(1-v)}{1-v\left(\frac{2+\alpha}{4}\right)}\right\} - 2$$

$$\doteqdot \alpha - v$$

Similarly we can investigate the effect of misclassification. Consider the model whereby the 'A' phenotype is mistakenly classified as 'a' in a proportion w of cases.

Then the estimate of α becomes

$$\hat{\alpha} = 4\left[\left(\frac{2+\alpha}{4}\right) + w\left(\frac{2-\alpha}{4}\right)\right] - 2$$

$$= \alpha + 2w$$

The effect on α of numerical non-disjunction may also be calculated. The expectations given by Catcheside (1956, Table 3) are used in these calculations. Complete viability of monosomic and trisomic gametes is assumed. Results of the three series of calculations are summarised in Table 3.

ESTIMATION PROCEDURE	SIMPLEX B.C.	SIMPLEX I.C.	DUPLEX B.C.	DUPLEX I.C.	SIMPLEX X DUPLEX	TRIPLEX B.C.	TRIPLEX I.C.
VIABILITY	$-v$	$-\frac{3}{4}v$	$-\frac{5}{12}v$	$-\frac{35}{144}v$	$-\frac{11}{30}v$	$-\alpha v$	$-\frac{\alpha}{2}v$
MISCLASSIFICATION	$2w$	$3w$	$\frac{5}{2}w$	$\frac{35}{4}w$	$\frac{22}{5}w$	$4w$	$\frac{8}{\alpha}w$
NUMERICAL NON-DISJUNCTION	$-\frac{\alpha}{2}x$	$-\frac{\alpha}{2}x$	$\frac{1}{4}x$	$\frac{1}{4}x$	$\frac{1}{5}x$	$\frac{1}{2}x$	$\frac{1}{2}x$

Table 3. Effect on estimate of α of viability, misclassification and numerical non-disjunction

Summing up from Tables 2 and 3, we may conclude that the triplex backcross is the most efficient and the least

affected by viability differences, but is somewhat sensitive to misclassification of the type considered here and numerical non-disjunction. Of the remaining crosses considered the duplex backcross is in most respects the most satisfactory and the simplex backcross perhaps least so.

3 THE DETECTION OF LINKAGE

The detection of linkage in diploids may be achieved by backcrossing either the coupling or repulsion double heterozygote. Linkage is detected with equal efficiency by both of these backcrosses. With tetraploids, however, a much larger number of genotypes with two segregating loci is possible, all of which may be used to detect linkage, but not with equal efficiency. The efficiencies of the various backcrosses will be considered for a start.

1 Efficiency of various backcrosses

Considering two linked loci each with two alleles, nineteen genotypes can be formed capable of segregating at both loci (Fisher, 1947, 1950). Of these nineteen ten are triplex at one or both loci, and if dominance is complete these will give a very small amount of segregation. Clearly these ten can only be used to detect linkage with very low efficiency and may thus be ignored in the present discussion. The remaining nine genotypes are listed in Table 4.

NO.	GENOTYPE	DESIGNATION
1	AB/ab/ab/ab	Bisimplex coupling
2	Ab/aB/ab/ab	" repulsion
3	AB/Ab/ab/ab	Duplo-simplex coupling
4	Ab/Ab/aB/ab	" repulsion
5	AB/aB/ab/ab	Simplo-duplex coupling
6	Ab/aB/aB/ab	" repulsion
7	AB/AB/ab/ab	Biduplex coupling
8	Ab/Ab/aB/aB	" repulsion
9	AB/Ab/aB/ab	" neutral

Table 4. Digenic genotypes simplex or duplex at two loci

As mentioned previously eleven parameters are needed to specify the segregation of two linked loci in tetraploids. This number is reduced to five if double reduction is assumed to be negligible. It appears necessary to make this assumption in order to derive the expected efficiencies. It seems very unlikely that the assumption would introduce a large error into the calculations.

The five modes of gamete formation not involving double reduction are numbers 1, 3, 6, 9 and 11 of Fisher (1947) viz. A_1B_1/A_2B_2, A_1B_1/A_2B_3, A_1B_3/A_2B_4, A_1B_3/A_2B_1,

A_1B_2/A_2B_1, where the parental genotype is $A_1B_1/A_2B_2/A_3B_3/A_4B_4$. Since we are concerned only with the results of a first backcross, gametes produced by the first and fifth, and the second and fourth of these modes will be indistinguishable. Thus to specify the phenotype of the gamete produced only three parameters need be introduced. These are given in Table 5.

PHENOTYPIC CLASS	MODES OF GAMETE FORMATION	GAMETIC PHENOTYPE	FREQUENCY
1	$A_1B_1/A_2B_2 + A_1B_2/A_2B_1$	A_1A_2, B_1B_2	p
2	$A_1B_1/A_2B_3 + A_1B_3/A_2B_1$	A_1A_2, B_1B_3	q
3	A_1B_3/A_2B_4	A_1A_2, B_3B_4	r

Table 5. Gametic phenotypic classes from $A_1B_1/A_2B_2/A_3B_3/A_4B_4$ parents

The backcross expectations for all nine parental genotypes listed in Table 4 may be written down from the Tables of Fisher (1947) or calculated from Table 5. For the bisimplex coupling and repulsion genotypes for example

the phenotypes and expected frequencies are given in Table 6.

PARENTAL GENOTYPE	BACKCROSS PROGENY (PHENOTYPE)	EXPECTED FREQUENCY
AB/ab/ab/ab	AB	$\frac{1}{2}p + \frac{1}{4}q = \frac{1}{2}(1 - z)^{*}$
	Ab	$\frac{1}{4}q + \frac{1}{2}r = \frac{1}{2}z$
	aB	$\frac{1}{4}q + \frac{1}{2}r = \frac{1}{2}z$
	ab	$\frac{1}{2}p + \frac{1}{4}q = \frac{1}{2}(1 - z)$
Ab/aB/ab/ab	AB	$\frac{1}{6}p + \frac{1}{4}q + \frac{1}{3}r = \frac{1}{6}(1 + z)$
	Ab	$\frac{1}{3}p + \frac{1}{4}q + \frac{1}{6}r = \frac{1}{6}(2 - z)$
	aB	$\frac{1}{3}p + \frac{1}{4}q + \frac{1}{6}r = \frac{1}{6}(2 - z)$
	ab	$\frac{1}{6}p + \frac{1}{4}q + \frac{1}{3}r = \frac{1}{6}(1 + z)$

$^{*}\ z = \frac{1}{2}q + r$

Table 6. Backcross expectations from bisimplex coupling and repulsion genotypes

The statistic χ^2 may now be used to judge the depar-

ture from random association of the two loci. The component of χ^2 corresponding to linkage detection may be calculated as explained by Mather (1957, Chap. IV). However the same results may be obtained more simply in the present case where expected values are used by calculating χ^2 in a 2x2 contingency table. The χ^2 value obtained is not strictly the expected value of χ^2, but presumably would not differ appreciably from this value.

The calculation of χ^2 is illustrated in Table 7 for the bisimplex coupling and repulsion genotypes. To have

	A	a	
B	$\frac{n_1}{2}(1-z)$	$\frac{n_1}{2}\cdot z$	$\frac{n_1}{2}$
b	$\frac{n_1}{2}\cdot z$	$\frac{n_1}{2}(1-z)$	$\frac{n_1}{2}$
	$\frac{n_1}{2}$	$\frac{n_1}{2}$	n_1

AB/ab/ab/ab

	A	a	
B	$\frac{n_2}{6}(1+z)$	$\frac{n_2}{6}(2-z)$	$\frac{n_2}{2}$
b	$\frac{n_2}{6}(2-z)$	$\frac{n_2}{6}(1+z)$	$\frac{n_2}{2}$
	$\frac{n_2}{2}$	$\frac{n_2}{2}$	n_2

Ab/aB/ab/ab

$$\chi^2 = \frac{n_1\left(\frac{n_1}{4}(1-z)^2 - \frac{n_1^2}{4}z^2\right)^2}{\frac{n_1}{2}\cdot\frac{n_1}{2}\cdot\frac{n_1}{2}\cdot\frac{n_1}{2}} \qquad \chi^2 = \frac{n_2\left(\frac{n_2^2}{36}(1+z)^2\frac{n_2^2}{36}(2-z)^2\right)^2}{\frac{n_2}{2}\cdot\frac{n_2}{2}\cdot\frac{n_2}{2}\cdot\frac{n_2}{2}}$$

$$= n_1(1-2z)^2 \qquad\qquad = \frac{n_2}{9}(1-2z)^2$$

Table 7. χ^2 calculations for bisimplex coupling and repulsion genotypes

approximately equal chances of detecting a significant departure from random association in these two cases we must have $n_2 = 9n_1$. Thus the efficiency of the bisimplex coupling backcross is approximately nine times that of the repulsion backcross, no matter what the strength of linkage.

The phenotypic expectations and expected contingency χ^2's for all nine genotypes are given in Table 8. From this table it may be seen that considering only the first six genotypes, linkage is most efficiently detected by the backcross of the bisimplex coupling genotype. Further conclusions cannot be drawn without introducing models to specify the likely magnitude of p, q and r. As

a first approximation these frequencies will be investigated under the model of bivalent formation.

PARENTAL GENOTYPE	PROGENY PHENOTYPE				CONTINGENCY χ^2 (per unit offspring)
	AB	Ab	aB	ab	
1. AB/ab/ab/ab	$\frac{1}{2}(1-z)$	$\frac{1}{2}z$	$\frac{1}{2}z$	$\frac{1}{2}(1-z)$	$(1-2z^{*})^2$
2. Ab/aB/ab/ab	$\frac{1}{6}(1+z)$	$\frac{1}{6}(2-z)$	$\frac{1}{6}(2-z)$	$\frac{1}{6}(1+z)$	$\frac{1}{9}(1-2z)^2$
3. AB/Ab/ab/ab	$\frac{1}{6}(3-z)$	$\frac{1}{6}(2+z)$	$\frac{1}{6}z$	$\frac{1}{6}(1-z)$	$\frac{1}{5}(1-2z)^2$
4. Ab/Ab/aB/ab	$\frac{1}{6}(2+z)$	$\frac{1}{6}(3-z)$	$\frac{1}{6}(1-z)$	$\frac{1}{6}z$	$\frac{1}{5}(1-2z)^2$
5. AB/aB/ab/ab	$\frac{1}{6}(3-z)$	$\frac{1}{6}z$	$\frac{1}{6}(2+z)$	$\frac{1}{6}(1-z)$	$\frac{1}{5}(1-2z)^2$
6. Ab/aB/aB/ab	$\frac{1}{6}(2+z)$	$\frac{1}{6}(1-z)$	$\frac{1}{6}(3-z)$	$\frac{1}{6}z$	$\frac{1}{5}(1-2z)^2$
7. AB/AB/ab/ab	$\frac{1}{6}(4+p)$	$\frac{1}{6}(1-p)$	$\frac{1}{6}(1-p)$	$\frac{1}{6}p$	$\frac{1}{25}(6p-1)^2$
8. Ab/Ab/aB/aB	$\frac{1}{6}(4+r)$	$\frac{1}{6}(1-r)$	$\frac{1}{6}(1-r)$	$\frac{1}{6}r$	$\frac{1}{25}(1-6r)^2$
9. AB/Ab/aB/ab	$\frac{1}{24}(16+q)$	$\frac{1}{24}(4-q)$	$\frac{1}{24}(4-q)$	$\frac{1}{24}q$	$\frac{1}{100}(2-3q)^2$

$$^{*}\ z = \tfrac{1}{2}q + r$$

Table 8. Backcross expectations and expected contingency χ^2's

Under this model chiasmata are assumed to form independently in the two bivalents, and in terms of the recombination frequency y

$$p = (1-y)^2 \qquad q = 2y(1-y) \qquad r = y^2 .$$

Under this model $z = \frac{1}{2}q + r = y$, but in general z is not equal to the recombination frequency. It should also be noted that the assumption that double reduction is absent is not necessary under the model of bivalent formation, so that all results given are exact under this model.

Introducing the above values of p, q and r it is easily seen that the contingency χ^2 for biduplex coupling is greater than those of the biduplex repulsion and neutral genotypes for all values of y. Clearly then the greatest contingency χ^2 must be that associated with either the bisimplex or the biduplex coupling genotype. Now

$$\chi^2(\text{bisimplex}) - \chi^2(\text{biduplex})$$

$$= (1 - 2z)^2 - \frac{1}{25}(6p - 1)^2$$

$$= (1 - 2y)^2 - \frac{1}{25}(6(1 - y)^2 - 1)^2$$

$$= \frac{4y}{25}(5 - 11y + 3y^2)(1 - 3y)$$

In the range $0 \leqslant y \leqslant \frac{1}{2}$ only the last term of this expression changes sign, so that in the range $0 < y < \frac{1}{3}$ the bisimplex backcross is the most efficient, while the biduplex backcross is the most efficient in the range $\frac{1}{3} < y \leqslant \frac{1}{2}$.

The contingency χ^2's for the nine parental genotypes under the bivalent formation model are given in Fig. 3 for

Figure 3. Efficiency of various backcrosses for detecting linkage

(1) Bisimplex coupling

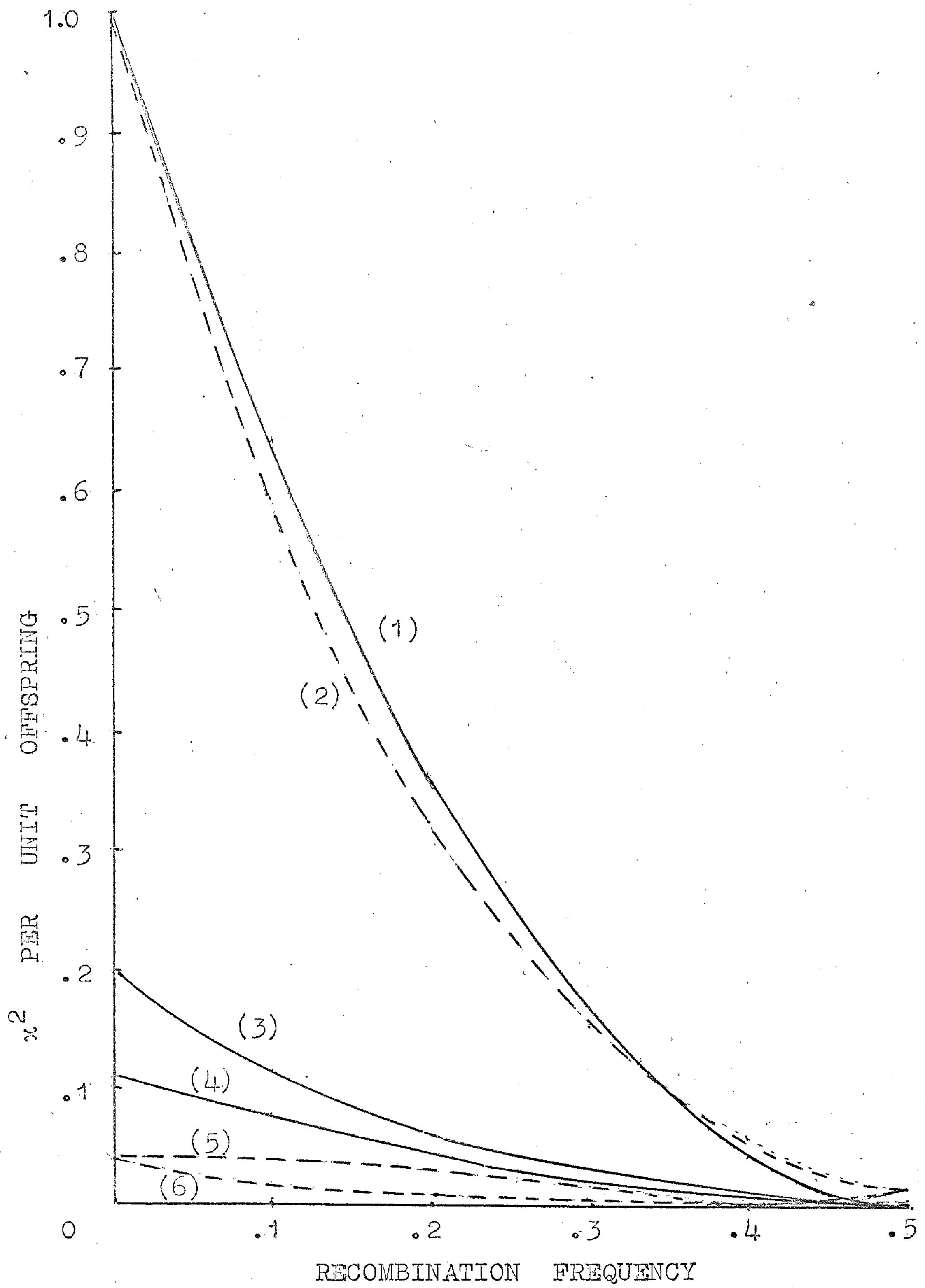
(2) Biduplex coupling

(3) Duplo-simplex genotypes

(4) Bisimplex repulsion

(5) Biduplex repulsion

(6) Biduplex neutral


1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
.1
.2
.3
.4
.5
(1)
(2)
(3)
(4)
(5)
(6)
χ^2 PER UNIT OFFSPRING
RECOMBINATION FREQUENCY

the range $0 \leqslant y \leqslant \frac{1}{2}$. One surprising result, which is in essence the same finding as obtained by de Winton and Haldane (1931), is that with bivalent formation no matter how widely two genes are separated they will not segregate independently. The limiting frequencies for the three classes of Table 5 are obtained for this case by substituting $\frac{1}{2}$ for y, giving

$$p = \frac{1}{4}, \qquad q = \frac{1}{2}, \qquad r = \frac{1}{4}$$

The limiting frequencies for independent genes on the other hand are

$$p = \frac{1}{6}, \qquad q = \frac{2}{3}, \qquad r = \frac{1}{6}$$

These deviations from independent segregation are however masked in all except the biduplex backcrosses.

No substantial changes to the shapes of the curves of Fig. 3 are expected when the possibility of quadrivalent formation is taken into account. The range of the ordinate must however be extended since y may now take values up to any point between 0.50 and 0.75 (see Part V). The values of $\varkappa^2$ at y=0 are not altered, but the values for the biduplex curves are reduced at the upper end of the range. In fact if the possibility of double reduction is taken into account it can be shown that these values may be

reduced to zero. Despite this result it does not follow that a configuration equivalent to that given by independent genes is possible with one partner exchange. Truly independent behaviour of two loci is only possible with an infinite number of partner exchanges and crossovers.

ii <u>Optimal procedures for the detection of linkage</u>

Evidently if the bisimplex or biduplex coupling genotypes are available these could be most efficaciously used in the detection of linkage. However these will not necessarily be available since in most cases the two genes will be obtained from separate stocks, and will in the first case be present in the repulsion phase. Even if the two genes are obtained from a single equilibrium random mating population, as shown by Bennett (1954) the bisimplex repulsion genotype will be approximately three times as frequent as the coupling genotype.

Whereas in diploids the coupling double heterozygote can easily be selected from the progeny of a repulsion heterozygote backcross, in tetraploids the coupling progeny from a bisimplex repulsion backcross cannot be separated from the repulsion progeny except by a second backcross. In the progeny of a bisimplex repulsion backcross, if bivalent formation is operative, we expect approximately

$\frac{y}{6}$ coupling and $\frac{1}{6}$ repulsion progeny. The ratio of coupling/repulsion genotypes will be slightly higher if quadrivalents are formed. Thus in cases where y is low, almost all bisimplex progeny from such a backcross will be of the repulsion form. Fortunately in such cases linkage may be detected relatively easily by backcrossing the repulsion genotype. In the opposite case where y is high and detection by the repulsion backcross is difficult, up to one-third of the bisimplex progeny will be of the coupling type. Provided that the genes are not too widely separated, such genotypes should be detectable by backcrossing. The complete programme may nevertheless be of some considerable magnitude.

More efficient procedures for obtaining a bisimplex coupling genotype may under some circumstances be possible. For instance in a test of independent segregation of two genes A and B, if B is known to be linked or allelic to a third gene C, a stock may readily be made up containing all three genes with B and C linked in repulsion. The stock may now be backcrossed, and plants containing all three genes selected for backcrossing. Provided that B and C are still linked in repulsion, which will be tested by the backcross, if A is linked to B and C it must be now linked in coupling with either B or C.

If di-tetrasomic rather than tetrasomic inheritance is operative, the efficiencies of all backcrosses with the exception of the bisimplex coupling backcross must be reconsidered. For instance in the bisimplex repulsion backcross, if the genes are linked on preferentially pairing chromosomes the genetic behaviour approaches that of disomic inheritance, where the coupling and repulsion genotypes may be used equally efficiently. On the other hand the efficiency of the bisimplex repulsion backcross becomes lower if the genes are linked on preferentially non-pairing chromosomes, and in the limit allelic genes may segregate independently.

4. TESTS FOR ALLELISM

As discussed in the previous section a simple although inefficient test for linkage is the backcross of a bisimplex repulsion individual. In diploids such a backcross is also a test for allelism, since the appearance of a single recessive individual is sufficient to demonstrate non-allelism (ignoring the possibility of intra-genic recombination). However with tetrasomic inheritance a proportion of recessive individuals is expected regardless of whether the genes are allelic or independent (Table 9).

		A_1A_2	A_1 +	+ A_2	+ +
Tetrasomic inheritance	independent	$(2-\alpha_1)(2-\alpha_2)$	$(2-\alpha_1)(2+\alpha_2)$	$(2+\alpha_1)(2-\alpha_2)$	$(2+\alpha_1)(2+\alpha_2)$
	Allelic	$2(1-\alpha)$	$4-\alpha$	$4-\alpha$	$2+4\alpha$
Disomic inheritance	Allelic	.	1	1	.

Table 9. Phenotypic expectations from backcross of A_1A_2 individuals

The differences between the allelism and independence expectations for tetrasomic inheritance are sufficiently large to ensure that either the hypothesis of allelism or

the hypothesis of independence may be rejected relatively easily. However the test between the alternative hypotheses of close linkage or allelism is obviously extremely insensitive. The sensitivity is greatest when all four phenotypic classes of Table 9 may be distinguished, and least when only the last class may be distinguished from the other three. The case where all four classes are distinguishable has the additional advantage that the single gene segregations may be tested separately for agreement with 1:1 ratios in order to test for viability differences or misclassification which otherwise might invalidate the test. An intermediate case, where the first two classes are confounded but distinguishable from the third and fourth classes, is encountered several times in Part III. The frequency of ++ amongst non-A individuals is used in this case as the most sensitive indicator of linkage or allelism.

While the test described above is insensitive for the case of tetrasomic inheritance, it may become misleading as well for an organism in which di-tetrasomic inheritance has been demonstrated. An approximate 1:2:2:1 ratio such as expected for allelism and tetrasomic inheritance may now be attributed to the absence of

duplex segregation combined with a recombination frequency of one-third, or to any situation intermediate between these two extremes. A second backcross of the A_1A_2 progeny would be needed to distinguish between these possibilities. The other type of misleading result possible with di-tetrasomic inheritance is for allelic genes to become located on non-pairing chromosomes, and thus behave as unlinked genes.

On the other hand under some circumstances the occurrence of di-tetrasomic inheritance may assist in testing for allelism. The case where a bisimplex repulsion backcross gives a 0:1:1:0 ratio may be interpreted as showing both a low duplex segregation frequency and a low frequency of recombination. Provided that no recombinant individuals are found, this test for allelism is equal in sensitivity to the test in diploids.

A critical test for allelism in tetraploids may be made which is not subject to the uncertainties described above and is analogous to the test used in diploids. Two stocks each homozygous for a mutant gene (i.e. quadruplex in this case) are intercrossed, and the resulting product backcrossed. The appearance of a single segregant individual indicates non-allelism whether inheritance is

tetrasomic or di-tetrasomic. The duplex stock for this test may be made up directly instead of through intercrossing quadruplex stocks if the products of the two mutant genes are recognizably different, but even in this case a large programme would be required to acquire the desired stocks. The test is also very insensitive compared with diploids. For example if no segregation is found when eighteen hundred progeny from such a backcross are grown, the hypothesis of linkage greater than 10% may be rejected at the 95% level of probability. Only fifty-nine such progeny would be needed for the equivalent test in diploids.

As was the case with the detection of linkage discussed in the previous section, much more efficient tests are possible when three genes with recognizably different products are involved. The joint hypothesis of allelism of three genes may be tested by backcrossing a stock containing all three genes. The appearance of offspring containing none of the three genes does not constitute evidence against allelism since such an individual may arise by double reduction. However the production of offspring containing all three genes is not possible under the hypothesis of allelism unless trisomic gametes have been produced, which may be tested for by backcrossing the offspring concerned.

5. ESTIMATION OF THE RECOMBINATION FREQUENCY

The efficiencies of the various possible procedures for estimating the recombination frequency will be considered in this section. It should first be recalled that the frequency of recombination is only one of a number of parameters that may be estimated for two linked loci in tetraploids. However with the exception of the frequencies of double reduction for the two loci, which may be estimated more efficiently from other programmes, and the recombination frequency, it is difficult to see which mode frequencies or combinations of mode frequencies are most useful in describing the segregation of the two genes. The recombination frequency is useful if for no other reason than that it supplies the basis for comparison with results from the diploid. It seems worthwhile therefore to attempt to find which genotypes can be used most efficiently to estimate the recombination frequency, while remembering that other parameters may also prove to be useful in other contexts.

It is assumed that linkage is estimated, as described by Fisher (1947), by backcrossing one of eighteen doubly digenic genotypes. The biduplex neutral genotype, although it may conceivably be used to detect linkage, is

the only one of the nineteen genotypes which may not be used in estimating the recombination frequency. The ten genotypes not considered in section II 3 through being triplex at one or more loci must be considered here.

The eighteen genotypes may be grouped into four partitional types as shown by Fisher. The genotype AB/ab/ab/ab for example has three equivalent genotypes, viz. Ab/Ab/Ab/aB, Ab/aB/aB/aB and AB/AB/AB/ab. Other partitional types may be represented by Ab/aB/ab/ab which also belongs to a set of four; AB/Ab/ab/ab and AB/aB/ab/ab each belonging to a set of four, the two sets being equivalent when the A and B loci are interchanged; and finally the AB/AB/ab/ab and Ab/Ab/aB/aB genotypes forming together a set of two. Since genotypes of the same partitional type may be used with equal efficiency in any second backcross programme, only four genotypes need be considered, viz. bisimplex coupling and repulsion, duplo-simplex coupling and biduplex coupling.

The method to be used in the present treatment rests on the demonstration that although in Fisher's complete analysis of gametic data both strands in the gamete must be considered together, nevertheless if only the recombination frequency is under consideration, the same estimate

may be obtained by considering strands separately. This seems intuitively reasonable, and has in fact been demonstrated for the bisimplex and biduplex coupling and repulsion genotypes. However the reasoning is somewhat involved and it does not seem worthwhile to enter into such detail here.

Considering only single strands, the gametic output for genotypes representing each of the four classes is given in terms of the recombination frequency y in Table 10 (Bennett; 1954, Table 2).

TYPICAL PARENTAL GENOTYPE	GAMETIC OUTPUT				DIVISOR	i
	AB	Ab	aB	ab		
AB/ab/ab/ab	1-y	y	y	3-y	4	$\frac{3-2y}{2y(1-y)(3-y)}$
Ab/aB/ab/ab	y	3-y	3-y	6+y	12	$\frac{3+2y}{2y(3-y)(6+y)}$
Ab/Ab/ab/ab	3-2y	3+2y	2y	6-2y	12	$\frac{9+12y-8y^2}{y(6-2y)(9-4y^2)}$
AB/AB/ab/ab	3-2y	2y	2y	3-2y	6	$\frac{2}{y(3-2y)}$

Table 10. Single strand gametic expectations for two linked loci

The amount of information per observation for the maximum likelihood estimate of y for each of the four parental genotypes may be calculated as explained by Mather (1957, Chap. V). For the bisimplex coupling genotype for example

$$i = \Sigma\frac{1}{m}\left(\frac{\partial m}{\partial y}\right)^2 = \frac{4}{1-y}\left(\frac{1}{4}\right)^2 + \frac{4}{y}\left(\frac{1}{4}\right)^2 + \frac{4}{y}\left(\frac{1}{4}\right)^2 + \frac{4}{3-y}\left(\frac{1}{4}\right)^2$$

$$= \frac{3-2y}{2y(1-y)(3-y)}$$

The values of i are given in the last column of Table 10. The relative efficiencies in terms of the biduplex backcross results are given in Fig. 4.

It was stated previously that the estimate of the recombination frequency was the same whether strands were considered separately or together. Nevertheless it is not necessarily true that the variances of the two estimates are equal. It appears that the variances would be equal if recombinant and non-recombinant strands are combined at random in the gametes. This asumption is perhaps not too unreasonable, and could if data were available be tested since under the assumption we expect

Figure 4. Efficiency of various procedures for estimating the recombination frequency

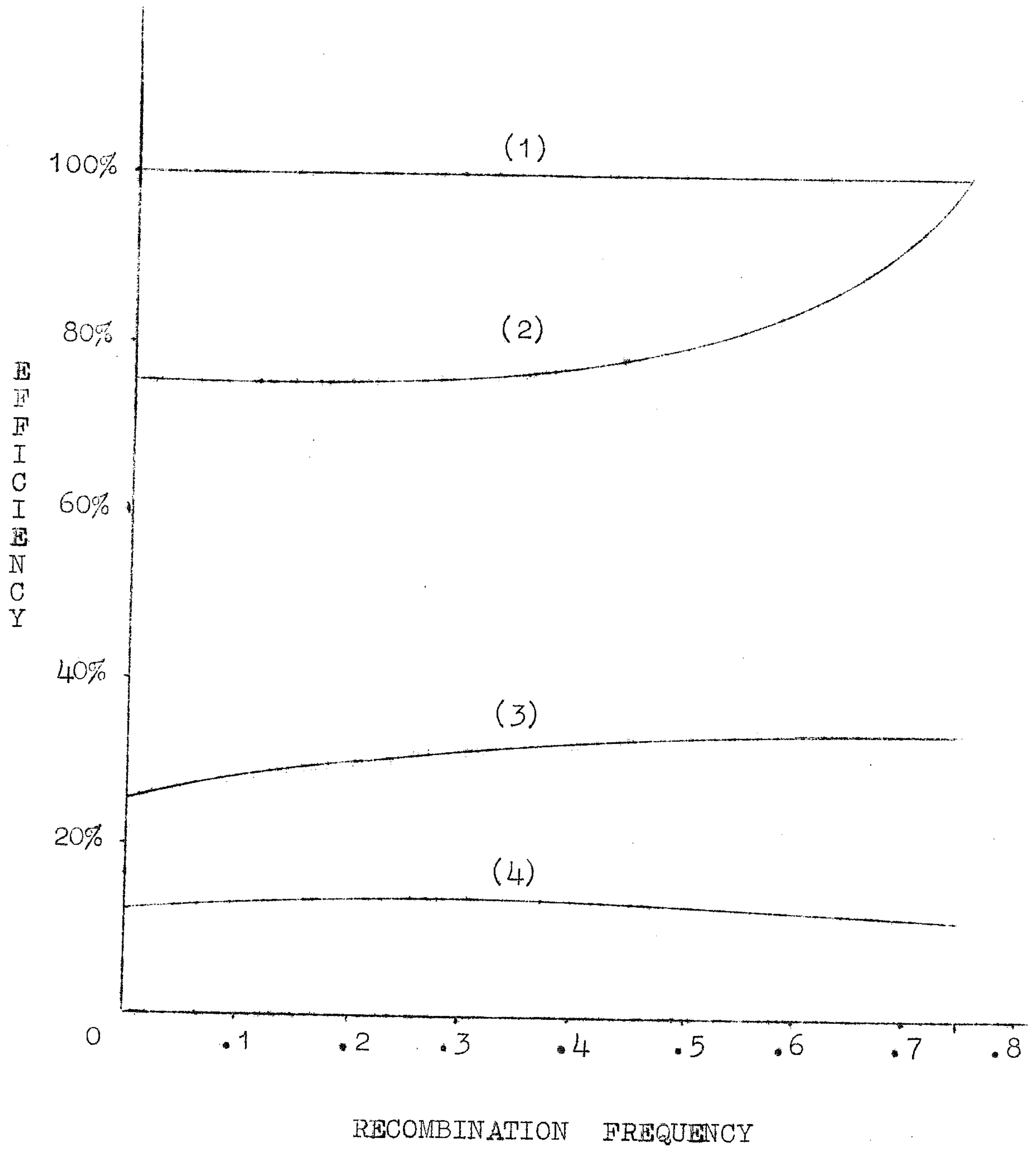
(Biduplex genotypes taken as unity)

(1) Biduplex coupling & repulsion

(2) Bisimplex coupling & similar genotypes

(3) Duplo-simplex & similar genotypes

(4) Bisimplex repulsion & similar genotypes



100%
80%
60%
40%
20%
0
(1)
(2)
(3)
(4)
EFFICIENCY
•1
•2
•3
•4
•5
•6
•7
•8
RECOMBINATION FREQUENCY

$$4(f_1+f_2)(f_6+f_7+f_8+f_9+f_{10}+f_{11}) = (f_3+f_4+f_5)^2$$

where the f's refer to the eleven modes of gamete formation listed by Fisher (1947).

The above treatment has not taken into account one aspect of the manner in which the data are obtained. In the backcross of the bisimplex coupling parents, for instance, a proportion of double recessive progeny is expected, ranging from one-half if the genes are closely linked to one-quarter if they are loosely linked. For these progeny it is not necessary to use a second backcross to identify the genotype. Obviously therefore with the same number of second backcrosses a larger number of progeny from the bisimplex coupling backcross (but not for instance from the bitriplex coupling backcross) can be classified than from the biduplex backcrosses. If this point is taken into account it is seen that the efficiency of the bisimplex coupling backcross may be slightly greater than that of the biduplex backcrosses.

No attempt has been made to find the most efficient programme for estimating linkage when in order to reduce the effect of viability, only the phenotypically AB

offspring are used in the second backcross programme (see Fisher, 1949a). In view of the results given in Fig. 4 however it seems likely that such a programme in which the biduplex coupling and repulsion genotypes are used would be the most efficient.

III GENETICAL STUDIES IN THE TETRAPLOID *OXALIS PES-CAPRAE*

1. MATERIALS AND METHODS

i Description of *O. pes-caprae*

Two chromosome races of *Oxalis pes-caprae*, L. occur in South Australia (Oram, 1956). The commonly occurring form is a sterile, clonally propagated plant known as the weed soursob. It is short-styled, with little variation in colour pigments, and has a chromosome number of thirty-five. However small populations of *O. pes-caprae* have been located with considerable variation in pigments and style forms. The chromosome number of this form was shown to be twenty-eight (Oram, 1956). The two forms have been shown by the author to cross freely, giving rise to viable offspring.

The distribution of the two forms has been studied by Michael (1964), who states that *O. pes-caprae* was introduced from South Africa to Australia possibly via nurseries in England. In fact it seems quite likely that the local thirty-five chromosome variety was developed in England (Lower, 1963). In many areas in South Australia

and elsewhere where the twenty-eight chromosome variety occurs, a closely related species O. compressa, Lf. has been recorded. The chromosome number of the South Australian form of this species has been determined as fourteen. Attempts by the author and by several other writers (quoted by Michael, 1959) to cross O. compressa to O. pes-caprae have been unsuccessful. However Lower (1963) has claimed to have found in South Africa many plants morphologically intermediate between these two forms, although chromosome counts were unfortunately not made. Nevertheless there seems to be sufficient evidence that the two forms of O. pes-caprae are tetraploid and pentaploid races of a polyploid series closely related to the diploid O. compressa. The genetical studies have have been carried out on the twenty-eight chromosome race, which is described in the chapter heading as a tetraploid purely on the basis of its chromosome number.

Cytological observation in O. pes-caprae has proved difficult. Quadrivalents have been seen, but the standard of preparation has not been sufficiently high to allow their frequency to be estimated. One cell is shown (Fig. 5) which is interpreted as having five quadrivalents and four bivalents, although the number of quadrivalents

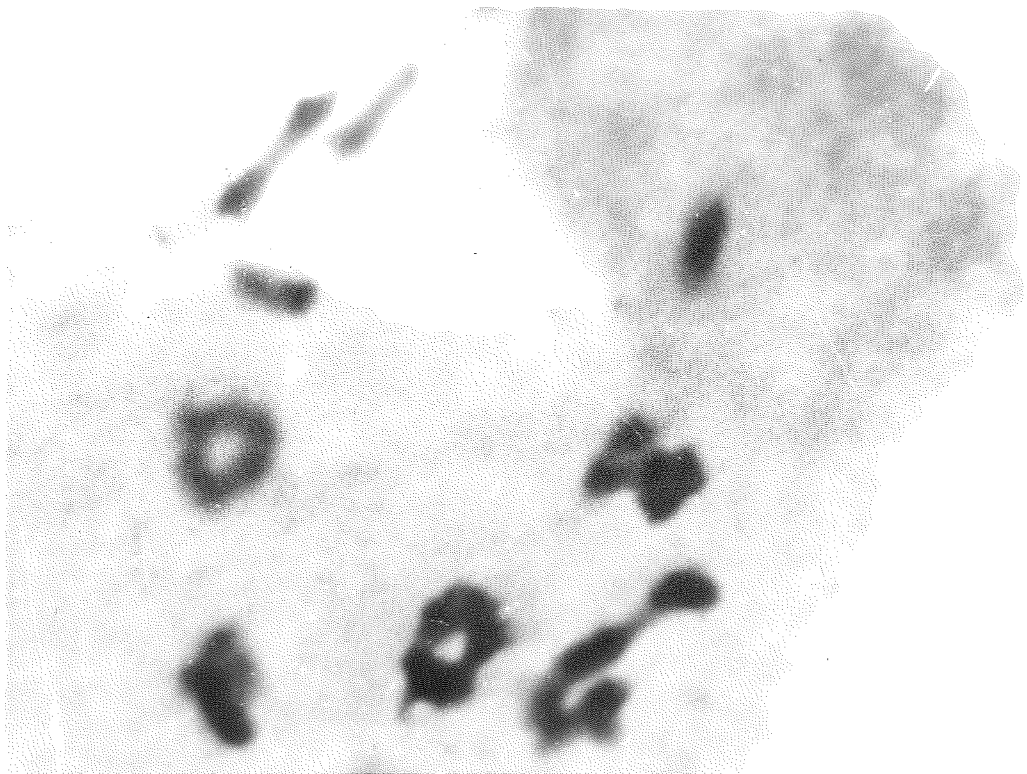


Figure 5. First division metaphase of meiosis in O. pes-caprae

in this cell appeared to be exceptionally high. Two pentaploid X tetraploid hybrid plants were studied and found to have thirty chromosomes. Meiosis was apparently normal, and two pentavalents in a cell were seen on at least one occasion.

The growth of the tetraploid O. pes-caprae is extremely variable, clearly depending on both genotype and environment. An average plant would perhaps have ten or eleven leaves and three or four flowering stalks, each stalk having up to twenty flowers throughout its cycle. The normal season for O. pes-caprae in South Australia is throughout the colder months, i.e. approximately May to October.

The tetraploid form of O. pes-caprae sets seed freely in the field (as does the pentaploid form when compatible pollen is available), and the majority of these seeds are fertile. However the plants produced, especially in the seedling stages, are far less viable than plants grown from bulbs. Tetraploid populations tend to occur in clones in the field, which further suggests that vegetative propagation is generally the rule for this species.

Both bulbs and seed are subject to a dormancy lasting from seven to eight months. After this time bulbs sprout naturally, while the seeds germinate when soaked.

ii Outline and scope of genetical studies

The first collections of *O. pes-caprae* were made in the years 1954-56 by Mr. R.N. Oram, then of the Genetics Department, University of Adelaide. Mr. Oram described the style and pigment variants and made the first trial crosses. More concerted genetical studies were commenced by Mr. O.R. Byrne in the years 1957-1959. In 1960 all studies were taken over by Mr. I.R. Franklin, who continued in 1961 to work on the inheritance of style length as part of the general study of incompatibility in *O. pes-caprae*. The inheritance studies of the present author were made in the years 1961-1964, the studies in 1961 being in co-operation with Mr. Franklin.

Having limited glasshouse space available, Mr. Byrne investigated the possibility of raising plants in outside plots. Although the tetraploid *O. pes-caprae* grows successfully from bulbs in the field, it appears that the seed form is not sufficiently hardy, and only a few per cent

of these plants were successfully grown to maturity. The confinement of the study to glasshouses reduced it in scope considerably, until 1963 when larger improved glasshouses became available. In addition, expression of pigments in the old glasshouse was far from ideal, due apparently to restriction on the amount of light entering. When the new glasshouses were first used in 1963, the expression of pigments was more distinct and less variable than previously. Subdivisions of several existing markers became evident, necessitating much re-classification, as described in Sec. III 3.

Some four hundred plants were grown in each of the years 1958 and 1959. No plants were grown from seed in 1960, but in 1961 over fifteen hundred were grown. Due to a large scale failure of plants in 1962, only one hundred and fifty complete classifications were made. Portion of the information lost in this failure was recovered in 1963, since although plants failed to grow to maturity, a large number left at least one bulb, allowing classification of over four hundred additional plants in the 1963 season. The failure of the 1962 season was also felt in 1963, since the scarcity of seed did not permit the glasshouses to be used at full capacity. Nevertheless some fifteen hundred

plants from over forty crosses were grown to maturity, and over forty thousand seed obtained from crosses in that year. Of this number approximately five thousand plants were grown to maturity in 1964, and large numbers of seed were again obtained.

iii <u>Methods</u>

As mentioned previously <u>O. pes-caprae</u> seed is subject to a dormancy lasting for several months, after which time seed can be germinated by soaking. Many efforts were made to eliminate the dormancy period, which if successful would permit two or more generations to be grown per year, and would also assist in synchronizing the bulb and seed plants. A number of treatments were tried; heating, cooling, freezing and various combinations of the three; dissection; leeching; CO_2 and activated charcoal; reduced light intensity and 550μ light; and various chemicals, but none were successful.

In 1961 seed germination was carried out in sandpots, and plants were transplanted after approximately one month. This method was somewhat unsatisfactory due to loss of plants and setbacks after transplanting, and from 1962 onwards a method of germinating in petrie dishes was used.

From the petrie dishes plants were set out in standard flats (approx. 21" x 12"), from twenty-four to thirty-five plants per flat. Classification of plants was carried out in these flats, and the information recorded on specially printed sheets. Plants to be used as parents in crosses were transplanted to individual pots and transferred to insect-proof crossing cages, short-, mid- and long-styled plants being stored separately (see Sec. III 2 for description of tristyly). Crossing was effected by removing with tweezers one of the five stamens from the appropriate level and rubbing pollen on to the style. In this way up to ten flowers could usually be pollinated with the pollen from one flower. Seed capsules were soon initiated and were bagged, and twelve to fourteen days later the capsules dehisced, giving under good conditions some twenty to thirty seeds per capsule. An open-pollination plot as used by Fisher and Mather (1943) was maintained by Mr. Franklin in 1960, but the complexities of the crossing programme did not permit its use in later years.

Bulbs were kept wherever possible from all plants used in the crossing programme. Unfortunately transplanting to individual pots for crossing appeared to hinder bulb

formation, and in some cases no bulbs were produced. Nevertheless through the medium of bulbs a fairly complete set of all plants used in crosses from 1960 to 1964 is now available.

The system of nomenclature in use at present is the same as that initiated by Messrs. Oram and Byrne. Plants receive a designation showing the style length, year when grown from seed, and number. As an example the first short plant used in 1961 would be designated as S61.01. The fifteenth progeny in the cross between this plant and L60.14 (with S61.01 used as the female parent) would be S61.01 x L60.14-15. If used as a parent in the 1962 crossing programme this progeny, a mid say, might then be redesignated as M62.08. Wherever possible both designations have been included in the text, and a table from which the ancestry of all plants mentioned can be traced is included as an appendix.

2. THE INCOMPATIBILITY SYSTEM

An account of early studies on incompatibility in tristylic species has been given by Darwin (1877, Ch. IV). Darwin himself showed in Lythrum salicaria that the fertilization of each style form using pollen from an anther of the same height produced a much greater number of seed on the average than did fertilization with pollen from a different height. The two types of pollination were labelled legitimate and illegitimate respectively (Fig. 6). A similar type of incompatibility was found in several other species, the most extensive study reported being that in Oxalis valdiviana.

That a similar system of incompatibility was present in O. pes-caprae was readily demonstrated. Mr. Franklin studied pollen tube growth in legitimately and illegitimately pollinated styles, and found clear differences of growth between the two types. A few illegitimate pollinations made by the author in 1961 failed to produce any seed. This plus the fact that seeding was on no occasion found in flowers left unpollinated in the crossing cages suggested that the incompatibility reaction was very strong in O. pes-caprae.

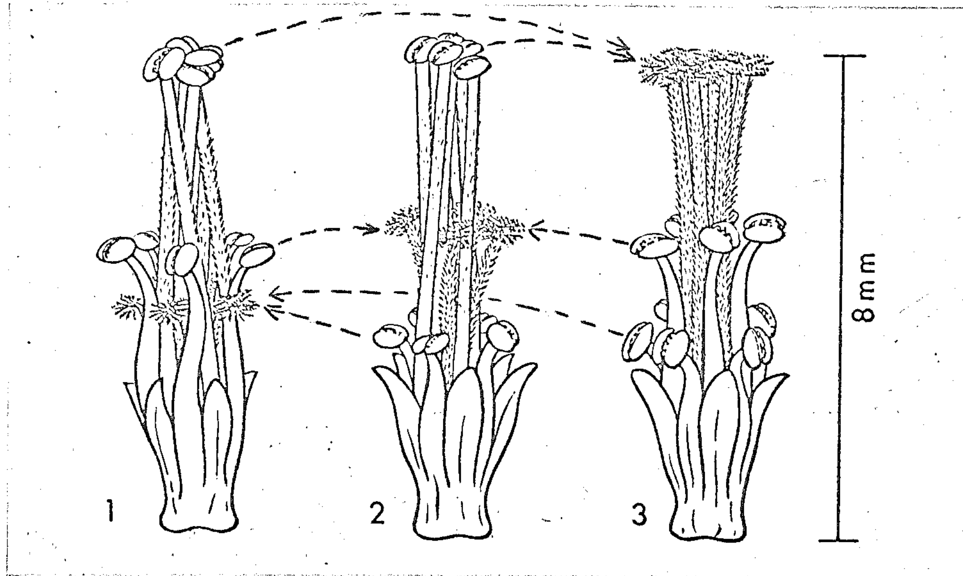


Figure 6. The three style-length forms of *Oxalis pes-caprae*, with legitimate pollinations indicated. (From Michael, 1964)

However larger programmes were undertaken in 1964 in attempts to produce seed from illegitimate pollinations. The majority of such pollinations produced at least one or two seed, while two or three plants were found whose pollen consistently gave up to half as much seed in illegitimate pollinations as normally given by legitimate pollinations. It appears that the reason why self-fertilization is rarely observed in crossing cages is due to the pollen and styles being effectively isolated.

While cases of partial breakdown of the incompatibility system have been found previously (Darwin, loc. sit.), no detailed investigation of the nature of the breakdown appears to have been made. However by comparing the relative numbers of seed produced from various illegitimate pollinations with pollen from these plants, useful information concerning the nature of the incompatibility system can be obtained. A preliminary investigation of this kind has been made, but a detailed discussion of the results does not seem appropriate here.

The sizes of pollen grains from different levels were studied by Mr. Franklin and the author. Clear differences

between pollen from the three levels were found, those from the longest level being the largest, and from the shortest level the smallest. No differences in size were found between the semi-compatible pollen mentioned previously and pollen from the same level of normal plants.

One apparent case of a breakdown in the tristyly mechanism is worthy of description in some detail. The plant S60.15 x L60.01-7 was classified in 1961 as short-styled and transplanted into a pot for use in the crossing programme, as S61.39. The plant was later used as a female parent in two crosses but the records show that no seed was obtained, which would not be unexpected in view of the hot weather conditions late in the season when these crosses were made. Still later in the season the last flower from S61.39 opened and was noticed to be a mid. Five flowers from a long plant were pollinated using the long pollen from this plant, while the short pollen was used on two mid and three short flowers. Six days later capsules were observed forming on the long and short plants but not on the mid. However only ten seed were eventually obtained, all from the short plant, S61.37. Plants were raised to flowering in subsequent years from four of these seed, and one was classified by backcrossing (see Sec. III 3 i c) as having two short

genes, strongly suggesting that genotypically a short X short cross had in fact been made. In addition the pigment markers possessed by these four plants were compatible with those possessed by the plants S61.37 and S61.39.

The classification of the original plants and the observations of capsule formation etc. were made at an early stage in the work on O. pes-caprae when the author was somewhat unfamiliar with the material. However records of all pollinations and classifications have been kept, and none of these contradict the facts as given above. In addition bulbs from S61.39 are still available although those from S61.37 have been lost. Sixty-four pollinations of two independent short plants were made using S61.39 pollen under good conditions in 1964 and nineteen seed were obtained. This is less than the number produced in most illegitimate short pollinations, showing that S61.39 pollen is normally quite incompatible on short styles.

These observations have been made in some detail because the implications appear to have an important bearing on the theory of the genetical control of style length. It appears that in the above case a morphological transformation occurred, due presumably to heat stress, and this morphological transformation was accompanied by a transformation

in the incompatibility properties. This suggests that the morphological and incompatibility systems are connected physiologically, rather than through genetic linkage. A similar conclusion was drawn by Beale (1939) in experiments on distylic <u>Primula sinensis</u>. As Beale and others have pointed out the mere fact that in a tristylic plant two types of pollen are produced from the one genotype also supports the first theory considered above. It seems that the evidence for the genetic linkage theory, based principally on the work of Ernst (1936), should be critically re-examined.

One further observation of interest in the present discussion is the classification of a 'homostyle' plant during the 1964 season. The plant S61.12 x L63.01-246 was classified as having styles at the mid level and pollen at the mid and long levels. This plant was highly self-fertile, and both pollen and style behaved in different crosses as expected from their morphological positions. In addition the pollen grain sizes at the lower level were those to be expected of mid but not short pollen. A number of crosses have been made with this plant, and the results may be of considerable interest from the point of view of style length inheritance in general.

3. GENETICAL STUDIES

i. The inheritance of style length

a Introductory

After several crosses involving small numbers had been made in the years 1954-1956 by Mr. R.N. Oram, the first sizable progenies were obtained by Mr. O.R. Byrne in the years 1958 and 1959. In that time Mr. Byrne made some thirty-six crosses of mid-styled plants by long-styled plants, using nineteen different, although not unrelated mid parents and a similar number of longs. The results of all these crosses are homogeneous ($\chi^2_{35} = 39.17$, $0.20 < p < 0.30$), and the totals, viz. 367 mid:403 long are in agreement with a 1:1 ratio ($\chi^2_1 = 2.51$, $0.10 < p < 0.20$). Three short X long crosses were also scored in 1959, two of which gave a total of 19 short:23 mid, while the third gave out of a total of five plants, three shorts and two mids. Two crosses between short and mid gave small progeny sizes, having between them plants of all three style lengths in the progeny.

It is seen from the above results that short plants were on no occasion found amongst the progeny of mid X long crosses, but that long plants were produced by short X mid

crosses and mid plants by short X long crosses. Thus it appears that the genetic control of the short phenotype is epistatic to the control of the mid and long phenotypes. In crosses involving a short parent all results were in agreement with a 1 short:1 non-short ratio. Thus it appears that a single gene difference is sufficient to explain the short:non-short difference. Furthermore the non-appearance of shorts in a large series of mid X long crosses favours the hypothesis that short is dominant to non-short. The 1 mid:1 long ratio found in the mid X long crosses suggests that the mid:long difference is also controlled by a single gene difference. However the dominance relationships of the mid and long genes cannot be inferred from the data.

Thus the results may be explained by postulating a two-locus model, as proposed for several other cases of tristyly (Fisher and Mather, 1943; Fyfe, 1956). However the above results give almost no information on the linkage relationships between the two loci. They do not refute the possibility of tight linkage between the two loci, or the possibility that in the limit the inheritance of style length could be controlled by just one locus with three alleles, I^S, I^M and I^L, such that I^S is dominant to both

I^M and I^L.

In 1960 Mr. I.R. Franklin selected a series of short and mid plants from the progeny of the 1958 short X mid crosses (using in fact bulb plants arising from the progeny grown in 1959). These plants were crossed to a smaller number of mid and long plants also selected from the same progenies. The results of the short X long crosses are given in Table 11 and those of the short X mid crosses in Table 12. (see over page).

The results of the two series of crosses provide an immediate answer to the question of the dominance relationships at the mid-long locus. The results are clearly incompatible with a dominant long gene, but are compatible with the hypothesis of mid dominant to long. Strictly speaking however the dominance of the mid gene may only be demonstrated by identifying by backcrossing a mid plant containing more than one mid gene.

PROGENY PHENOTYPE / SHORT PARENT	SHORT	MID	LONG	TOTAL
S60.01	35	.	44	79
S60.04	8	.	12	20
S60.07	37	.	47	84
S60.09	20	.	14	34
S60.10	28	.	26	54
S60.12	15	.	18	33
S60.13	15	.	17	32
S60.14	13	.	8	21
S60.15	57	1	40	98
TOTAL	228	1	226	455
S60.02	12	15	3	30
S60.03	23	15	3	41
S60.05	37	27	10	74
S60.06	10	2	.	12
S60.08	54	36	20	110
S60.11	39	30	17	86
TOTAL	175	125	53	341
S60.16	146	55	90	291

Table 11. Results from 1960 short X long crosses

CROSS \ PROGENY PHENOTYPE	SHORT	MID	LONG	TOTAL
S60.04 X M60.05	21	5	13	39
S60.07 X M60.04	22	12	12	46
S60.09 X M60.03	17	16	13	46
S60.10 X M60.04	23	14	9	46
S60.12 X M60.03	14	6	8	28
S60.14 X M60.03	19	8	12	39
S60.15 X M60.03	20	7	14	41
TOTAL	136	68	81	285
S60.03 X M60.03	6	4	1	11
S60.06 X M60.02	21	11	5	37
S60.06 X M60.03	44	20	4	68
S60.08 X M60.06	16	17	5	38
TOTAL	87	52	15	154

Table 12. Results from 1960 short X mid crosses

On the hypothesis that short is dominant to non-short and mid is dominant to long, the cross of short to long constitutes a backcross for identifying the genotype at the two loci, called the short and mid loci respectively.

Shorts giving mid progeny when backcrossed to long are thus said to be short-carrying-mid, while those which produce no mid offspring in a sufficiently large backcross are said to be short-not-carrying-mid. Sufficient data were available from Mr. Byrne's records to classify in this way the short parents of the plants S60.01, S60.02, ... S60.16. The first fifteen of these were seen to come from short-not-carrying-mid X mid crosses, while S60.16 came from a short-carrying-mid X mid cross.

The backcrosses in Table 11 have been divided into three classes on the basis of the mid:long ratio. Those plants having no mid progeny have been placed in the first class. (The one mid progeny from S60.15 has been attributed to contaminant pollination.) Those plants giving an excess of mid to long, significant in four of the six cases, have been assigned to the second class. The remaining plant, S60.16, gave a significant excess of longs and constitutes the third class. In several cases the backcrosses were made to more than one long parent, but the results from each short parent have been pooled in Table 11 since in no case was significant heterogeneity found between the individual progenies. For Table 12

the same grouping of shorts as emerges from Table 11 has been retained.

The short:non-short ratio is homogeneous throughout the two tables ($\chi^2_{26} = 15.16$, $0.95 < p < 0.98$) and the totals, 772 short:754 non-short, are in agreement with a 1:1 ratio ($\chi^2_1 = 0.21$, $0.50 < p < 0.70$). However the mid:long results from the short-carrying-mid parents are clearly not in agreement with a 1:1 ratio. Within the second class of Table 11 the results are homogeneous ($\chi^2_5 = 5.80$) in showing a deficiency of longs ($\chi^2_1 = 14.6$, $p < .001$), while there is a significant deficiency of mids in the third class ($\chi^2_1 = 8.45$, $p < .01$).

Fifteen of the sixteen short parents come from short-not-carrying-mid X mid crosses, and if the mid parents contain one mid gene one half of these plants are expected to carry the mid gene and one half not. Altogether six of the plants were found to possess the mid gene and the remaining nine not, which is in agreement with the above expectations.

On the hypothesis of independent short and mid loci nearly equal numbers of mids and longs are expected in the backcross of a short-carrying-mid. The hypothesis

of linkage between the short and mid loci was put forward in an attempt to explain both the excess of mids in the second class and the excess of longs in the third. Evidently under this hypothesis those plants with the genes linked in coupling are expected to give an excess of longs to mids, and vice versa for the repulsion plants. The hypothesis fits in well with what is known of the ancestry of the short-carrying-mid plants. The plants giving an excess of mids, viz. S60.02, 03, 05, 06, 08 and 11, coming from short-not-carrying-mid X mid crosses, could only have the short and mid genes in the repulsion phase. S60.16 on the other hand, coming from a short-carrying-mid X mid cross, could have the genes linked in coupling or repulsion.

Despite these aspects which the linkage hypothesis explains satisfactorily, it does not provide a complete explanation of the results if tetrasomic inheritance is operative. S60.02, 03, etc. would then be assigned the genotype $Sm/sM/sm_2$, i.e. bi-simplex repulsion. Even if the genes are completely linked, only a 2 mid:1 long ratio is expected. Thus the mid:long ratio observed in these backcrosses, viz. 123 mid:53 long, could only be explained by a low frequency of recombination between the

two loci. On the other hand the ratio observed from the plant S60.16, 55 mid:90 long, may only be explained by a relatively high frequency of recombination if the genes are here linked in coupling.

Mr. Franklin was able to give expectations (Table 13) for the two backcross ratios in terms of p, a parameter dependent on the amount of recombination between the two loci and closely related to the recombination frequency. Professor J.H. Bennett showed that the mid:long

OFFSPRING / PARENT	SHORT	MID	LONG
Bisimplex coupling	$\frac{1}{2}$	$\frac{1}{2}p$	$\frac{1}{2}(1-p)$
" repulsion	$\frac{1}{2}$	$\frac{1}{2}(\frac{2}{3}-\frac{p}{3})$	$\frac{1}{2}(\frac{1}{3}+\frac{p}{3})$

Table 13. Backcross expectations for two classes of shorts-carrying-mid

expectations are infact exact provided that double reduction at the mid locus is ignored. The estimates of p calculated from the above two sets of data are significantly

different (S.N.D. = 4.28, $p < .0001$).

On the other hand on the hypothesis of disomic inheritance the frequency of recombination between the two loci can be estimated as a simple proportion, and the estimates obtained from the two sets of data are in good agreement (S.N.D. = 0.74, $p = 0.46$).

b. The mode of segregation at the mid locus

While the above evidence does not favour the hypothesis of tetrasomic inheritance for the mid and short loci, a more direct test for duplex segregation is evidently necessary. To this end Mr. Franklin selected short and mid plants from the progeny of the 1960 short-carrying-mid X mid crosses, which he backcrossed to long and intercrossed. The backcrosses of those mid plants containing two mid genes were expected to give the critical evidence for duplex segregation at the mid locus. The backcrosses of the shorts however could not give conclusive evidence for duplex segregation (at the mid locus) owing to the possible complications introduced by linkage and epistasis between the two loci.

Unfortunately at the stage at which the initial

crosses were carried out the procedures outlined in Sec. II 1 for critically testing for duplex segregation were not fully appreciated. While the three short plants and two of the three mid plants used, viz. S60.03, 06, 08, M60.02 and M60.03 all came from the same mid X short-not-carrying-mid cross, viz. M57.17 X S58.07, the mid plant M60.06 was of independent origin. Thus in those short X mid crosses where M60.06 was involved it was not possible to tell whether or not the two mid genes came from a common source.

Very few results were obtained in 1962 when for the first time the plants were under the sole care of the present author. As related in Sec. III 1 ii, portion of the information was salvaged by classifying in 1963 plants grown from small bulbs left by some of the 1962 plants. In addition it was possible to repeat some of the backcrosses in 1962. The results from all backcrosses are pooled in Table 14.

ORIGINAL NO.	NEW NO.	YEAR CROSS MADE	SHORT	MID	LONG	TOTAL
S60.03 x M60.03-13	M61.01	1961		6		6
S60.03 x M60.03-16	M61.02	1962		13	16	29
S60.06 x M60.02-10	M61.03	1961		15	2	17
S60.06 x M60.03-41	M61.04	1962		8	7	15
S60.08 x M60.06-21	M61.05	1961		21	2	23
S60.08 x M60.06-22	M61.06	1962		26	22	48
S60.08 x M60.06-32	M61.07	1961		14	15	29
S60.08 x M60.06-35	M61.08	1962		8	12	20
S60.03 x M60.03-1	S61.01	1962	15	15	3	33
S60.03 x M60.03-2	S61.02	1962	29	13	13	55
S60.06 x M60.03-16	S61.03	1961	20	14	4	38
S60.06 x M60.03-18	S61.04	1961	2		5	7
S60.06 x M60.03-109	S61.05	1961	19	11	1	31
S60.08 x M60.06-9	S61.06	1961, 1962	14	10	6	30
S60.08 x M60.06-41	S61.07	1961	9	3	3	15
S60.08 x M60.06-16	S61.09	1962	9		10	19
S60.08 x M60.06-28	S61.10	1962	17	11	4	32

Table 14. Backcross results for 1961 shorts and mids

Five of the mid plants backcrossed have given results in agreement with a 1 mid:1 long ratio and evidently contain one mid gene. M61.01, 03 and 05 on the other hand have given significant excesses of mid and are thus classified as having more than one mid gene and very probably two. The appearance of the long

plants in the progeny of M61.03 constitutes critical evidence for duplex segregation at the mid locus, since the mid genes possessed by this plant are known to be identical by descent. The long plants observed in the M61.05 progeny constitute supporting evidence for this point.

Owing to the small numbers of plants involved there seems little point in attempting to draw inferences about the duplex segregation frequency from these results. Neither may inferences regarding duplex segregation be drawn from the results of the short crosses, since the excesses of mid to long in some crosses may be due to the effects of linkage rather than to the parent having two mid genes.

Much more satisfactory numbers were obtained for two new and one repeated backcross made in 1963 (Table 15).

PARENT (OLD NUMBER)	PARENT (NEW NUMBER)	PROGENY: SHORT	MID	LONG	TOTAL
S61.34 x M61.23-2	M63.25		68	1	69
S61.06 x M61.08-17	M62.30		94	1	95
S60.08 x M60.06-21	M61.05		103	5	108

Table 15. Backcross results from various mids

The plants S61.34 and M61.23 are from the short-not-carrying-mid X simplex mid cross S60.15 X M60.04. Thus unless double reduction has occurred to produce a triplex individual the plant M63.25 must possess two mid genes identical by descent. Duplex segregation is apparently occurring, but at a low frequency. The repetition of the M61.05 backcross has given a similar picture, with an estimated duplex segregation frequency equal to $\frac{5}{108} = 0.046$, which differs significantly from the lowest value expected for tetrasomic inheritance, viz. one-sixth ($\chi_1^2 = 11.3$, $p < .001$)

Further evidence of the same type is presented by the backcross of M62.30, both parents of which have one mid gene, the two being not necessarily identical. Portion of the backcross results of M62.30 were actually obtained in the 1963 season when the results were recorded as 38 mid:1 long. This was interpreted at the time as a possible triplex backcross ratio. In order to test this interpretation five of the thirty-eight mid progeny were themselves backcrossed to long on the expectation that approximately one-half of the progeny of a triplex back-cross should be duplex. The results, Table 16, show that four of the plants contain only one mid gene, but are

PROGENY / PARENT OLD NUMBER	NEW NUMBER	SHORT	MID	LONG	TOTAL
L62.12 x M62.30-2	M63.31		9	14	23
-19	M63.32		7	3	10
-24	M63.33		13	14	27
-30	M63.34		18	16	34
-32	M63.35		10	14	24

Table 16. Second backcross for M62.30

equivocal regarding the plant M63.32. Five simplex plants are needed to show at the 95% significance level that M62.30 is not triplex mid, so that these results are not in themselves critical. However combined with the backcross results of M61.05 and M63.25, and knowing that a priori the probability of double reduction occurring is low, these results point to a duplex genotype for most if not all of the three plants under discussion.

A further possible explanation for the high mid:long

ratio observed from duplex plants would be trisomy for the chromosome carrying the mid locus. The genotype at the mid locus under these circumstances would be MMm, and a low viability of monosomic gametes could lead to a low frequency of longs being observed. Amongst the mid progeny one-third would be expected to be duplex in such a case, so that the failure to find one amongst four mid progeny from M62.30 does not constitute decisive evidence against the trisomy hypothesis. Several further backcrosses of M62.30 progeny have been made in an attempt to obtain this evidence in 1965.

Cytological evidence is useful on this point since the demonstration that the plant has the tetraploid number of chromosomes reduces considerably the likelihood of trisomy. Although the standard of preparation was not good (Fig. 7), two or three cells from M62.30 were found showing twenty-eight chromosomes at first division anaphase of meiosis. M63.25 was similarly classified as having twenty-eight chromosomes but M61.05 has not so far been studied.

A large number of further mid plants were backcrossed in 1963, and several (Table 17) yielded significant excesses of mid in the progeny. As may be seen from the

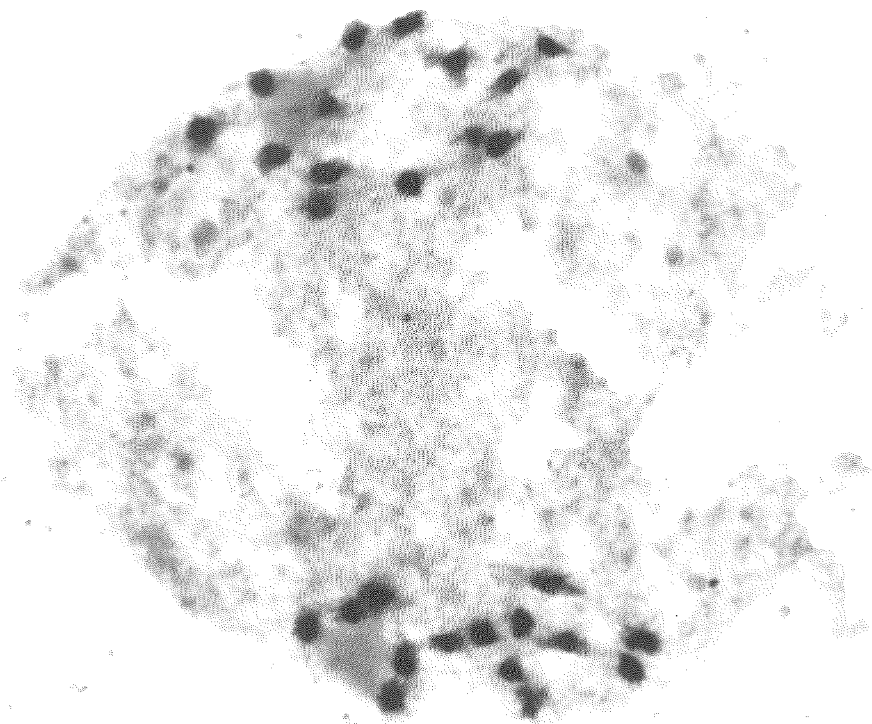


Figure 7. First division anaphase of meiosis in Plant No. M62.30

PARENT / PROGENY OLD NUMBER	NEW NUMBER	SHORT	MID	LONG	TOTAL
M61.03 x S61.05-18	M63.15		35	7	42
M61.03 x S61.05-20	M63.16		48	22	70
M61.03 x S61.05-23	M63.17		21		21
M61.05 x S61.03-18	M63.22		26	7	33
S61.03 x M61.03-16	M62.22		50	1	51
M62.25 x S62.21-18	M63.27		82	15	97
M62.25 x S62.21-20	M63.28		63	3	66
L61.14 x S61.11-30	M63.10		106	18	124
S61.11 x M61.11-4	M63.11		87	22	109
S61.11 x M61.11-7	M63.13		72	9	81

Table 17. Backcross of miscellaneous 1963 mids

backcrosses of parents from which these plants are derived (see Table. 14 and p. 105) in none of these cases are the parents both known to be simplex for the mid gene. In fact it seems likely that most of these plants come from duplex X simplex crosses, except for M63.10 which comes from a duplex X nulliplex cross. The tetrasomic expectations from a duplex X simplex cross are approximately 1 nulliplex:5 simplex:5 duplex:1 triplex, so that the

possibility that some of the mids in Table 17 are triplex cannot be ignored. Most would however be classified as duplex on the basis of their backcross ratio. It seems clear that there is a good deal of variability in the duplex segregation frequency, since the results from at least six of the above backcrosses are in agreement with tetrasomic inheritance expectations, while one or two others, plus the three in Table 15 clearly indicate di-tetrasomic inheritance. This point will be further discussed in Part IV.

The plant M63.16 is of some interest since the backcross ratio, 48 mid:22 long, deviates significantly from a 1:1 ratio ($\chi^2_1 = 8.23$, $p < .01$), but is also difficult to explain as a duplex backcross ratio. At a 95% confidence level a value of $\alpha > 0.153$ is needed to explain such a high frequency of longs if tetrasomic inheritance is operative. Therefore the possibility must be considered that M63.16 contains two mid genes which lie on chromosomes which pair only rarely, a situation which would lead to an approximate 3:1 ratio. Any mid genes possessed by M63.16 are known to be identical by descent, so such a situation could only have arisen through crossing-over removing one of the M genes from

its original chromosome within the previous two or three generations. Further seed have been obtained from backcrosses of M63.16 in order to verify the segregation ratio found.

A summary has been made of the results of all backcrosses of plants classified as simplex mid. A problem of ascertainment arises here, since most classifications must be made on the basis of the backcross ratio. Some simplex plants may be omitted from the classification through giving by chance results agreeing with duplex but not simplex expectations, while some duplex plants may be spuriously included. The two errors are to a certain extent compensatory. A minimum progeny size of ten has been adopted in an effort to minimize this type of error. Few cases have then been found where plants could not be unambiguously classified as either simplex or duplex.

Thirty-one crosses have been classified as simplex mid backcrosses, and have given a total of 446 mid:481 long. The results of these crosses are not homogeneous ($\chi^2_{30} = 57.02$, $p < .01$). The majority of the heterogeneity is attributable to one cross, L63.22 x M63.06,

which gave 12 mid:43 long. The results of this cross were obtained in two groups, one of which suffered little mortality while the other suffered about 50% mortality. However the results are quite comparable in the two cases, 7 mid:23 long and 5 mid:20 long respectively. Testing for a 1:1 ratio gives for the totals $\chi_1^2 = 17.5$, $p < .0001$. However since we are considering a single extreme cross selected from thirty-one crosses, a significance level of .0001 for this case would be approximately equivalent to a significance level for a single cross of $1 - (.9999)^{31} = 0.0031$. Thus the results from L63.22 x M63.06 may be considered as significant only at the 1% level. The very large deviation from a 1:1 ratio nevertheless suggests that the possibility of another segregating major gene affecting style length cannot be ignored(see also p.110).

c. The mode of segregation at the short locus

The programme for building up stocks for the short locus is made more difficult by virtue of the incompatibility system which hinders the making of short X short crosses. However under circumstances which have been described in Sec. III 2, ten seed were obtained from the

cross S61.37 x S61.39. Plants from four of these seed grew to maturity and gave three short and one mid. One of these short plants, S63.43, was used in backcrossing to long, while seed produced by open pollination was obtained from S63.44. Assuming that the incompatibility reaction is operative, the open pollination is equivalent to a backcross for the short locus, since pollen from short plants would be incompatible, or at least retarded compared to pollen from the short level of mid and long plants. S63.43 gave altogether 3 short:6 long and was classified as simplex for the short gene. S63.44 however gave 17 short:1 mid:1 long and was classified as having two short genes.

While duplex segregation at the short locus was demonstrated by the above results, the demonstration is unsatisfactory for several reasons. The plants S61.37 and S61.39 were unrelated, and thus duplicate short genes could be involved. Larger numbers would have been desirable to test more accurately for a 3 short:1 non-short ratio in the cross S61.37 x S61.39, partly to confirm that this cross had in fact been made. A more accurate estimate of the duplex segregation frequency would be desirable. In addition the seed from the plants S63.43

and S63.44 were obtained not from the original plants, which did not flower, but from bulbs produced by these plants. Finally the open pollination backcross introduces a further uncertainty factor.

The plant S63.44 has been lost, but further information may be expected from a second backcross programme, in which all seventeen short plants from S63.44 have themselves been backcrossed. In addition, as related in Sec. III 2, several short X short crosses have been made in 1964 for building up stocks for more critical tests of the mode of segregation at the short locus.

A summary of all crosses involving a short parent has been made. No plant excepting S63.44 gave results suggesting that it contained more than one short gene. Altogether one hundred and twenty-two crosses with more than ten offspring are included, involving eighty-one different short parents. The totals are 2928 short : 3192 non-short. However the individual results are highly heterogeneous ($\chi^2_{121} = 181.46$, $p < .001$). There is no evidence that this heterogeneity is due to differences between the short parents, since testing for heterogeneity between different backcross results from the same

short parent yields $\chi^2_{41} = 81.12$, $p < .001$. This heterogeneity could possibly be due to some contribution from the non-short parent, but seems more likely due to viability differences such as found by Fisher and Mather (1943) between different style forms of Lythrum salicaria.

d Linkage between the short and mid loci.

Two programmes were carried out in order to test more extensively the hypothesis of linkage between the two style length loci. The first of these was concerned with the plants and descendants of the plants of the second group of Table 14, i.e. those tentatively classified as bi-simplex repulsion for the short and mid genes. The second was concerned with descendants of S60.16, i.e. that plant classified as having the short and mid genes in coupling.

(1) First programme

The principal aim of this programme was to try to produce a 'coupling' plant from a 'repulsion' plant, i.e. to identify a short plant giving an excess of longs to mids on backcrossing amongst the backcross progeny of a short plant giving an excess of mids to longs. If the map distance between the two loci is p, then if tetrasomic inheritance is operative the expected frequency of coupling

plants in a repulsion backcross would be approximately $\frac{p}{3}$, while the expected frequency of repulsion plants would be a little less than $\frac{1}{3}$. Preferential pairing of the chromosomes containing the S and M genes would however raise the former frequency and lower the latter. In either case a larger number of backcrosses than was practicable at the time would have been necessary to be reasonably certain of achieving the aim of the programme.

Fourteen short plants were selected from the backcrosses of the short plants S61.01, 02, 06 and 10, all four of which were thought to be bisimplex repulsion individuals. One or two of these plants had given large excesses of mid:long in the progeny (Table 14), and a further aim of the programme was to confirm the classifications of simplex for mid.

PARENTAL CROSS					
OLD NUMBER	NEW NUMBER	SHORT	MID	LONG	TOTAL
L62.25 x S61.01-7	S63.01	49		49	98
-8	S63.02	22		14	36
-13	S63.03	41		42	83
-29	S63.04	8		12	20
-34	S63.05	54		65	119
S61.02 x L61.14-4	S63.06	78	63		141
-29	S63.07	18		13	31
-35	S63.08	62	80	4	146
S61.06 x L62.25-4	S63.09	79		62	141
-29	S63.10	30		23	53
L61.12 x S61.10-12	S63.11	51		73	124
-49	S63.12	34		56	90
-53	S63.13	68	63	25	153
-67	S63.14	3	2		5

Table 18. Backcross results for first programme

The backcross results for the fourteen short plants are given in Table 18. Looking first at the results of plants from the L62.25 x S61.01 cross it is seen that all five plants are short-not-carrying-mid. Clearly S61.01 does not carry two mid genes. But L62.25 x S61.01 gave a higher frequency of mid:long (15 mid:3 long) than any of the other three parental crosses (Table 14). The results

as summarized in Table 19 show conclusively that the short and mid genes are not independent, since the probability of results such as these on the hypothesis of independent segregation is approximately 1 in 300.

		MID	
		+	−
SHORT	+	.	5
	−	15	3

Table 19. Genes received by the offspring of S61.01

The second outstanding aspect of the results of Table 18 is the very high ratio of mid:long given by the shorts-carrying-mid. As remarked earlier, under the model of tetrasomic inheritance the backcross of a short-carrying-mid can give at most a ratio of 2 mid:1 long if the short and mid genes are completely linked. The results from the backcross of S63.13 are in good agreement with these expectations. From S63.06 and S63.08 however the results clearly indicate a much higher mid:long ratio. Such a

ratio could be explained on the basis of di-tetrasomic inheritance, if the two loci are linked closely in repulsion on two preferentially pairing chromosomes.

While it is difficult to visualize any alternative explanation for the above results, one result is difficult to reconcile with the hypothesis advanced. Since the short and mid genes possessed by S63.06 and S63.08 were both received from S61.02, it must therefore be supposed that two chromosomes of similar pairing constitution were received from this parent. A mid:long ratio of 2:1 or greater would therefore be expected from backcrossing S61.02. The results observed however, 13 mid:13 long in addition to one short-not-carrying-mid and two shorts-carrying-mid, are seen to be equivalent to a mid:long ratio of 14:15, which deviates significantly from a 2:1 ratio ($\chi_1^2 = 4.42$, $p < .05$). But the results are in agreement with expectations for independently segregating short and mid genes, which suggests that the genes in S61.02 are situated on chromosomes which do not pair or pair rarely. A larger backcross of S61.02 would evidently be desirable to confirm the trends shown, especially in view of the possible heterogeneity of mid:long segregations mentioned earlier.

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If the chromosomes containing the short and mid genes in a particular plant were known to pair in a purely disomic manner, then the frequency of longs amongst the non-short plants would be a direct estimate of the frequency of recombination between the short and mid loci. However in a bisimplex backcross there is no way of detecting, or estimating the frequency of, duplex segregation for the portion of the chromosome involved. It is not known whether any particular long plant is produced through duplex segregation or through recombination between the two genes. In the present case the frequency of longs may be taken as an upper bound of the recombination frequency.

Two alternative estimates for the upper bound are possible from the data of Table 18, depending on the assumptions made. It might be argued that since the results from S63.06 and S63.08 do not differ significantly ($p = 0.21$), they should therefore be pooled, giving an estimate of the upper bound of the recombination frequency as $\frac{4}{147}$. The second line of argument rests on the assertion that the frequency of duplex segregation is not expected to be the same from one plant to another, as for

example shown by the results of Collins and Longley (1935). Thus equal frequencies of longs are not to be expected from backcrossing two different short plants, and since we are interested in estimating the least upper bound the lower value may be used as an estimate. Thus the estimate in this case would be $\frac{0}{63}$, and the 95% upper limit may be derived exactly or from Table VIII, Fisher and Yates (1957) as 0.056. The comparable value for the first estimate is only slightly higher, viz. 0.068.

Further plants are obviously needed from S63.06 and S63.08 backcrosses to establish whether the frequency of longs tends to the same value in both cases. However this frequency will evidently be relatively low for both plants, and this circumstance should be exploited to obtain as accurate an estimate of the recombination frequency as possible. It should be mentioned that the above argument is all based on the supposition that S63.06 and S63.08 are simplex for the mid gene, which seems likely in view of their ancestry. However all or nearly all of the short backcross offspring should be short-not-carrying-mid on this hypothesis, and backcrosses have been made to test this in a few cases.

The overall programme failed in the stated aim of detecting a coupling short-carrying-mid plant. In view of the unexpectedly low recombination frequency revealed by the new data this is not surprising. In fact the numbers of progeny grown for the ten second backcrosses not segregating for mid would not be sufficient to be at all certain of detecting such a coupling plant should it occur.

(2) Second programme

The finding that the frequency of recombination between the short and mid loci may be very low obviously casts doubt on the classification of S60.16 as bisimplex coupling. The estimate of the recombination frequency from this plant would be at least $\frac{55}{145}$, which is clearly incompatible with all estimates of the recombination frequency obtained from bisimplex repulsion backcrosses.

A second backcross programme for S60.16 was undertaken by the author in 1961, when eleven short plants from the cross S60.16 x L60.01 were themselves backcrossed to long. In the backcross of a SM/$(sm)_3$ individual a high proportion of SM/$(sm)_3$ offspring is expected but a low proportion of Sm/sM$(sm)_2$. The formation of a heterozygous

repulsion gamete is dependent on a number of factors, viz. a change of partners, a crossover between the two loci, adjacent chromosome disjunction at the first division of meiosis, and the correct disjunction of chromatids at the second division of meiosis. Thus the great majority of short-carrying-mid progeny from S60.16 x L60.01 would be expected to have the bisimplex coupling genotype, and to give a deficiency of mid to long when backcrossed. The programme was carried out principally to test this expectation.

Of the eleven short plants backcrossed, progeny from only four were obtained. Two of the backcrosses, viz. those of S61.11 and S61.12, were repeated in 1962, and the combined results are given in Table 20. The results from

CROSS	OFFSPRING			
	SHORT	MID	LONG	TOTAL
L61.14 x S61.11	14	47		61
S61.12 x L61.20	11	6	20	37
S61.14 x L61.20	4		9	13
S61.15 x L61.20	3		2	5
S61.16 x L61.20	1		5	6
L61.11 x S61.23	13	2	13	28

Table 20. Backcrosses for various S60.16 x L60.01 shorts

S61.12 and S61.23 could perhaps be considered as in agreement with the expectations given previously. However from S61.11 the results could scarcely be further from expectation. Instead of the expected excess of longs to mids there is a complete absence of longs. In addition there is a large deficiency of shorts:non-shorts (χ^2_1 for 1:1 ratio = 17.9, $p < .0001$). Partial confirmation of the unexpected results of L61.14 x S61.11 comes from the cross of S61.11 to the simplex mid plant M61.11, which gave 8 short:22 mid:0 long.

At the stage at which the anomalous results were obtained in 1963 the parent plants had already passed the flowering stage. Fortunately the backcrosses of four S60.16 x L60.01 progeny, viz. S61.11, 12, 13 and 20 had already been repeated in connection with another programme. The results are given in Table 21. No further results for S61.23 could be obtained.

SHORT PARENT	SHORT	MID	LONG	TOTAL
S61.11	90	88	10	188
S61.12	104	62	61	228*
S61.13	66	82	18	166
S61.20	31		59	90

* Including one 'homostyle' (see Sec. III 2)

Table 21. Repeat backcrosses of some S60.16 X L60.01 plants

It is clear firstly that for S61.11 the large deficiency of shorts obtained in 1963 is not substantiated. There is however a small deficiency in S61.11 and similar deficiencies in the three other progenies. The overall ratio, 291 short:380 non-short differs from a 1:1 ratio ($\chi^2_1 = 11.8$, $p < .001$), the individual crosses being homogeneous in this respect ($\chi^2_3 = 5.81$, $.1 < p < .2$). In view of the overall heterogeneity in the short:non-short ratio found earlier, which is attributable to other segregations besides the above, it is difficult to attach any special significance to these results, or even to the short:non-short ratio of L61.14 x S61.11.

The mid:long ratio given by S61.11 is again very high, although this ratio differs significantly from the 1964 value ($p = .034$). The results from S61.13 are comparable to those from S61.11. However the mid:long ratio from S61.12 is clearly lower than those from S61.11 and S61.13, and as for S61.11 is also significantly different from the 1963 value ($\chi^2_1 = 5.41$, $p < .05$).

One possible explanation of the large deficiency of longs in the backcross of S61.11 and S61.13 is linkage in repulsion such as postulated for S61.01, S63.06, etc. Under

this hypothesis the majority of the short backcross progeny would be shorts-not-carrying-mid. This expectation was tested by backcrossing nine short plants from L61.14 x S61.11 (see Table 22). Clearly all nine plants contain

SHORT PARENT	SHORT	MID	LONG	TOTAL
S63.21	15	10	10	35
S63.22	35	19	23	77
S63.23	36	31	16	83
S63.24	7	6	3	16
S63.25	28	22	9	59
S63.26	14	2	4	20
S63.27	32	23	15	70
S63.28	17	6	6	29
S63.29	22	15	14	51
TOTAL	206	134	100	440

Table 22. Backcross of various L61.14 x S61.11 plants

mid, thereby ruling out the hypothesis of linkage in repulsion. In view of the demonstration that nearly all the progeny from S61.11, whether short or non-short, contain the mid genes it seems clear that S61.11 could not be simplex for the mid gene but is very likely duplex. A similar genotype would also seem likely for S61.13.

The backcross results of S60.16, obtained from crosses to two different long plants which gave homogeneous results and a total of 146 short:55 mid:90 long, clearly suggest that S60.16 does not contain more than one mid gene. Then double reduction, which a priori must be considered unlikely, must be invoked in two cases to explain how both S61.11 and S61.13 inherited two mid genes. An alternative explanation, based on the possibility that these two plants arose through S60.16 selfing rather than backcrossing to long, could be put forward. The cross S60.16 x L60.01 was made in an open pollination plot at a time when it was not realized that selfings could occur in a limited number of cases. Favouring this hypothesis is the result that the ratio of half red-brown (a marker possessed by S60.16, Sec. III 3 iii): unpigmented, viz. 140:109, showed a significant excess of half red-brown ($\chi_1^2 = 3.86$, $p < .05$), which might be expected if a limited amount of selfing took place. The ratio of short:non-short, 130:119, also shows a slight, but non-significant excess of shorts over expectation. Against this hypothesis is the fact that only one obvious contaminant plant was found in several hundred progeny produced from the open pollination plot, so that the frequency of illegitimate short X short crosses could not be high.

Secondly the plants S60.16, L60.01 and S61.11 have been classified by backcrossing as simplex, duplex and triplex respectively for the gene red tip (Sec. III 3 ii a), so that double reduction at this locus would need to be invoked to make these results compatible with the hypothesis of selfing.

Two further results which may not be unrelated to the question under discussion come from crosses involving the plants S60.15 x L60.01 - 25 and 7, i.e. S63.45 and S61.39. Since S60.15 gave on backcrossing 57 short:1 mid:40 long, provided the one mid plant is attributed to contamination neither S63.45 nor S61.39 would be expected to contain the mid gene. However S63.45 gave on backcrossing 15 short: 10 mid:7 long. Unfortunately some doubt exists about the identity of the plant S63.45, since this plant was left outside in a box with other S60.15 x L60.01 progeny for two years. However the presence of anthocyanin markers characteristic of S60.15 x L60.01 plants (see Sec. III 3 ii) makes it unlikely that S63.45 was a contaminant plant. The plant S61.39 gave three short and one mid offspring on crossing to the plant S61.37 (see Sec. III 2), which should also be a short-not-carrying-mid.

All four anomalies mentioned above could be resolved by postulating that the plants concerned received a mid gene from L60.01. This hypothesis is extremely speculative, especially as L60.01 was involved in five crosses to short plants which did not segregate for mid. The hypothesis is put forward principally because it explains the puzzling phenomena mentioned above, but also because a finding of this kind might not be altogether unexpected. It seems likely from the demonstration of di-tetrasomic inheritance that _O. pes-caprae_ is of hybrid origin. Therefore the style length system in _O. pes-caprae_ is presumably the result of the interaction of two tristyly systems of related diploid ancestors. Little is known about such interactions, and the hypothesis could be put forward that the style length determinants from one species are, in some circumstances at least, epistatic to those from the other species, leading to a masking of mid genes in some long plants. This explanation could also account for the large deficiency of mid plants in the cross L63.22 x M63.06.

While the broad hypothesis of interaction between two style length systems appears feasible, a more specific hypothesis to explain all the above unexpected results has

not been found. (The 1 short:3 non-short ratio observed in L61.14 x S61.11 could perhaps be included amongst these results awaiting explanation, especially as both of the parents came from the cross S60.16 x L60.01.) In an attempt to clarify the situation, several crosses involving S60.16, L60.01, S61.11 and L61.14 have been repeated, and results should be available in the 1965 season. Also backcrosses have been made of four or five short progeny from crosses involving L60.01, none of which progeny would be expected to carry the mid gene unless they received it from L60.01.

The mid:long ratio from the cross L61.14 x S61.11 is compatible with the hypothesis that the short and mid genes are closely linked, and that S61.11 contains one S and two M genes. The fact that all nine short L61.14 x S61.11 offspring contain mid could be interpreted as showing preferential pairing between the two chromosomes containing the mid genes. However the plant L61.14 x S61.11 - 30(M) contains two mid genes (Table 17) which would have to be ascribed to the occurrence of duplex segregation. The finding of a short plant among the L61.14 x S61.11 progeny containing two mid genes would be particularly detrimental

to the hypothesis of close linkage, but none of the nine short plants would appear to be of this kind.

The results from the backcrosses of S61.11 and S61.13 of Table 21 are not in agreement with the hypothesis of close linkage between the short and mid loci. With complete linkage between the two loci, long plants from the backcross of a Sm/sM/sM/sm plant could only arise by double reduction, regardless of whether tetrasomic or di-tetrasomic inheritance is operative.

Two explanations for the high frequency of longs may be put forward, both of them feasible on the notion that the style length determinants of O. pes-caprae have come from two different sources. First it is possible that either the S or the M genes possessed by S61.11 and S61.13 are loosely linked or unlinked to the S and M genes of S61.02, S63.06, etc. In this case an approximate 5 mid:1 long ratio would be expected which is in agreement with the observed results of Table 21. The second explanation is that the long plants from S61.11 and S61.13 actually contain the mid gene, but are long plants for reasons such as previously suggested for L60.01. This explanation has the advantage of having been invoked previously to explain

others results, as well as providing a possible explanation for the different results from the two backcrosses of S61.11. Furthermore the excess of mid:long plants in Table 22 could be explained under the hypothesis of close linkage between the short and mid loci, which favours the second rather than the first explanation.

There seems little point in speculating further on these two or other possible hypotheses at the moment. The results which will be obtained in 1965 should either decisively reject or substantiate the second hypothesis. In addition crosses have been made to produce stocks containing all possible pairs of the S and M genes possessed by S61.12 and S61.02. The backcrosses of these stocks should allow a comprehensive test of the first hypothesis.

e Discussion

A low frequency of recombination between the short and mid loci, in some plants at least, has been shown. Linkage between the short and mid loci has been found previously by Fyfe (1950) in the diploid *Oxalis valdiviensis*, where the two loci are linked with a recombination frequency of approximately 5.7%. The linkage may be tighter than this figure in *O. pes-caprae*. In fact it

might be argued that since crossing-over between the S and M genes has not been definitely demonstrated, it is simpler to postulate that multiple alleles at a single locus rather than two alleles at two loci control the inheritance of tristyly. However taking into account the two-locus control of tristyly demonstrated for a number of other species the single locus model must be considered as somewhat unlikely.

It seems more than a coincidence that the two loci controlling style length should be so closely linked. If if it not by chance, then two explanations appear feasible to account for the linkage. It is possible that the two loci have evolved together under the influence of selection. Alternatively the close linkage could be a reflection of the fact that the two loci were originally adjacent or very close. This could in turn be due to the fact that one gene was produced from the other by duplication and subsequent divergence of function. Such duplication and divergence has been well documented, for example in _Drosophila melanogaster_ by Lewis (1951).

Theoretically the conditions for the evolution of close linkage appear to be fulfilled. The two genes at both loci are kept at high frequency in the population,

and Fisher (1949b) has shown that the effect of linkage would be to reduce the frequency of matings between close relatives and thus afford protection against deleterious inbreeding. It does appear however that the selective advantage attched to close linkage is probably very low. Fisher has shown that the effect of complete linkage would be to leave the frequency of parent-offspring mating unchanged from the case of independent loci, but to lower the frequency of compatible full-sib mating by some two per cent. For the case of half-sib mating the reduction in frequency of compatible matings appears to be less than one-tenth of two per cent, so that this type of mating may be ignored in the present discussion. (A diploid organism is assumed in these calculations since such evolution of close linkage would presumably have taken place at the diploid stage.) To calculate the selective advantage possessed by a system with complete linkage over one with independent loci, the figure of two per cent must be multiplied by the frequency with which a plant is normally exposed to pollen from full sibs, and by a quantity representing the loss in fitness of the inbred plants over the outbred plants. The resulting quantity may be very

low, and although Fisher (1930) has shown that small selective pressures may over a long time be very effective, it is felt that the alternative explanation for the close linkage in terms of a duplication should be strongly considered.

ii <u>Leaf Markers</u>

a Individual segregations

(1) Red Tip

Presence and absence of pigments in the leaf indentations were recognized by Mr. Byrne as quite distinct (Fig. 8 (a) shows the present condition, Fig. 8 (c) the absent condition). He postulated the existence of a gene Rt controlling the production of this pigment, the absent condition, green tip, being the result of a homozygous rt genotype. The amount of variability of expression shown between different plants containing the pigment suggested that dominance might not be complete, and that the phenotype would be a good indicator of the number of Rt genes possessed by a plant.

The cross S60.16 x L60.01 made by Mr. Franklin in 1960 was expected to be useful for building up stocks for investigation at this locus since both parents showed

Figure 8. Leaf markers in *O. pes-caprae*

Left to right, top to bottom

(a) Red tip

(b) Light crescent-red tip

(c) Outer crescent

(d) Inner crescent

(e) Inner crescent-red tip

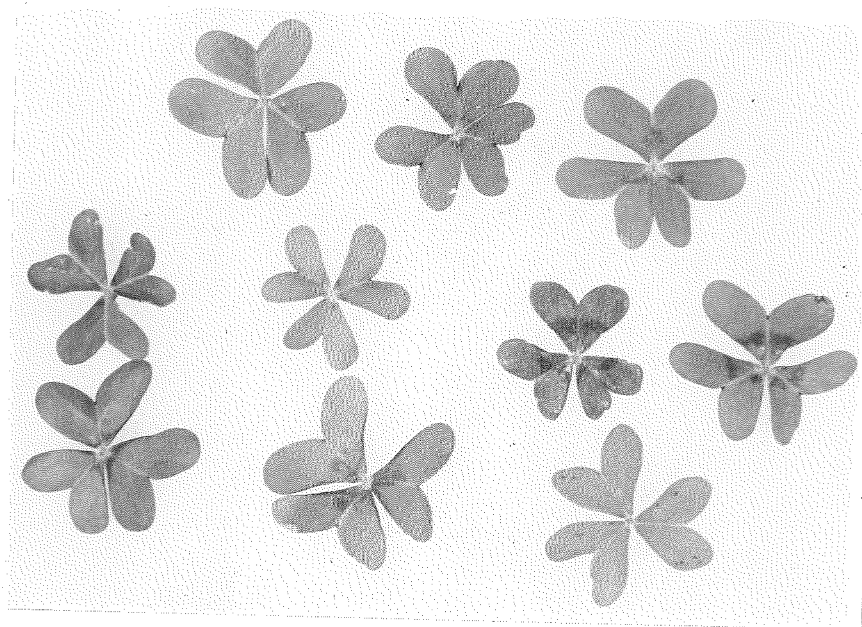
(f) Wide crescent

(g) Wide crescent-red tip

(h) Red crescent

(i) Wide crescent and red crescent

(j) Leaf fleck



strong expression of the red tip pigment. Both parents were also backcrossed to green tip plants. The backcross of L60.01 gave 63 red tip:13 green tip, indicating that this plant possessed two Rt genes, and giving results in close agreement with the hypothesis of tetrasomic inheritance. However the backcross of S60.16 gave results in good agreement with a 1 red tip:1 green tip ratio showing that this plant possessed only a single gene for red tip. From the cross S60.16 x L60.01, 226 red tip:29 green tip were observed, in agreement with the ratio predicted from the individual backcross ratios.

Nineteen plants containing red tip from these progeny were backcrossed to green tip by the author, principally with the aim of identifying a triplex Rt plant. Some of the nineteen plants were chosen at random from those available, while others were chosen especially for their strong expression of red tip. The nineteen plants were ranked subjectively in order of their strength of expression of red tip.

Small progeny sizes were obtained in 1962 and again in 1963 from progeny grown from bulbs. Several of the backcrosses were later repeated as recorded in Table 23.

PARENT	YEAR CROSS MADE	RANK ON PHENOTYPIC SCALE	RED TIP	UNPIGMENTED (GREEN TIP)	TOTAL
S61.22	1961	1	10	10	20
S61.21	61	2	12	17	29
S61.20	63	3	51	45	96
L61.15	61	4	2	3	5
S61.19	61	5	*		
S61.18	61	6	13	8	21
S61.17	61	7	4	5	9
S61.16	61	8	4	5	9
TOTAL			96	93	189
S61.15	61	9	19	6	25
S61.14	61	10	31	10	41
L61.14	62	11	48	7	55
S61.13	63	12	145	30	175
L61.13	61	13	17	2	19
M61.12	61	14	19	5	24
L61.12	62	15	65	12	77
L61.11	61	16	44	5	49
M61.11	61	19	19	4	23
TOTAL			407	81	488
S61.12	63	17	263	2	265
S61.11	63	18	193	0	193

* Numbers not recorded, but recorded as being in agreement with a 1:1 ratio

Table 23. Backcross ratios for red tip plants selected from S60.16 x L60.01

Plants ranked in the first eight on the phenotypic scale (i.e. weakest expression) gave results which are homogeneous ($\chi_6^2 = 2.80$) and in agreement with a 1:1 ratio. The remaining plants all gave significant excesses of red tip progeny. Two gave exceedingly large ratios of red tip:unpigmented, while the remaining nine gave results which are homogeneous ($\chi_8^2 = 5.75$) and in good agreement with a 5:1 ratio.

The results of Table 23 are in agreement with the model of a single tetrasomic gene controlling the production of red tip. Plants in the first class would be classified as $Rtrt_3$, and those in the second as Rt_2rt_2. The results from S61.12 are consistent with a parental genotype Rt_3rt, while S61.11 could be either Rt_3rt or Rt_4. However in view of the finding of di-tetrasomic inheritance for the mid locus the possibility that S61.11 and S61.12 are duplex for the Rt gene must be considered. This possibility may be discounted for S61.12 since one of the red tip progeny was backcrossed and gave 23 red tip:8 unpigmented. The progeny may thus be assigned the genotype Rt_2rt_2 which strongly favours the hypothesis that S61.12 is triplex for the Rt gene. Intercrosses amongst other red tip progeny from S61.12 have shown one plant to be duplex and four or five

others to be simplex. The results are not as clear cut for S61.11 although in one case it has been shown that one offspring received only one Rt gene, thus ruling out the possibility that S61.11 is Rt_4. Other results favour the hypothesis that the correct genotype is Rt_3rt, but this has not been shown conclusively.

It is clear from Table 23 that a dosage effect for the red tip gene has been established. The red tip phenotype may be used as a guide for selecting plants of a particular genotype, although especially in the upper ranges of expression it is not a completely reliable guide.

While the results of the above series of crosses are in accord with the model of tetrasomic inheritance there has been no critical demonstration of duplex segregation, and the possibility of duplicate factors has not been definitely excluded. In fact it was at one stage thought that a light leaf crescent (Fig. 8(b)) was associated with the red tip phenotype in some crosses but not in others, which would have strongly suggested the existence of duplicate factors. However it is now clear that this crescent is extremely variable, and seems to be found with low frequency in most if not all crosses involving red tip.

A more precise programme to investigate the mode of segregation at this locus would nevertheless seem desirable.

Such a programme was carried out through backcrossing plants from the cross M62.25 x S62.21 in which it was known that both parents contained an identical Rt gene. One of these plants, M63.27, gave an excess of red tip, viz. 122 red tip:6 unpigmented. Duplex segregation has thus occurred with an estimated frequency of 0.047, which differs significantly from the value one-sixth expected with tetrasomic inheritance.

A study of the overall segregation of red tip: unpigmented in simplex backcrosses has been made as a check for viability differences, misclassification, etc. Twenty-two crosses have been classified as simplex X nulliplex for the red tip gene (or perhaps genes). These results are homogeneous ($\chi^2_{21} = 19.24$, $0.50 < p < 0.70$), and the totals, 492 red tip:558 unpigmented, differ significantly from a 1:1 ratio ($\chi^2_1 = 4.14$, $p < .05$). These details are given in the first line of table 24, which contains similar summaries for all leaf markers.

The slight excess of unpigmented plants is not

necessarily an indication that red tip is being misclassified or is at a selective disadvantage. Double reduction may be expected to bias the ratio slightly in favour of the unpigmented plants. The results from S61.11 and S61.12 suggest that the frequency of double reduction is only of the order of 2%, but if all chromosomes are not completely homologous, as suggested by the finding of di-tetrasomic inheritance, the frequency could be higher in other crosses. Another factor which could lead to an excess of recessives is numerical non-disjunction. Finally as explained in conjunction with the simplex mid backcrosses, erroneous ascertainment of simplex backcrosses could bias these results.

For the purposes of demonstrating di-tetrasomic inheritance it is only necessary to show that the deficiency of recessives from $\frac{1}{6}$ in the duplex backcross is not matched by an equivalent deficiency from $\frac{1}{2}$ in the simplex backcross. Since in the present case no deficiency of recessives is found in the overall simplex segregations, the observed backcross ratio from M63.27 is clearly not explicable on tetrasomic expectations. As was the case with the mid: long segregations it is difficult to rule out the possibility of disomic inheritance and incomplete penetrance, since the

simplex segregations are compatible with a penetrance of the gene Rt of 122/128.

GENE	NO. OF CROSSES	HETERO-GENEITY χ^2	PROBA-BILITY	PIG-MENTED PROGENY	UNPIG-MENTED PROGENY	TOTAL PROGENY	χ^2 FOR 1:1 RATIO	PROBA-BILITY
Rt	22	19.24	.5-.7	492	558	1050	4.14	.05-.01
Ic(r) (1)	12	13.90	.2-.3	199	225	424	1.59	.2-.3
(2)	19	15.19	.5-.7	596	558	1154	1.25	.2-.3
Ic	7	5.02	.5-.7	209	192	401	0.72	.3-.5
Wc(r) (1)	15	3.97	> .99	587	580	1167	0.04	.8-.9
(2)	8	13.46	.05-.1	239	263	502	1.15	.2-.3
Wc	5	5.79	.2-.3	155	142	297	0.57	.3-.5
Rc (1)	5	7.02	.1-.2	144	179	323	3.79	.05-.1
(2)	6	15.94	.001-.01	122	159	281	4.87	.02-.05
Oc	45	73.57	.001-.01	813	1004	1817	19.68	< .0001
Lf	3	5.77	.05-.1	62	55	117	0.42	.5-.7

Table 24. Summary of 'simplex' backcrosses for leaf markers

(2) Inner Crescent

This crescent, Fig. 8 (d), is apparently the same as appearing in several crossed made by Mr. Byrne, and designated as leaf crescent. In all plants used by Mr. Franklin in 1960 in which this crescent was present it was associated with a pigment indistinguishable from that produced by the gene Rt. In all progeny classified by the author in 1961 as having the inner crescent pigment, the red tip pigmentation was also present. However in three or four progeny of crosses involving inner crescent but where the gene Rt was not segregating, the red tip pigment was scored as present and inner crescent as absent. Isolated cases of this are also found in Mr. Byrne's data. Since no further cases of this type have been found in 1963 or 1964 when plants were grown under improved conditions, and since the reciprocal type, i.e. inner crescent with no red tip, was not found, it seems that theese cases may be attributable to poor expression of the inner crescent pigment. Whether the red tip pigment involved is the same as that produced by the gene Rt and the two pigments are associated through close linkage of two genes, or whether the red tip pigment is a pleiotropic effect of the gene

producing the inner crescent pigment is not obvious. The point will be considered in some detail in Sec. III 3 ii b.

In field collections during 1962 a pigment was found similar to inner crescent but lacking the associated red tip pigmentation. When grown in glasshouses under the same environment the two crescents were not distinguishable (Fig. 3 (d) and (e)). Both have been given the name inner crescent, and the genes controlling the two crescents have been designated Ic(r) and Ic respectively.

The results for all crosses involving the gene Ic are homogeneous and in agreement with a 1:1 ratio (Table 24, line 4). The results for Ic(r) have been divided into two classes. In the first are those crosses where the presence of the red tip pigmentation can be definitely assigned to the gene Ic(r). As mentioned previously it appears for practical purposes that the presence or absence of Ic(r) can be most accurately determined by the red tip pigmentation. As shown in Table 24 line 2 these results are homogeneous and in agreement with a 1:1 ratio. In the third line the results are given for those crosses where red tip cannot be used in classification. Once again the results are in agreement with simplex expectations, showing that

either the crescent or the red tip pigment should usually be satisfactory for scoring for the gene Ic(r).

Four plants have given sufficient excesses of inner crescent on backcrossing to be classified as duplex. For three of these plants where it is not known whether the two genes are identical by descent, the numbers of inner crescent:unpigmented are 30:13, 47:13 and 113:23 respectively. The fourth, S62.13, has given 30 inner crescent: 4 unpigmented, and since only one parent of this plant, viz. S60.15, contained the Ic(r) gene and gave on backcrossing 46 inner crescent:55 unpigmented, double reduction must have occurred in gametogenesis in this parent. Unfortunately a little uncertainty exists in this case owing to the fact that S60.15 contained also the leaf crescents red crescent and outer crescent. The above demonstrations of double reduction and duplex segregation rest on the assertion that the different pigments have been correctly distinguished.

(3) Wide Crescent

The situation with regard to this pigment appears exactly analogous to that for inner crescent. Two indistinguishable pigments are found, one invariably associated with a red tip pigmentation and the other not (Fig. 8 (f)

and (g)). The simplex segregations for Wc and for both classes of Wc(r) are homogeneous and in agreement with a 1:1 ratio. However for several crosses the scoring of the wide crescent pigment was obviously unsatisfactory since many plants evidently contained the Wc(r) gene as evidenced by the red tip pigmentation, but expressed the crescent very weakly or not at all. In all these crosses plants probably received insufficient light due to the extra shading required at the end of the season to keep plants alive in excessive heat. For one of these plants in the second class of the Wc(r) segregation (Table 24, line 6), the results, 67 wide crescent:105 unpigmented, showed a significant deficiency of wide crescent ($\chi^2_1 = 8.20$, $p < .01$). This result accounts for over half of the heterogeneity χ^2 in the second Wc(r) class.

No results from duplex crosses have been obtained for either of the genes Wc or Wc(r).

(4) Red Crescent

All plants containing this crescent are descended from the plant S60.15. This crescent (Fig. 8 (h), 8 (i) with wide crescent) is invariably associated with a red tip pigment, although the pigmentation in this case is obviously

weaker than that produced by the genes Rt, Ic(r) and Wc(r). The crescent is very weak, and the results of simplex backcrosses (Table 24 line 9) show that misclassification may be frequent when the red tip pigmentation cannot be used for classifying for this gene. The results are on the borderline of significance for the classification using the associated red tip, the large value of χ^2 being due principally to a cross grown late in the season which gave 46Rc:76+. This indicates that classification may be unreliable under conditions of bad lighting even in crosses where the red tip pigmentation may be scored.

One backcross has been made involving a plant duplex for the Rc gene. The plant M63.25, coming from a simplex Rc intercross, gave on backcrossing 78Rc:0+. M63.25 was previously mentioned as being duplex for the mid gene, and giving on backcrossing 68 mid:1 long.

(5) Outer Crescent

The results for this crescent, pictured in Fig. 8 (c), are heterogeneous and show a deficiency of pigmented plants. This result might have been anticipated in view of the difficulty of classification in many crosses. The

inheritance maybe postulated as controlled by a single gene, Oc, with incomplete penetrance.

Three backcrosses have given large excesses of outer crescent, One from a plant of unknown origin gave 112 outer crescent:3 unpigmented. The other two, M63.27 and M63.28, sib plants both of which were known to possess two Oc genes identical by descent, gave respectively 110 outer crescent:18 unpigmented and 67 outer crescent:5 unpigmented. The second of these results is significantly different from a 5:1 ratio ($\chi^2_1 = 4.9$, $p < .05$), and thus suggestive of di-tetrasomic inheritance. However in view of the heterogeneous results obtained with this marker, it seems unwise to try to draw more specific conclusions from these results.

(6) Leaf Fleck

This marker, Fig. 8 (j), gives a very variable pattern of pigmentation, and may be due to a mutable gene such as Dt in maize. It has been involved in few crosses, all of which have given results in agreement with simplex expectations. A single gene Lf is postulated to control this pigment. The pentaploid form of <u>O. pes-caprae</u> contains a similar pigment, which when backcrossed to the unpigmented tetraploid has also given results in agreement with a 1:1 ratio.

b Joint Segregations

In view of the phenotypic similarity of the crescents produced by the genes Ic and Ic(r), and also by Wc and Wc(r), and of the red tip pigmentations produced by Rt, Ic(r) and Wc(r), the hypothesis might be put forward that the gene Ic(r) is actually a complex of the genes Ic and Rt, and Wc(r) a complex of Wc and Rt. This would demand that all three genes Ic, Wc and Rt be closely linked or allelic. A slightly different hypothesis might be to suppose that two duplicate Rt genes occur, one closely linked to Ic and one to Wc.

An alternative hypothesis may be put forward that red tip is a pleiotropic effect of the Ic(r) and Wc(r) genes. The leaf indentation might be thought of as a region of preferential pigmentation. The occasional appearance of the light crescent produced by the gene Rt (Fig. 8 (b)), suggests that under this hypothesis the gene Rt could be better labelled as the gene Lc(r) thus making it quite analogous to the genes Ic(r) and Wc(r). In support of this hypothesis is the phenotype produced by the gene Rc. Here the red tip phenotype is clearly weaker than that produced by the gene Rt , thus showing that more

than one red tip phenotype is possible. Further evidence supporting the hypothesis might be obtained by showing that the red tip pigments produced by the genes Ic(r), Wc(r) and Rt are not the same. A preliminary investigation using chromatography and spectrophotometry was made, but it was evident that a much more sensitive investigation would be needed to detect small differences between the pigments, should such differences exist. A weakness of the second hypothesis is that it does not explain why if red tip pigmentation should be a pleiotropic effect of some crescent genes, it should not be a pleiotropic effects of others producing practically identical crescents.

Segregational data may be used as evidence to decide between the two hypotheses. Several close linkages are predicted by the first hypothesis, whereas none are necessary under the second. As remarked in Section II 4, the test for close linkage or allelism of two genes in a tetraploid is very unreliable, especially when it is suspected that di-tetrasomic inheritance is operative. Perhaps the most that can be expected from backcrosses of the markers in pairs is an indication as to whether the two genes concerned lie on chromosomes of the same or different sets.

No data are as yet available on the joint segregation of the genes Ic and Ic(r). However three plants containing the genes Wc and Wc(r) were backcrossed, and the results are given in Table 25. Large numbers of extra plants were grown late in the season to supplement these results,

PROGENY PHENOTYPE / PARENT	WIDE CRESCENT RED TIP	WIDE CRESCENT GREEN TIP	NO WIDE CRESCENT RED TIP	NO WIDE CRESCENT GREEN TIP	TOTAL
L63.16	38	27		11	76
L63.18	20	13		9	42
L63.19	39	29		7	75
TOTAL	97	69		27	193

Table 25. Joint segregation of markers Wc and Wc(r)

but as related previously scoring for presence of the crescent was very difficult, and large numbers of plants were classified as lying in the third class of Table 25. However results for the red tip phenotype appeared to be in good agreement with expectations. The results from the later batch of plants have been disregarded in considering the

simultaneous segregation of the two markers.

A large excess of wide crescent:unpigmented is apparent amongst the green tip plants of Table 25, the results being homogeneous in showing this excess (χ^2_3 = 3.13, $0.2 < p < 0.3$, χ^2_1 for 1:1 ratio = 18.38, $p < .0001$). Thus it appears that the genes Wc and Wc(r) do not segregate independently. The results are however in agreement with a 2 wide crescent:1 unpigmented ratio (χ^2_1 = 1.17, $0.2 < p < 0.3$), which is the approximate expectation with close linkage or allelism when tetrasomic inheritance is operative. In the present case the likelihood of di-tetrasomic inheritance is diminished by the finding that three plants, although full sibs, have given homogeneous results for the crescent:unpigmented ratio where such homogeneity is not necessarily to be expected with di-tetrasomic inheritance.

The joint segregations of Ic(r) and Rt and of Wc(r) and Rt have been studied. Those for Ic, Rt and Wc, Rt are not as yet available. For the Ic(r), Rt segregations the results are unfortunately very conflicting. The first four parents are from the same cross, and have given homogeneous results (Table 26), the totals

153(Ic(r)Rt and Ic(r)+) : 68+Rt : 73++

PROGENY PHENOTYPE / PARENTS	INNER CRESCENT RED TIP	INNER CRESCENT NO RED TIP	NO INNER CRESCENT RED TIP	NO INNER CRESCENT NO RED TIP	TOTAL
L63.45	71		34	27	132
L63.42	49		21	27	97
L63.43	13		9	9	31
L63.41	20		4	10	34
L63.07	7		6	5	18
S63.45	18		18	4	40
L63.05	29		11	15	55
L63.05	22		19	7	48
L63.05	32		13	12	57
L63.05	11		9	2	22

Table 26. Backcross results from Ic(r), Rt plants

being in agreement with a 2:1:1 ratio expected for independent genes. The results from S63.45 however show significant deviations from expectation for unlinked genes, but the results could be explained by postulating linked or allelic genes. However since the parent contributing the Rt gene to S63.45, viz. L60.01, contained two Rt genes the possibility that S63.45 also contains two Rt genes cannot

be neglected. The last four results from Table 26, all from the parent L63.05, are heterogeneous. The heterogeneity is not due to the inner crescent:no inner crescent comparison, for which $\chi^2_3 = 1.22$, but is restricted to the red tip:green tip amongst the non-inner crescent comparison, for which $\chi^2_3 = 8.00$, $p < .05$. The results from two of the four crosses are in agreement with the expectations for unlinked genes, while those from the other two show excesses of red tip as would be expected for linked or allelic genes.

It is difficult to draw any positive overall conclusions from the results of Table 26. No deviations from independent segregation can be said to have been definitely established, although some of the results are very difficult to explain on the hypothesis of independence. On the other hand all results are more or less in agreement with the hypothesis of close linkage or allelism given that with di-tetrasomic inheritance variable segregation frequencies may be expected, and independent segregation of allelic genes is also possible.

The results for the simultaneous segregation of the genes Wc(r) and Rt are much more readily interpretable.

Only one plant with the required genotype, viz. L63.11 was backcrossed in this case. Two batches of results were obtained, the first giving

31 wide crescent red tip:28 red tip:8unpigmented.

The red tip:unpigmented ratio is clearly significantly different from a 1:1 ratio ($\chi_1^2 = 11.1$, $p < .001$) but in agreement with a 2:1 ratio ($\chi_1^2 = 2.00$, $0.10 < p < 0.20$). The second batch was grown much later in the season when, as related previously, the wide crescent pigmentation was difficult to score. The overall red tip:green tip ratio may however still be used to give information on the joint segregation of the two genes, although less efficiently than if the three classes were distinguishable. The results, 93 red tip:13 green tip, differ significantly from the 3:1 ratio expected for independent genes ($\chi_1^2 = 7.14$, $p < .01$), but are in agreement with a 5:1 ratio expected for allelic genes and tetrasomic inheritance ($\chi_1^2 = 1.48$, $0.20 < p < 0.30$). While the two sets of results are individually in agreement with the hypothesis of allelism and tetrasomic inheritance, there is a deficiency of unpigmented plants from expectation in both cases. An overall test for the deficiency of unpigmented plants from

tetrasomic expectations in the two cases, using maximum likelihood to obtain a best overall estimate of the frequency of unpigmented, gives $\chi^2_1 = 4.41$, $p < .05$. Thus the results indicate that preferential segregation may be occurring, which in turn casts doubt on the hypothesis of allelism.

The simultaneous segregation of Ic and Wc(r) was studied in the backcross of two full sib plants, L63.34 and S63.39. L63.34 was backcrossed to two different plants and each of the three progenies was grown in two batches, making six batches in all. The six batches are homogeneous for the four classes ($\chi^2_{15} = 11.2$) and give altogether

42 IcWc(r) : 90 Ic+ : 101 +Wc(r) : 35++.

The two genes evidently do not segregate independently, but the results are in agreement with a 1:2:2:1 ratio expected for allelism and tetrasomic inheritance. Once again however a slight suggestion of preferential pairing may introduce complications. The sum of the first and fourth classes accounts for 28.7% of the total, whereas a minimum of 33.3% is possible with random pairing. The difference is however not significant, although nearly so on a one-tail test ($\chi^2_1 = 2.55$, $0.10 > p > 0.05$).

The joint segregations with the markers Oc and Rc also give evidence concerning the linkage relationships of Ic, Wc and Rt. The joint segregation of the genes Wc and Rc was tested in the backcross of L63.38. The results were obtained in two batches which were homogeneous ($\chi^2_3 = 2.40$) and gave totals of

25 WcRc : 74 Wc+ : 49+Rc : 39++.

The test for independent segregation of the two loci gives a highly significant result ($\chi^2_1 = 16.79$, $p < .001$). The results also differ significantly from a 1:2:2:1 ratio ($\chi^2_3 = 8.22$, $p < .05$). Although it is difficult to make a meaningful orthogonal partition of χ^2 in this case, it seems certain that the significance is due principally to a departure of the red crescent segregation from a 1:1 ratio, for which $\chi^2_1 = 8.13$, $p < .01$. The results are in agreement with the hypothesis of close linkage or allelism and tetrasomic inheritance if the penetrance of the Rc gene is assumed to be incomplete.

(The simultaneous classification for some markers such as Rc and Wc poses some difficulty (see Fig. 8 (i)). In order to be certain that the apparent association between the leaf markers is due to linkage rather than to some type

of interaction of expression, the above results could perhaps be compared with results from a cross Rc X Wc. The results from a small cross of this kind are

8 WcRc : 4 Wc+ : 5 +Rc : 3++,

from which no association between the two markers is evident. Unfortunately no large progenies have been grown from crosses of this type.)

Further results suggest that the gene Ic(r) is closely linked to both Rc and Oc. Backcrossing the plant L63.08, i.e. S60.15 x L60.01-39, which contained Ic(r) and Rc, gave

9 Ic(r)Rc : 29 Ic(r)+ : 26 +Rc : 10++.

Similarly the plant S61.35, which is unrelated to L63.08, contained the genes Ic(r) and Oc, and the backcross progeny were classified as

8 Ic(r)Oc : 22 Ic(r) : 30+Oc : 16 ++.

In both cases the segregation of the two genes is not independent ($\chi_1^2 = 15.58$, $\chi_1^2 = 9.31$ respectively), but both have given results in agreement with a 1:2:2:1 ratio ($\chi_3^2 = 2.18$ and $\chi_3^2 = 3.90$ respectively).

The joint hypothesis of allelism of three or more

genes with distinguishable products may be subjected to a much more rigorous test than possible for two genes (Sec. II 4). If in the backcross of a stock containing three markers, progeny are found containing all three markers, then provided the possibility of trisomic gametes may be ruled out the hypothesis of allelism is rejected. A backcross programme has been carried out for the three genes thought from evidence given above to be closely linked or allelic, viz. Ic(r), Rc and Oc. The backcross of S60.15, classified as having all three genes, was repeated in 1963, and gave the results of Table 27. Classification of the

Ic(r) Rc Oc	Ic(r) Rc +	Ic(r) + Oc	Ic(r) + +	+ Rc Oc	+ Rc +	+ + Oc	+ + +	TOTAL
11	14	11	10	10	22	22	1	101

Table 27. Backcross results for S60.15 leaf markers

three markers simultaneously involved considerable difficulty. Some evidence for the correctness of the classification is given by the agreement of the three individual segregations

with 1:1 ratios (Table 28, Cols. 1, 2 and 3).

COMPONENT OF χ^2 DUE TO	Ic	Rc	Oc	IcRc	IcOc	RcOc	IcRcOc	TOTAL
χ_1^2	0.80	1.67	0.49	0.25	1.20	13.55	8.33	26.29

Table 28. Partition of χ^2 for S60.15 results

However the significance of the second order interaction χ^2 is difficult to explain except on the basis of misclassification.

The χ^2's testing for independence of pairs of markers are non significant for both pairs involving Ic(r) but significant for the RcOc pair. Thus there is no evidence from these data that Ic(r) is even linked to the other two genes, which is very surprising in view of the fact that the Ic(r) and Rc genes which gave results in a previous cross suggesting close linkage are descended from the same genes.

An extremely large proportion, viz. 11/101, of the

offspring are classified as having all three markers. However the irregularities in the data as indicated by the significant interaction χ^2 give rise to the suspicion that this proportion is spuriously high. The frequency of +++ plants would be expected to be roughly the same as the frequency of Ic(r)RcOc plants, or slightly higher if double reduction is occurring. However only one +++ plant was found, which is no more than might be expected through double reduction alone. The nature of the markers being classified makes it much more likely that the frequency of Ic(r)RcOc plants is overestimated through misclassification rather than that the frequency of +++ is underestimated.

The classification of the Ic(r)RcOc plants might have been tested through backcrossing, but at the time the importance of these plants was not appreciated since the evidence for the close linkage of Ic(r) and Rc, and Ic(r) and Oc had not yet been obtained. Bulbs have however been obtained from three or four of these plants so that these backcrosses may eventually be made. In addition the backcross of S60.15 has been repeated during the 1964 season. Until the results of these crosses are available it would seem wiser not to speculate on the possible meaning of the high frequency of Ic(r)RcOc plants found.

The only leaf marker* not so far considered in joint segregations is leaf fleck. The joint segregation of this marker with red tip has been studied in three backcrosses of tetraploid plants, together with four backcrosses of pentaploid plants which contain what appear to be the same two markers. The results from the tetraploid crosses are homogeneous ($\chi^2_6 = 2.33$), giving a total of

25 RtLf : 35 Rt+ : 37+Lf : 20++.

The two loci do not appear to be segregating independently ($\chi^2_1 = 5.44$, $p < .05$) but once again have given results in agreement with a 1:2:2:1 ratio, thereby suggesting possible close linkage. The results from the pentaploid are homogeneous ($\chi^2_9 = 10.44$) giving altogether

16 RtLf : 28 Rt+ : 51 +Lf : 33 ++.

There are again suggestions of linkage (χ^2_1 for independence= 4.64, $p < .05$), but the results are complicated by a large deficiency of red tip progeny, which may perhaps be due to some peculiarity of segregation in pentaploids.

Results of the joint segregations of some leaf markers with the style length markers are available (see appendix for summary). A large number of parental genotypes are

possible for each pair of markers, and if di-tetrasomic inheritance is operative no two backcrosses would necessarily have the same expectations. The only possible procedure in analysing these results appears to be to pool all backcross results for each plant, and calculate a one degree of freedom chi-square for independence for each plant and pair of markers. The chi-squares are then added for a test of independence for each pair of markers. Although Yates' correction has been used in calculating chi-square values in individual 2 X 2 contingency tables throughout the thesis, it has not been used in the calculation of chi-squares in the appendix. A small experiment using randomly generated 2 X 2 tables on a computer showed that the sum of a number of independent values of chi-squares not using the correction is reasonably close to, and perhaps slightly less than, its expected value. However the use of Yates' correction grossly reduced the average chi-square, typical values obtained being 0.3 - 0.4 per degree of freedom. The omission of the correction when considering a series of values is in line with the recommendation of Finney et al. (1963).

A rough overall test for linkage of style and leaf

markers appears reasonable in the present case. Summing the chi-square values for all such pairs of markers gives $\chi^2_{57} = 76.82$, $0.05 > p > 0.02$. There is therefore some evidence of linkage between the style and leaf markers. Moreover in only one case where the χ^2_1 value is reasonably large has it been possible to show that the parental genotype suggested by the backcross results is incompatible with the known genotype. Nevertheless in view of the wide range of material involved, and of the non-independence of a few χ^2's in the overall test, it is felt that the significance of χ^2 should be interpreted very cautiously. The finding of a significant association between two specific markers in a specially designed programme such as suggested in Sec. II 3 would be a much more conclusive demonstration of linkage. Such programmes have been designed, using the suspected linkage of the leaf markers to facilitate the selection of bisimplex coupling plants (see p. 49).

c Discussion

With respect to the markers Rt, Ic, Ic(r), Wc and Wc(r), definite indications of close linkage or allelism

have been found for most pairs studied. Thus the segregational results must be interpreted as favouring the first hypothesis given in p. 130, viz. that Ic(r) is a complex of Ic and Rt, and that Wc(r) is a complex of Wc and Rt. These conclusions are of course by no means definite. A much more rigorous test of allelism than from the segregation of pairs of markers is possible through backcrossing stocks containing three markers. It is hoped that the RtIcWc stocks for this test will be available in 1965.

The finding that the markers Rc, Oc and possibly even Lf may also be closely linked to Rt, Ic and Wc is somewhat surprising, and casts a little doubt on the special interpretation of the possible close linkage between Rt, Ic and Wc suggested above. A multiple allelic series for leaf markers may perhaps not be unexpected in view of the postulation of such a series controlling chlorophyll deficient mutants in the leaf of *Trifolium repens* (Brewbaker, 1955). An hypothesis of an allelic series of genes controlling anthocyanin pigments in the leaf of *T. repens* has also been put forward (Carnahan et al., 1955), although no evidence of joint segregations for these markers has been given to support this hypothesis.

iii Calyx Markers

The marker red apex (Fig. 9b) has segregated in a large number of crosses, although no programme has been carried out specifically to determine the mode of segregation at the locus or loci controlling this pigment. In fact visual indications have been found during the past season that there may be more than one marker classified as red apex.

The results from simplex backcrosses of red apex (Table 29) show overall agreement with a 1:1 ratio. In addition eleven backcrosses have given large excesses of red apex plants. Nine of these have given homogeneous results ($\chi^2_8 = 6.59$), and totals of 392 red apex:93 unpigmented which are in agreement with tetrasomic duplex backcross expectations. The remaining two backcrosses have given a total of 129 offspring all having red apex. In neither case, unfortunately, is it known how many genes for red apex the parent possesses. Second back-crosses have been made for some of these progeny which should give some information on this point.

Figure 9. Calyx markers in *O. pes-caprae*

Left to right, top to bottom

(a) Orange apex

(b) Red apex

(c) Red margin and red apex

(d) Half red-brown

(e) Half red-red base

(f) Half red-small red base

(g) Half red

GENE	NO. OF CROSSES	HETERO-GENEITY χ^2	PROBA-BILITY	PIG-MENTED PROGENY	UNPIG-MENTED PROGENY	TOTAL PROGENY	χ^2 FOR 1:1 RATIO	PROBA-BILITY
Ra	24	28.29	.2-.3	399	401	800	0.005	.90-.95
Rm	17	20.01	.2-.3	484	529	1013	2.00	.1-.2
Hrb	33	33.68	.3-.5	834	793	1627	1.03	.3-.5
Hr	8	6.77	.3-.5	117	143	260	2.60	.05-.1
Hr(r) Hr(r⁻)	16	10.26	.8-.9	376	380	756	0.02	.8-.9
Rb	1			32	39	71	0.69	.3-.5

Table 29. Summary of simplex backcrosses for calyx markers

The overall simplex segregations for the marker red margin (shown in Fig. 9c with red apex) are homogeneous and in agreement with a 1:1 ratio. However the portion of these results obtained prior to 1963 show a slight but significant deficiency of red margin (231 red margin:278 unpigmented, $\chi^2_1 = 4.34$, $p < .05$). The deficiency was not unexpected in view of the difficulties experienced in scoring for the marker in the glasshouse used in the early

years of the study.

Nine backcrosses have given a significant excess of red margin in the progeny. Eight of these crosses have given homogeneous results ($\chi^2_7 = 1.78$, $.95 < p < .98$) in agreement with duplex expectations, viz. 268 red margin:61 unpigmented. The remaining plant, M63.25, has given no unpigmented amongst 69 progeny. The genotype of this plant at the Rm locus is not known for certain, but the genotypes of **its** ancestors are known and it may be calculated that this plant has a 94.6% chance of being duplex and only a 5.4% chance of being triplex or quadruplex for the Rm gene. It should be recalled that this plant has also given indications of low duplex segregation frequencies, viz. $\frac{1}{69}$ and $\frac{0}{78}$ respectively, for the genes M and Rc.

Four sepa**ls** containing the marker <u>half red</u> are pictured in Fig. 9. The four are differentiated by the pigmentations of the lower half of the sepal. In Fig. 9(g) the marker half red occurs with a normal green coloured sepal. The gene controlling this pigment is designated as Hr. The pigment depicted in Fig. 9(d) appears to diffuse lightly through the lower half of the sepal, giving

it a brownish appearance. The marker is given the name <u>half red-brown</u>, and the gene the symbol Hrb. The distinction between this marker and the marker half red cannot without great difficulty be made in individual plants. However crosses involving the two markers separately have always been phenotypically distinguishable, although this does not constitute definite proof that the two genes are non-identical since differences of background genotype could possibly account for the differences between crosses. An additional difference however is that the marker half red-brown appears to be associated with a light often poorly manifested petal pigment, pink edge. On the other hand the marker half red, and the remaining two calyx markers with half red, are associated with another petal pigment, red line.

The two half red pigments pictured in 9(e) and 9(f) are both invariably associated with a pigment known as <u>red base</u>, the first having a strong red base and the second a weak red base. The two, which are also difficult to distinguish with certainty in every case, are given the symbols Hr(r) and Hr(r^-) respectively. A red base pigment very similar to that segregating with the gene Hr(r), is

also found separately. The gene controlling this pigment has been designated as Rb. Unfortunately no plants of this genotype were available for inclusion in Fig. 9.

As seen from Table 31 all the simplex segregations for the half red markers and for red base are in agreement with expectation. The results from Hr(r) and Hr(r^-) have been pooled in this table since the two may not always have been correctly distinguished in the past.

Four plants classified as having two Hrb genes have been backcrossed. The results from the four are homogeneous and have given a total of 195 half red brown: 44 unpigmented, which is in agreement with tetrasomic duplex backcross expectations. In all four cases the two genes are known to be identical by descent, and for one of them, S61.23, i.e. S60.16 x L60.01-134, the two parents have been classified as simplex and nulliplex for the Hrb gene respectively. Therefore double reduction must have occurred in the production of S61.23, unless the plant is actually produced by selfing as suggested in Sec. III 3 i d (2). Two possible duplex backcrosses of Hr(r) plants have produced a total of 46 pigmented:10 unpigmented,

while one such backcross of Hr(r^-) has given 11 pigmented: 2 unpigmented.

Several joint segregations have been studied, all of which point to the fact that the genes Hrb, Hr, Hr(r), Hr(r^-) and Rb may form an allelic series. The markers Hrb and Rb have given on backcrossing L63.27.

7 HrbRb : 23 Hrb+ : 25 +Rb : 16 ++.

χ^2_1 testing for independence gives 8.45, $p < .05$. The results are in agreement with the 1:2:2:1 ratio expected for close linkage or allelism with tetrasomic inheritance ($\chi^2_3 = 3.56$).

Two Hr(r)Hr plants, viz. M63.23 and M63.24 have been backcrossed. The results are homogeneous and total 116 half red, red base : 88 half red : 36 unpigmented. The ratio of half red:unpigmented amongst the plants not containing red base (i.e. not containing the gene Hr(r)) differs from the 1:1 expected for independent Hr(r) and Hr genes ($\chi^2_1 = 21.8$, $p < .0001$). However the results are in agreement with a 2:1 ratio expected for allelism and tetrasomic inheritance. ($\chi^2_1 = 1.03$, $.3 < p < .5$).

The simultaneous segregation of Hr(r) and Hrb from the plant L63.05 has given

69 half red, red base : 51 half red : 24 unpigmented

from which similar conclusions to the above may be drawn. Finally the joint segregation of Hr(r^-) and Hrb has given 54 half red:11 unpigmented, where only the two classes have been classified. The results suggest a 5:1 ratio, but do not differ from a 3:1 ratio expected for independent genes ($\chi^2_1 = 2.26$, $.10 < p < .20$).

The above results suggest that an allelic series of genes may be responsible for the production of the five markers mentioned. Unfortunately this hypothesis cannot be tested as rigorously as can the similar hypothesis for the leaf markers, since it is not possible to recognize any three of the calyx markers together.

In joint segregations of calyx pigments and other markers (Appendix, Table 1), only the markers red margin and outer crscent have shown significant association. Further evidence is needed to substantiate this finding, especially as these two markers are perhaps the least reliable of the leaf and calyx markers mentioned.

In addition to the markers described in this, as well as previous sections, a number of calyx and other markers have been investigated (e.g. orange apex, Fig. 9a). Generally the expression of these markers has not been as good as those described in the text, and in consequence not enough is known about their inheritance to make their inclusion worthwhile.

4. SUMMARY

One of the most striking features of the results in O. pes-caprae is the suggestion of di-tetrasomic inheritance in a number of cases, but the finding of ratios in agreement with tetrasomic inheritance in others. A summary has been made of all segregations of all markers classified as coming from probable duplex backcrosses. Forty-five such backcrosses have altogether been ascertained, involving ten different markers and some three thousand classifications. The overall results are highly heterogeneous (χ^2_{44} = 131.9, $p < .001$).

There appears to be some evidence of bi-modality in the distribution of the tetrasomic segregation frequencies. Eight of the forty-five results (Table 30) show significant deficiencies of recessives from one-sixth, thereby suggesting the occurrence of preferential pairing. No overall heterogeneity can be demonstrated in these data (χ^2_7 = 12.70, $0.05 < p < 0.10$) although the results from M63.25 differ significantly from the other five results (χ^2_1 = 5.97, $0.01 < p < 0.02$). The remaining thirty-seven crosses are homogeneous (χ^2_{36} = 31.3, $0.50 < p < 0.70$), and

DUPLEX PARENT	MARKER	NO. OF DOMINANTS	NO. OF RECESSIVES	TOTAL	χ^2_1 FOR 5:1 RATIO
M61.05	M	103	5	108	11.27
M62.30	"	94	1	95	16.68
M63.25	"	68	1	69	11.50
M63.28	"	63	3	66	6.98
M63.25	Rc	78	-	78	15.60
M63.27	Rt	122	6	128	13.23
M63.28	Oc	67	5	72	4.90
M63.25	Rm	69	-	69	13.80
TOTAL		664	21	685	91.24

Table 30. Summary of duplex backcrosses having significant deviations from tetrasomic expectations

the totals, 1887 dominant:404 recessive, are in good agreement withtetrasomic duplex backcross expectations. While the lack of heterogeneity in the two sets of data does not conclusively demonstrate the bimodal nature of the distribution, this finding is strongly suggested. This would be interpreted as meaning that chromosomes show either a considerable degree of preferential pairing or very little

or no preferential pairing.

There is a preponderance of mid backcrosses showing preferential segregation (Table 30), since altogether only ten of the forty-five segregations involve the mid locus. Thus there is some suggestion that certain chromosomes may tend to show preferential pairing more often than others. However one or two factors must be taken into account when considering this possibility. More attention was paid to designing crosses in the style length programme than in programmes for other markers. A number of the duplex mid backcrosses were thus made using two genes identical by descent, whereas this was not often the case with other markers. For reasons which will be discussed more fully in the following sections, such programmes for building up duplex stocks may favour the production of genotypes showing preferential pairing. In addition a number of possible cases of low duplex segregation frequencies amongst the pigment markers could not be included since insufficient was known of their ancestry to rule out the possibility that they were triplex rather than duplex backcrosses. There is therefore little evidence to

contradict the assertion that a similar type of genetical behaviour is being shown by genes throughout the genome.

If O. pes-caprae arose on a single occasion, or equivalently arose on several occasions from the same diploid ancestor(s), then the results from the duplex backcrosses could be interpreted in two ways. First, if O. pes-caprae arose as a hybrid tetraploid, preferential pairing could be the primitive rather than the derived condition. The results would then support the suggestion that there is a tendency for preferential pairing to be lost during the evolution of the species. The second interpretation possible is that preferential pairing has in some way been derived during the evolution of the species, possibly being favoured by selection acting to produce a more stable genetic system. These questions will be considered in Part IV where arguments strongly favouring the first interpretation will be put forward.

IV INHERITANCE IN NATURALLY OCCURRING TETRAPLOIDS

1 INTRODUCTION

Whilst the nature of inheritance in newly synthesized allotetraploids has been well studied (see Sec. I 2 i b), only the experiment of Collins and Longley (1935) has been designed to investigate the manner in which this inheritance is modified in subsequent generations. Such modification is not expected in all tetraploids, but only in those having sufficient homology between the two genomes for at least occasional heterogenetic pairing to occur, i.e. those showing di-tetrasomic inheritance. Clearly the results of Collins and Longley show that in tetraploids of this type the genetical expectations may be altered considerably in subsequent generations. Collins and Longley refrained from speculating at length on the reasons for these alterations in behaviour. However it seems that an elementary discussion of the expected changes may be quite useful, particularly in view of several relevant results from studies carried out since 1935.

Before embarking on such a discussion it may be worthwhile to quote briefly from a discussion by Clausen, Keck and Hiesey (1945, p. 71) of a closely related topic, viz. the balance between the two genomes possessed by a newly synthesized tetraploid.

> ". . . In general the balance (between specifically distinct genomes) is attained through the interplay between two groups of antagonistic processes. One group tends to destroy the identity of duplicated chromosomes by (1) interchange of genes or chromosomes between parental genomes, and (2) chromosomal rearrangements. Either or both may take place either before or after doubling of the chromosomes. The other group tends to preserve the identity of the genomes by (1) preferential pairing (due to closer homology) between duplicate chromosomes of one parent, as compared with pairing between them and their nearest homologues from the other parent, and (2) elimination of unsuccessful combinations resulting from occasional interchange between unlike genomes."

The attention of Clausen et al is given mainly to the way in which the original genomes of the tetraploid are modified, and in consequence what relationship the evolved tetraploid bears to its diploid ancestors. In the present chapter interest is directed more towards the course of events occurring in the tetraploid and less towards the relationships with the diploid ancestors. The aim in particular is to give a more detailed description of the evolution of genetical behaviour of the tetraploid,

especially in the initial generations after its synthesis. Some changes from the approach adopted by Clausen et al seem desirable.

The quantity of prime interest in the present discussion is the intensity of preferential pairing, since to a large extent this determines the genetical behaviour of an allotetraploid. Some processes which tend to decrease the intensity of preferential pairing, i.e. to modify the genetical behaviour towards tetrasomic inheritance, are considered in Section IV 2. These processes are not dependent on selection, and are similar to the first group of processes of Clausen et al. Those processes tending to increase the intensity of preferential pairing are considered in Section IV 3, the conclusions being similar to those of Riley (1960). Finally an attempt is made to interpret further the genetical results from _O. pes-caprae_.

2 FORCES TENDING TO REDUCE PREFERENTIAL PAIRING

i Classification

The possibility that inter-genomal crossing-over will tend to reduce preferential pairing has been recognized by several authors. The possible effect of segregation of chromosomal regions on preferential pairing however does not appear to have been similarly appreciated. To illustrate such segregation it may be useful to consider one chromosome set, AABB, of a newly synthesized tetraploid, in which it is assumed that the chromosomes are inherited as a whole and not broken up by recombination. Since we are interested in this case only in the pairing propoerties of the chromosome, it is really only necessary to assume that these properties are determined by a single region of the chromosome which cannot be broken up by recombination. If preferential pairing is occurring the large majority of gametes will be of the constitution AB. However if preferential pairing is not complete, then provided that there is no mechanism which ensures that disjunction from quadrivalents at the first division of meiosis is always equational, some AA and BB gametes are expected. Then regardless of the mating system, a number

of tetraploids having the constitution AAAA, AAAB, ABBB and BBBB will be produced. Preferential pairing is not expected in any of these genotypes. This is clearly so for the AAAA and BBBB genotypes although not so obvious for AAAB and ABBB. For AAAB for instance, at any point pairing amongst the three A strands takes place at random, and any one of the three A strands is equally likely to be left to pair with the B strand.

The simple model of chromosome behaviour chosen leads eventually in a random mating population to a stable situation in which a proportion of individuals show preferential segregations for genes on the particular chromosome set, and a proportion show genetical behaviour indistinguishable from tetrasomic inheritance. The proportion of individuals showing preferential pairing would be dependent on the strength of the preferential pairing. However the assumption on which these results are based, viz. indivisible pairing properties, is clearly an oversimplification. The only mechanism of pairing compatible with this assumption is one where pairing is initiated at a single site on the chromosome, and then proceeds serially along the entire pairing length. This type of

pairing cannot account for the formation of multivalents and therefore cannot be considered as a likely mechanism.

The possibility of breakdown of pairing properties through crossing-over is clearly likely to be very important. It is necessary to have some model of chromosome pairing on which to base arguments of the likely affect of such recombination. A model will be introduced in the following section, where its applicability will be considered in some detail.

Before introducing the model, a third factor relevant to the process of preferential pairing must be mentioned. This is the possibility that the pairing properties of a particular chromosome set may be influenced by factors other than the structure of the set, viz. the background genotype and environment. The possible importance of such factors was indicated by the discovery (Riley and Chapman, 1958; Sears and Okamota, 1958) that monosomic plants in wheat lacking chromosome V showed hitherto undetected associations between homeologous chromosomes (Riley & Kempanna, 1963), throughout the genome. This discovery indicated that the structure of chromosomes might be less important in determining their pairing properties than

previously realized (Darlington, 1937; Stebbins, 1950). It does not necessarily follow from this discovery that in newly synthesized allotetraploids the structure of individual chromosomes is not important in determining their pairing properties. Indeed the opposite is suggested by several results (Swaminathan, 1956; Gerstel, 1960, 1963; see Section I 2 i b). A simpler interpretation is that some aspect of the pairing process is potentially subject to overall control, and that selection has acted in this case to produce a controlling mechanism to stabilize the genetical behaviour (Riley, 1960).

ii A model for chromosome pairing

A systematic study of metaphase configurations made by Morrison and Rajhathy (1960a, b) in a number of tetraploid species has given results suggesting that the process of pairing may be quite regular over a large range of species. Approximately two-thirds of the chromosomes were associated in quadrivalents in all species studied. The distribution of the number of quadrivalents per cell was stated to follow the binomial distribution, thereby indicating that the probability of forming a quadrivalent

was roughly the same for each chromosome set within the different species.

John and Henderson (1962) have shown that a simple model of pairing in which pairing is initiated independently at two sites on the chromosome will lead to a quadrivalent frequency of two-thirds. However these authors have severely criticized some aspects of the work of Morrison and Rajhathy, and expressed doubts as to the generality of such a model. A detailed examination of these criticisms will not be given here. It is sufficient to mention that the criticisms seem inappropriate for the plant material studied by Morrison and Rajhathy (which is the type of material of interest in the present discussion).

While the overall results of Morrison and Rajhathy are roughly in agreement with the model of John and Henderson, in one or two cases the frequency of quadrivalents is significantly lower than two-thirds and in two or three cases significantly higher. The low quadrivalent frequencies may readily be attributed to the failure of strands to initiate pairing at one site in a proportion of

cases, or to the failure of chiasma forming to maintain the pairing. The high quadrivalent frequencies are a little more difficult to explain. For instance the frequency in maize is 0.78 which is of the same order as the estimates of Gilles and Randolph (1951) and Venkateswarlu (1950). Two partner exchanges per quadrivalent have also been found by the latter author in a number of cases. It seems necessary therefore to invoke the existence of an extra initiation site in some cases. Clearly a completely regular process of quadrivalent formation cannot be inferred from the overall data. Nevertheless the fact that many chromosomes in a large number of species apparently form roughly two-thirds quadrivalents can scarcely be fortuitous.

As well as testing the model on cytological data in autotetraploids, some predictions on the nature of preferential pairing in allotetraploids may be made. In particular the expected relationship between cytological and genetical behaviour may be derived.

A diagrammatic representation of one set of the chromosome complement of a newly synthesized tetraploid is given in Fig. 10. The two initiation sites may be

assumed to occur at any point of the chromosome in the

A M X
____

A M X
____

B m Y
____

B m Y
____

First Init. Site   Second Init. Site

Figure 10. Model chromosome set with two pairing initiation sites

following argument provided pairing is initiated independently at the two sites. The gene configuration at the M locus does not affect the pairing relationships. The location of the locus is also not important in the following argument.

Two types of pairing may be initiated at the first

site, viz. (AA)(BB), with probability $1 - \gamma_1$, and (AB)(AB) with probability γ_1. The parameter γ_2 similarly specified the pairing at the second site. γ_1 and γ_2 may take any value from zero, as for very distantly related ancestors, to two-thirds, expected for closely related ancestors. It is assumed that sufficient chiasmata are formed so that quadrivalents do not fall apart. Evidence that this assumption may not be unrealistic is given by the findings mentioned previously of Riley et al. in monosomic wheat. These findings have been interpreted by Riley (1960) as showing that the inability of hemeologues to pair rather than to form chiasmata is the limiting factor in determining their genetical behaviour. The same evidence also shows that the values of γ_1 and γ_2 in any particular case are determined not only by the structure of the particular chromosomes but also by the background genotype and environment.

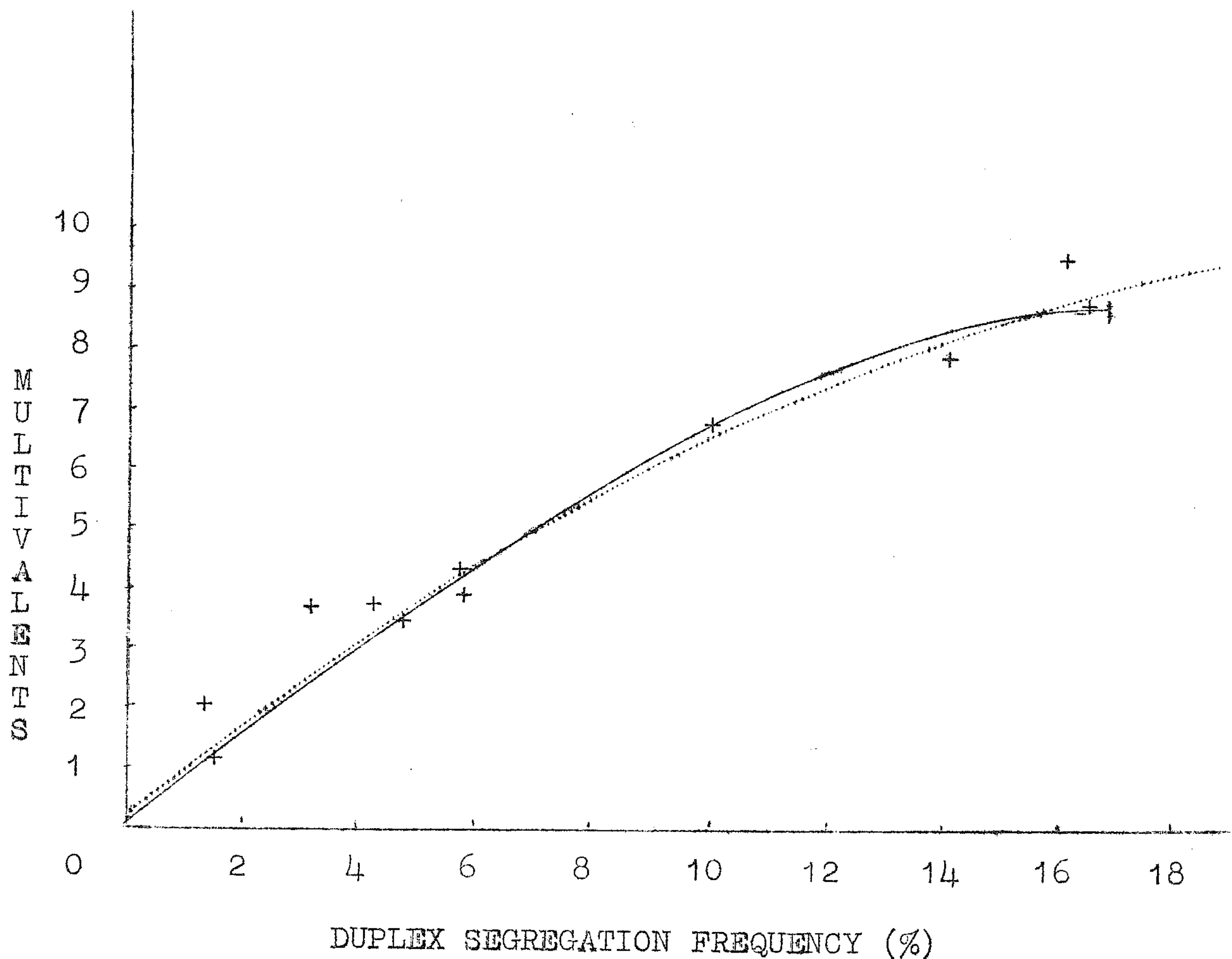
In a proportion $(1 - \gamma_1)(1 - \gamma_2)$ of cases bivalents with like pairing, i.e. homogenetic pairing, will be formed. All gametes arising from this configuration will have the genotype Mm. In a proportion $\frac{1}{2}\gamma_1\gamma_2$ of cases

bivalents with unlike, i.e. heterogenetic, pairing will be formed. One quarter of the gametes arising from such pairing would be mm. The remaining $\gamma_1 + \gamma_2 - \frac{3}{2}\gamma_1\gamma_2$ of cases would have quadrivalent pairing. Approximately one-sixth mm gametes may be expected in this case assuming random chromosome disjunction.

The total frequency of quadrivalents under the model is therefore $\gamma_1 + \gamma_2 - \frac{3}{2}\gamma_1\gamma_2$, while the frequency of recessives expected is $\frac{1}{6}\gamma_1 + \frac{1}{6}\gamma_2 - \frac{1}{8}\gamma_1\gamma_2$. These values define a family of closely related curves relating the frequency of quadrivalent formation and the duplex segregation frequency. Putting $\gamma_1 = \gamma_2 = \gamma$ which may be approximately expected in practice, reduces these to a single curve.

The data on segregation and quadrivalent formation in Nicotiana allotetraploids (see Phillips, 1964 for summary) may be used to test these expectations. All except one point are seen to lie close to the curve. derived above (Fig. 11), while the range of values is almost exactly that predicted by the model.

Phillips has proposed that pairing between unlike bivalents will tend to be incomplete, and that unpaired



—— Relation given in text

.... Regression line fitted by Phillips (1964)

Figure 11. Predicted relationship between duplex segregation frequency and multivalent frequency, fitted to data of Phillips (1964)

regions will then pair to form quadrivalents. This proposal is in essence not very different from that considered in the present section. Phillips has argued that this type of pairing will tend to produce a curvolinear relationship between the two frequencies, and he has fitted a quadratic regression line to give the line of best fit. This is seen (Fig. 11) to be almost coincident with the line given by the two-site initiation model. Clearly other arguments similar to that of the two-site initiation model could account for the relationship observed. Nevertheless the close fit given by the model constitutes further evidence for its acceptability.

A simple scheme may be put forward which could account for all the results considered above. Two definite points on the chromosome such as the telomeres are assumed to be attached to specific sites on the nuclear membrane, or perhaps the nucleolus, the two homologues of a diploid, or four chromosomes of a set in the tetraploid, being attached in close proximity to each other at both sites. This would imply the existence of a specific attraction between the chromosomes and a region of the nuclear membrane, rather than the long range attraction between homologous chromosomes which has often been

postulated. It is not necessary to assume that pairing is initiated at the actual site of attachment. A more likely hypothesis is that the attachment assures the close juxtaposition of distal regions, particularly when contraction occurs, and pairing is then usually initiated in these regions. Thus it is not necessary to assume a special mechanism for pairing initiation. The same type of local attraction which governs the 'zipping up' of homologous chromosomes once pairing is initiated could also account for the initiation. Under this scheme it is seen that a third point of initiation is possible even if the chromosomes are not attached at a third site. Pairing in the median regions could be considered as a race between the zipping up process initiated in the distal regions and the possibility of chance meeting of homologous regions initiating a new pairing. In other cases, such as pairing in an inversion-carrying diploid, pairing might be initiated normally but the zipping up process interrupted, leaving unpaired regions which could subsequently pair. The pairing in the inversion could be initiated near the centre where homologous regions are closest together.

Some attachment of the kind postulated could well be part of the mechanism whereby chromosome movement in the early stages of meiosis is controlled. The attachment could take place at a very early stage, perhaps immediately following the preceding mitotic division, when the chromosomes are still contracted and organized to some extent. This might help to explain the relative infrequency with which interlocking bivalents and quadrivalents are found. Alternatively it seems feasible that the chromosomes could be attached to some part of the nuclear membrane throughout the life cycle, perhaps at times only through fibres. It is however difficult to visualize a course of events during and following fertilization which is compatible with such attachment.

No cytological observations of the early stages of meiosis appear to be sufficiently accurate to enable the idea of attachment to the nuclear membrane to be decisively accepted or rejected. However observations such as those of Darlington (1935) do not appear to be inconsistent with this scheme. Darlington (1958, p. 15) has written "The chromosomes usually begin to pair near

the ends, but sometimes near their centromeres". Upcott (1939) has commented on the tendency for chromosome ends in Tulipa to be located near to the outside of the nucleus. The formation of 'bouquet' chromosomes (see Lewis and John, 1963; p. 14) in some insect species may also be interpreted as showing telomere attachment (see also Janssens, 1924).

The results from experiments of Doyle (1963) in inversion-carrying tetraploid maize are seen to be compatible with the scheme of telomere attachment. An inversion introduced by this author close to one end of the chromosome resulted in a reduction in the duplex segregation frequency. Telomere attachment in this case would tend to align non-homologous portions of the inversion over a long distance, and thus encourage preferential pairing at one end. Together with random pairing at the other end, and possibly some pairing initiation in the median regions, this would be sufficient to explain the observed deficiency of recessive gametes from tetrasomic expectations. A more critical test of the model put forward could perhaps be made with conbinations of suitably chosen inversions particularly in the distal regions.

While there appear to be few exceptions to the rule that synthesized autotetraploids show a large amount of multivalent formation, _Lotus corniculatus_ appears to be an example of a naturally occurring tetraploid showing tetrasomic behaviour but having a low frequency of multivalents. This example is not inconsistent with the two initiation site model. Natural selection in a tetraploid might tend to favour the gradual loss of one of the initiation sites in order to ensure almost exclusive bivalent formation to stabilize the chromosome behaviour. In fact the decline in the frequency of quadrivalents over a period of some generations found in autotetraploid _Zea mays_ and _Brassica campestris_ (Gilles and Randolph, 1951; Swaminathan and Sulbha, 1959) may illustrate the action of this process. The latter authors have interpreted the results as showing a decline in the frequency of heterogenetic pairing. This explanation will be considered further in Sec. IV 4.

iii _Loss of preferential pairing through recombination and segregation_

It was shown in Sec. IV 1 that if pairing was initiated at a single site on the chromosome, segregation

of preferentially pairing sites could lead to new chromosomal types. A similar result may be shown for the two-initiation site model, with the additional complication that the two preferentially pairing sites may be recombined through crossing-over. It is argued below that the combined effect of recombination and segregation of such preferentially pairing sites may be to reduce severely the incidence of preferential pairing within comparatively few generations following the synthesis of a new allotetraploid.

The same chromosome set as in Fig. 10 is again assumed. The initiation sites are assumed to be in the terminal regions and for the moment will be regarded as single points. If chiasmata are readily formed even between non-homologous strands, which as mentioned previously is suggested by Riley (1960), then the frequency of quadrivalent formation will be of the order of $\gamma_1 + \gamma_2 - \frac{3}{2}\gamma_1\gamma_2$. A typical quadrivalent configuration expected is depicted in Fig. 12, in which the minimum number of chiasmata necessary to hold the quadrivalent together is assumed. It may be seen that

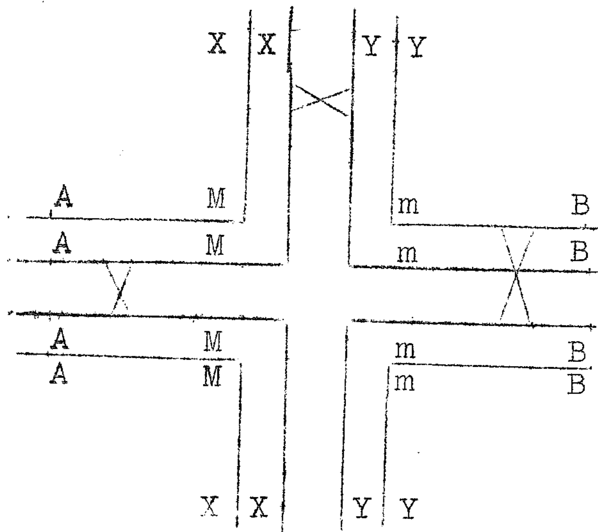


Figure 12. Typical quadrivalent configuration

disjunction from such a configuration may lead to segregation of entire chromosomes, and thus in the following generations to chromosome sets such as AX/AX/AX/BY. Preferential pairing is not expected in this genotype. However the genotype at the M locus in such a case would be MMMm, so that there would be no opportunity for estimating the duplex segregation frequency in such a genotype.

In additions to this type of segregation, if, as assumed in Fig. 12, chiasmata are not always localized distally to the initiation sites, then new chromosomal types such as depicted in Fig. 13 are expected. By the

A	M	X
A	M	X
B	m	X
B	m	Y

Figure 13. Model chromosome set after recombination

same argument as given in Sec. IV 1, it is seen that no preferential pairing is expected at the second initiation site in this genotype. Otherwise making the same assumptions as for Fig. 10, the expected frequency of recessive gametes at the M locus can be shown to be $\frac{1}{9} + \frac{1}{12}\gamma_1$. The occurrence of double reduction could raise this frequency still further. Even if γ_1 is low this frequency would not be very different from that expected with tetrasomic inheritance.

Although thirty-five chromosomal types are possible with two initiation sites, of these only the type AY/AY/BX/BX will show preferential pairing of comparable

intensity to the original AX/AX/BY/BY type. Five other types show comparable pairing behaviour to the set depicted in Fig. 13, viz. AX/AY/BX/BX, AY/AY/BY/BX, AX/AY/BY/BY, AX/AX/BX/BX and AY/AY/BY/BY, all giving $\frac{1}{9} + \frac{1}{12}\gamma_1$ recessive segregants. Similarly six types of which the set AX/AX/AY/AY is representative give $\frac{1}{9} + \frac{1}{12}\gamma_2$ recessive segregants. Of the remaining twenty-one types, all but one have lost the preferential pairing at both sites, giving comparable behaviour to that expected in an autotetraploid. The remaining type, having the configuration AX/AY/BX/BY, is expected to show preferential pairing between different chromosome pairs at the two sites, leading to a very high frequency of quadrivalent formation and a duplex segregation frequency of approximately $\frac{1}{6}$.

As with the case of the single initiation point model, with random mating and no selection an equilibrium distribution of pairing types will eventually be reached. The problem of determining the equilibrium frequencies is somewhat complex since there are thirty-five possible pairing types producing altogether ten gametic types. Owing to certain symmetries of the situation however the number of parameters needed to specify the gametic

frequencies at equilibrium may be reduced to two. The equilibrium frequencies may then be obtained from the relevant solution of a quartic equation. The results show that the equilibrium frequencies of the types AX/AX/BY/BY and AY/AY/BX/BX are quite low regardless of the values of γ_1 and γ_2. The maximum total frequency of these types is only 6.34% which occurs as γ_1 and γ_2 both approach zero. The minimum frequency is 4.69% when γ_1 and γ_2 approach two-thirds. This value, which in fractions is $\frac{3}{64}$, may also be obtained by a simple combinatorial argument. Since there is no preferential pairing at all in this case, the frequencies of the combinations AABB and XXYY will be $\frac{3}{8}$, i.e. $C^4_2(\frac{1}{2})^4$. Thus the frequency of chromosome types having both AABB and XXYY will be $\frac{9}{64}$. Only one-third of these will however be of the required type, the other two-thirds being of the type AX/AY/BX/BY. It should be noted that the above values are derived by a purely deterministic argument which assumes that selection or chance fluctuation do not change the frequencies of A, B, X and Y from 50%. However it is easily shown that any change of these frequencies from 50% will reduce the frequency of the preferentially pairing types, so the

values given above may be considered as upper limits for the frequency of preferential pairing.

The approach to the equilibrium situation may be quite rapid. In early generations, when there is a high frequency of AX/BY gametes, most gametes other than this type will combine with this type of gamete, leading to a chromosomal type in which preferential pairing is reduced or absent. The frequency of AX/BY gametes expected from this chromosomal type may be considerably less than one-half. Thus even in the almost complete absence of heterogenetic pairing in the original genotype, the frequency of AX/BY gametes may fall quite rapidly once some segregation and recombination has occurred.

The model considered so far could be inaccurate in two ways. The first and most important is that as argued in the previous section, pairing is probably not initiated at a fixed point. The arguments put forward indicate that pairing may tend to be initiated in the distal regions, but the possibility should be taken into account that initiation might occur over a much longer segment of the chromosome. Some idea of the effect of further subdivisions of the preferentially pairing sites may be obtained by considering a simple model in which the

first site may be subdivided by recombination into two regions. Then a similar argument to that given above shows that eventually only five or six per cent of the genotypes will show preferential pairing at the first site equal in intensity to γ_1. Further subdivision of the site will clearly lower the intensity of preferential pairing further.

The second way in which the model could be inaccurate is that pairing could be initiated at more or less than two sites. If pairing is initiated at more than two sites the opportunities for recombination and segregation would be increased. Only if pairing is regularly initiated at less than two sites, which does not appear to occur frequently in raw allotetraploids, would the ultimate incidence of preferential pairing be higher than predicted by the model. Even in this case however, recombination in the region in which pairing is normally initiated would be sufficient to lower the incidence of preferential pairing quite rapidly.

The results of Collins and Longley (1935) which are reproduced in Table 31, are the only ones available

Genetic group and percentage of waxy pollen (class value)	F_3 plants of indicated parentage									F_2 plants
	Progenies (upper) and percentages of waxy pollen (lower) of *Wx Wx wx wx*×self				Progenies (upper) and percentages of waxy pollen (lower) of *Wx wx wx wx*×self		Progenies (upper) and percentages of waxy pollen (lower) of *Wx wx wx wx* × *Wx Wx wx wx*			
	C_2 7.9	C_6 11.2	C_7 4.0	C_9 1.5	C_1 50.9	C_3 51.3	C_4 51.3×7.9	C_8 4.0×50.9	C_{10} 1.5×50.9	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Wx Wx Wx Wx Wx:										
0	0	0	0	0	0	0	0	0	0	1
Wx Wx Wx wx:										
0.1–0.9	3	1	4	2	0	0	1	0	2	2
Wx Wx wx wx:										
1.0–1.9	5	2	8	1	----	----	----	----	1	4
2.0–2.9	6	3	14	7	----	----	----	1	2	8
3.0–3.9	15	5	15	9	----	----	----	1	3	7
4.0–4.9	9	5	12	13	3	----	----	4	1	8
5.0–5.9	0	6	7	6	1	4	3	2	2	6
6.0–6.9	1	3	4	4	2	2	3	4	2	2
7.0–7.9	2	6	1	0	2	1	1	2	0	5
8.0–8.9	1	5	1	1	1	3	4	----	4	2
9.0–9.9	4	3	1	2	1	0	3	----	3	3
10.0–10.9	3	4	0	----	0	1	0	----	1	1
11.0–11.9	----	4	1	----	0	1	0	----	1	1
12.0–12.9	----	0	----	----	1	0	4	1	----	----
13.0–13.9	----	0	----	----	1	0	3	----	----	----
14.0–14.9	----	1	----	----	----	0	----	----	----	----
15.0–15.9	----	1	----	----	----	1	----	----	----	----
16.0–16.9	----	1	----	----	----	----	----	----	----	1
Plants..number	46	49	64	43	12	13	21	15	20	48
Mean waxy pollen....percent	4.7	7.2	4.0	4.5	7.6	8.1	9.4	5.9	6.5	5.2
Observed σ	2.7	3.5	2.0	1.8	3.0	2.8	2.9	2.3	3.0	3.1
σ_{50}	11.1	13.7	10.3	10.8	14.3	14.8	16.1	12.5	13.2	11.7
Expected σ	.95	1.16	.88	.93	1.19	1.22	1.31	1.06	1.10	.99
Wx wx wx wx:										
44.0	----	----	----	----	----	----	----	1	----	----
45.0	----	----	----	----	----	----	----	0	1	----
46.0	----	----	----	----	----	----	----	0	1	----
47.0	----	----	----	----	----	4	----	2	1	----
48.0	----	----	----	----	----	4	2	0	0	----
49.0	----	----	1	2	3	8	4	3	1	----
50.0	----	4	0	0	9	7	5	1	2	2
51.0	----	0	0	3	3	6	2	2	1	1
52.0	----	2	1	1	2	5	5	3	----	1
53.0	----	0	0	1	3	7	6	1	----	1
54.0	----	0	1	----	2	1	1	1	----	----
55.0	----	1	1	----	2	1	1	1	----	----
56.0	----	0	----	----	2	----	----	----	----	----
57.0	----	1	----	----	----	----	1	----	----	----
58.0	----	----	----	----	----	----	----	----	----	----
59.0	----	----	----	----	----	----	----	----	----	----
60.0	----	----	----	----	----	----	1	----	----	----
Plants..number	0	8	4	7	26	43	28	15	7	5
Mean waxy pollen....percent	----	52.5	53.0	51.4	52.2	50.9	52.2	5.08	48.8	51.7
Observed σ	----	2.7	2.6	1.5	2.2	2.1	2.7	2.9	2.3	1 3
Expected σ	----	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2

F_1 — 4.6 ± 0.94%

Table 31. Distribution of percentage of waxy pollen in F_1, F_2 and F_3 plants. (From Collins and Longley, 1935)

against which the predictions made in the present section may be tested directly. Independently of the effects of recombination and segregations it is first seen from these results that the background genotype and environment may exert a considerable influence on the segregation frequencies. The spread of values in the F_2 for instance (Column 10) is much greater than expected by chance, and must therefore be attributed either to change of pairing properties by recombination or to variation in the background genotype and environment. Breakdown of pairing properties due to recombination could however be invoked in only a small percentage of cases. Neither could such recombination easily account for a significantly increased frequency of preferential pairing which is found in several cases. In addition the genotype giving the second highest frequency of recessives has evidently been used as the parent for the C6 line of F_3 plants. Many plants in this line show comparable behaviour to the original allotetraploid, so that it seems unlikely that any appreciable breakdown of pairing properties has occurred in the plant C6. It is unfortunate that the single F_2 plant giving a

segregation frequency close to one-sixth was not also used as an F_3 parent, since some crossing-over is likely to have occurred in the production of this genotype.

While it is difficult to make a comprehensive statement regarding the agreement of Collins and Longleys' results with the model put forward, it seems that in a number of cases the breakdown of preferential pairing has not been as marked as predicted. In particular, strict application of the model would suggest that a high frequency, probably over one-half, of the plants in columns 5-9 would have a frequency of tetrasomic segregation of 12-16%. In fact only a small proportion show such a high frequency. The large background variation cannot account for this discrepancy. The most likely explanation appears to be that crossing-over has taken place close to the terminal region, leaving large segments of the chromosome in which pairing may be initiated, still linked to the Wx locus. Alternatively it is possible that chiasmata have been formed between heterogenetically paired strands in only a portion of cases. Despite these objections there is some evidence for a higher mean duplex segregation frequency of plants in

columns 5-9, so that some recombination and segregation of the type postulated is probably occurring.

It is unfortunate that, as explained in conjunction with Fig. 12, the effect of segregation of preferentially pairing sites cannot be detected except in the presence of recombination. Since all Wx genes come from the teosinte parent and all wx from the maize parent, those genotypes in which preferential pairing can be detected, viz. WxWxwxwx genotypes, are selected as having at least a portion of this chromosome set to which the two ancestors have contributed equally. It seems likely that if it were possible to investigate preferential pairing in those genotypes having 0, 1, 3 or 4 Wx genes such preferential pairing would be found to be considerably reduced. From this point of view Collins and Longleys' results tend to underestimate the amount by which preferential pairing has been reduced.

3. FORCES TENDING TO INCREASE PREFERENTIAL PAIRING

No account has so far been taken of the possible effect of selection in influencing the evolution of the tetraploid. Selection against multivalent-forming tetraploids could be of two kinds. It could first be directed against segregant products arising from multivalent configurations, due to favourable gene combinations being upset. Secondly it could be directed against the numerically unbalanced products formed from multivalents. Both these kinds of selection would presumably be most severe in allopolyploids formed from distantly related ancestors.

The net effect of either of these types of selection would be to favour a condition where all pairing is homogenetic. There are two views on the mechanism whereby such a conditions might be achieved. The earlier view, summarized by Stebbins (1950, p. 325) holds that

> ". . . selection would favour the further differentiation of these chromosomes by means of mutation and further chromosomal rearrangements and the elimination of multivalent formation".

A more recent discussion, based principally on the discovery of the control of pairing exercised by chromosome V in wheat, has been given by Riley (1960).

> "The diploidization of polyploids may also be achieved by the selection of genetic variants with modified chromosome pairing. The situation in T. aestivum is an example of this, with selection for fertility and genetical stability, in the original tetraploid, probably leading to the fixation of the mutant condition. Such a process, with a simply inherited control over the patterns of conjugation, would be rapid and precise, and the newly arisen polyploid would be exposed to the minimum period of reproductive inefficiency."

Evidence favouring a view similar to that proposed by Riley comes from Collins and Longleys' results. As mentioned previously the results indicate that considerable variation is possible in the pairing behaviour of structurally similar chromosomes. A high correlation between F_2 and F_3 genetical behaviour has been shown suggesting that some at least of this variation may be heritable. Recombination and segregation could also contribute to the size of this correlation, so that no estimate of the heritability can validly be made. However the overall results suggest that the strength of preferential pairing for any particular chromosome set is a quantitative

character under polygenic and environmental control, rather than under simple genetical control as suggested by Riley. This view is not incompatible with the finding of the importance of chromosome V in controlling meiotic behaviour, since many polygenically controlled quantitative characters are known where segregating major genes nevertheless play an important part. In addition the regularity in pairing behaviour could probably be acquired more easily with polygenic control than with simple genetical control, since some variation would always be present with polygenic control, and selection for regular homogenetic pairing could be effective in any tetraploid from its inception.

It should be noted that the results of Collins and Longley do not show whether all chromosome sets are affected in the same way by the genetical control of pairing. Some evidence on this point may be provided by the results of a programme undertaken in <u>O. pes-caprae</u> which is outlined in Sec. IV 5.

4. CONCLUSIONS

The overall conclusions from the arguments of the preceding sections do not differ essentially from those of Clausen, Keck and Hiesey (1945) mentioned on p. 160. Two types of naturally occurring tetraploids of hybrid origin are postulated. The first includes those in which the frequency of preferential pairing in the raw tetraploid was sufficiently low, and the selection against recombinant products sufficiently high, to allow complete homogenetic pairing to be developed before preferential pairing could be lost by the processes outlined in Sec. IV 2. The second includes those in which all preferential pairing has been lost, and inheritance has become tetrasomic.

Clausen et al have claimed that those plants in which the original genomes are not preserved intact would rarely give rise to products of evolutionary importance. While as a generalization this may be reasonable, nevertheless one or two possible exceptions should be pointed out. In particular *Medicago sativa* and *Solanum tuberosum* may have arisen from raw allotetraploids through processes

such as those outlined in Sec. IV 2, although the actual origin in both cases is still somewhat obscure.

It seems quite likely that, other things being equal, a tetraploid of hybrid origin which has evolved disomic inheritance will be better adapted than one which has evolved tetrasomic inheritance. Partly this is due to the advantages of what may be termed 'fixed heterozygosity' possessed by the diploidized species, and partly to the more regular meiotic behaviour of this type of species. In the species showing tetrasomic inheritance on the other hand any large chromosomal alterations between the two genomes will be broken down, probably leading to inviability. Even if such gross alterations do not occur, portions of the genome may persist for some time where heterogenetic pairing is not possible. Although some such segments could perhaps be tolerated, and the genetical behaviour of genes on such segments might not differ from other genes on the same chromosome (see Sec. I 3), nevertheless some meiotic irregularities might be expected if a large number of these segments occur. In addition quadrivalent formation, even with

complete pairing, may lead to numerical non-disjunction and genotypes of low selective value.

Despite the probable selective advantage of complete diploidization, the loss of preferential pairing through recombination and segregation may constitute a barrier which many tetraploids cannot overcome, owing to their having too high a frequency of heterogenetic pairing as raw tetraploids. Some may however evolve tetrasomic behaviour and a lower adaptive value than they might otherwise have had. Within this type of tetraploid selection for meiotic regularity may still be effective, through favouring the production of certain types of quadrivalent orientation or favouring a reduction in quadrivalent frequency (McCollum, 1958). *Lotus corniculatus* may represent an example where the selection for meiotic regularity has led to an almost complete absence of quadrivalents.

The exact point of division between the two classes of tetraploids described is a matter of some conjecture. However it is felt that the strength of the forces tending to break down preferential pairing may at times have been

underestimated. While a stabilizing effect has been established in wheat and other polyploids (Riley, 1960), none of the cases cited has been shown to have a high frequency of heterogenetic pairing in the putative raw polyploid.

The extreme viewpoint in arguments of the point of division between the two classes of tetraploids is taken by those writers who have postulated gradual diploidization of autotetraploids. There appears to be no authenticated case of a naturally occurring species of this kind. Swaminathan and Sulbha (1959) have claimed that the decline in quadrivalent frequency in selected autotetraploids in *Zea mays* and *Brassica campestris* shows a trend towards diploidization. However all of the arguments of Sec. IV 2 are opposed to an explanation of this kind, while as mentioned previously the decline can be explained without postulating any associated genetical changes towards disomic inheritance.

5. FURTHER DISCUSSION OF O. PES-CAPRAE RESULTS

The results in O. pes-caprae as summarized in Sec. III may readily be explained on the basis of the processes outlined in Sec. IV 2. This species could have arisen as an allotetraploid with a restricted amount of heterogenetic pairing, and be in the process of evolving tetrasomic behaviour. Furthermore the bi-modal nature of the tetrasomic segregation frequencies suggested in Sec. III is expected under the arguments put forward for the manner in which preferential pairing is lost.

If the arguments put forward in Sec. IV 2 are accurate it would appear that the inheritance in O. pes-caprae has not yet reached a stable state. Rather than supposing that the evolution is a very slow process, it seems simpler to postulate that evolution is retarded in this species because sexual reproduction occurs only rarely. As previously mentioned the rarity of sexual reproduction is suggested both by the tendency to clonal structure in the field, and by the relative difficulties of raising bulb and seed plants in the

laboratory. No estimates are available of the relative frequencies of seed and bulb plants in the field. A small experiment was started to give information on this point, but all plants were unfortunately lost. In any case it seems doubtful whether one experiment carried out in a single environment would be sufficient to enable useful conclusions to be drawn. Questions such as the relative ease of dispersal of bulbs and seed may also be important.

A further result of interest in this context comes from the plant M63.25, where possibly three chromosome sets show strong preferential pairing. This result may be interpreted as showing that this plant was produced from the original tetraploid in comparatively few sexual generations. The results from this plant suggest that the duplex segregation frequency in the original tetraploid was quite low, of the order of one per cent or less. It would be very useful to check this expectation if putative ancestors of O. pes-caprae could be located. As mentioned previously O. compressa may be one ancestor. However none of the other Oxalis species found in South Australia seems on morphological grounds likely to

be an ancestor, and the few crosses attempted to *O. compressa* have not been successful. Attempts have also been made to double the chromosome complement of *O. compressa* using colchicine, but so far no tetraploid products have been definitely identified. The problem of locating the ancestors of *O. pes-caprae* could probably be more advantageously approached in South Africa, where this species almost certainly originated (Michael, 1964).

Other explanations than the one considered so far could be put forward to explain the variation in duplex segregation frequencies. For instance it might be postulated that *O. pes-caprae* is an autotetraploid in the process of evolving diploid genetical behaviour. This explanation seems very unlikely on the basis of the argument put forward in Sec. IV 2, but cannot be excluded experimentally except by identifying the putative ancestors of *O. pes-caprae*.

A more feasible alternative explanation is that the variation in the duplex segregation frequencies of *O. pes-caprae* is due to the species not having arisen from a single pair of diploid species but being of heterogeneous origin. Most of the markers used in the present

study have been obtained from two small populations near Adelaide, both of which have a large number of the same conspicuous pigments segregating. Michael (1964) has shown that one of these populations was started in a nearby nursery, and a similar origin for the other population, in the grounds of Roseworthy Agricultural College, seems likely. It is therefore possible that although these plants now form small wild populations, they were originally gathered for cultivation from a number of different sub-species in South Africa. This could considerably complicate the interpretation of the overall results.

Clearly no amount of observation of a population at a single stage in time can give decisive evidence on the nature of the modification of preferential pairing. However it is hoped that information on this point will be given by a programme initiated in O. pes-caprae. The cross S61.34 x M61.23 from which the plant M63.25 was produced has been repeated. A number of the intercross progeny will be backcrossed, and it is also intended to intercross some progeny to establish lines with the three markers segregating. The programme conceived is

similar to that of Collins and Longley (1935), but with the important addition that more than one chromosome will be marked. (The possibility of linkage between the three markers will be tested as thoroughly as possible by programmes such as outlined in the concluding portion of Sec. II 3.)

The manner in which the three duplex segregation frequencies vary, both singly and jointly, will be of considerable interest. In addition the possibility of cytoplasmic influence on pairing behaviour may be investigated in this programme since the reciprocal cross M61.23 x S 61.34 has also been made. (However note that Gerstel (1963) failed to find such cytoplasmic influence.) It is hoped that as a long term project either the loss or retention of preferential pairing may be demonstrated.

V THE FREQUENCY OF RECOMBINATION IN AUTOTETRAPLOIDS

1. THE RELATIONSHIP BETWEEN THE RECOMBINATION FREQUENCY AND THE NUMBER OF CROSSOVERS

The recombination frequency between two loci may be expressed in the following form in both diploids and tetraploids:-

$$y = a_0q_0 + a_1q_1 + a_2q_2 + \ldots$$

where q is the frequency with which exactly i crossovers are found between the two loci, and a_i is the mean recombination frequency given by i crossovers. (It will be convenient to use the term "crossover" rather than "chiasma" in most places throughout this discussion. Each chiasma is assumed to be the cytological manifestation of a crossover, and to give rise to an exchange of material between two strands.) The a_i's of this relationship are determined solely by the configuration of these crossovers.

In diploids, since there are only two chromosomes involved, the a_i's depend only on the amount of chromatid interference. If chromatid interference is assumed to be absent, as will be assumed throughout this discussion, the relationship is a simple one (Mather, 1938)

$$a_0 = 0$$

$$a_i = \frac{1}{2} \text{ for } i = 1, 2, 3, \ldots$$

In tetraploids, besides the assumption of absence of chromatid interference, additional assumptions about chromosome pairing must be made for deriving the a_i's. Four models will now be considered.

Model 1 - bivalent formation. If there is no quadrivalent formation, no change is expected from the relationship derived for diploids, viz.

$$a_0 = 0 \qquad a_i = \frac{1}{2} \qquad i = 1, 2, 3, \ldots$$

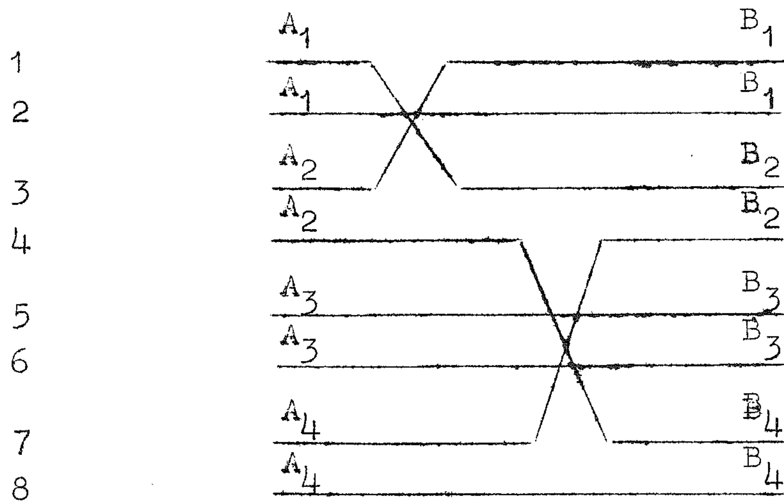
For this relationship the bivalents have been considered separately, and i refers to the number of crossovers per bivalent. The formation of chiasmata in one bivalent may not be independent of their formation in the other, since bivalent competition may occur (Mather, 1936b). However, this phenomenon cannot affect the relationship for a single bivalent, and for the moment can be ignored.

Model 2 - random change of partner. All eight strands must be considered together in this model and i refers to the total number of crossovers between the two loci per quadrivalent. Strands are associated pairwise

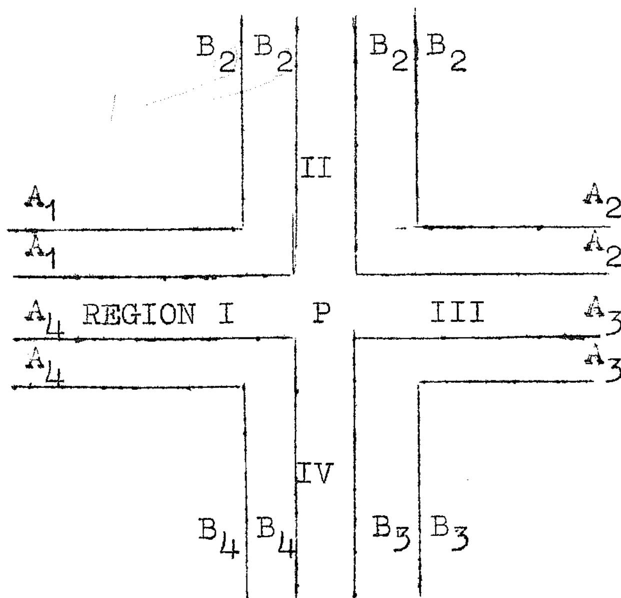
at all points, and any strand is equally likely to be paired with any other strand at a given point. A typical configuration may be depicted as in Fig. 14. A genotype with four distinctive A and B alleles is used in formulating expectations in this and the following tetraploid models. However, all conclusions derived using the tetragenic models still apply to the recombination frequency estimated from digenic material. The assumptions of random changes of partner and no chromatid interference taken jointly mean that each of the twenty-four possible non-sister crossovers is equally likely at any point.

Obviously a_0 must equal 0, and $a_1 = \frac{1}{4}$. By direct enumeration, i.e. by writing out all configurations possible with two crossovers, a_2 can be shown to be $\frac{5}{12}$, and similarly other a_i could be found. However, a recurrence relation can readily be found for the a_i by considering the fate of a single strand.

Regarding only one of the A alleles, e.g. A_1 on strand 1, consider the crossover configuration after i crossovers between the two loci, and the effect of adding another crossover adjacent to these. The probability that A_1 is connected to either B_2, B_3 or B_4 after i crossovers between the two loci is by definition a_i.



<u>Figure 14</u> <u>Diagrammatic representation of random change of partner model showing a typical configuration with two crossovers</u>



<u>Figure 15</u> <u>Diagrammatic representation of quadrivalent one change of partner per chromosome</u>

Then two of the twenty-four crossovers which could be added adjacent to the first i crossovers will alter this situation, i.e. restore the connection between A_1 and B_1, and the other twenty-two will not. If, for example, A_1 is connected to B_2 on strand 3, then the two crossovers leading to non-recombinant strands would be those involving strands 1 and 3, or strands 2 and 3. Similarly, if A_1 were connected to B_1 after i crossovers, with probability $1-a_i$, then six of the twenty-four possible crossovers would lead to a recombinant strand. Thus the probability that A_1 is connected to B_2, B_3 or B_4 after i+1 crossovers is

$$a_{i+1} = \frac{22}{24} \cdot a_i + \frac{6}{24}(1-a_i)$$

$$\therefore \quad a_{i+1} = \frac{1}{4} + \frac{2}{3}a_i$$

Thus a_i must be of the form $l + m(\frac{2}{3})^i$, and since $a_1 = \frac{1}{4}$ and $a_2 = \frac{5}{12}$, we can substitute to find l and m giving $l = -m = \frac{3}{4}$

$$\therefore \quad a_i = \frac{3}{4}\left(1 - (\tfrac{2}{3})^i\right).$$

One result from this calculation, which does not appear to have been reported previously, is that under this model the maximum recombination frequency in tetraploids is $\frac{3}{4}$. This seems intuitively reasonable since after an infinite number of crossovers the A's and B's could be regarded as oriented at random, giving a one in four chance that any A_j would be connected to B_j.

Model 3 - one change of partner, no interference. The model of random changes of partner, although mathematically tractable, is unlikely to be attained in practice, and the more realistic assumption of one change of partner per chromosome is considered here. This model, which is depicted in Fig. 15, assumes that chromosomes are associated in pairs at all points, with one point of partner change occurring at a homologous point on each of the four chromosomes. Cytological observation suggests that this type of pairing may occur frequently.

It may be seen from Fig. 15 that regions I and III and regions II and IV are equivalent. The ratio of region I to region II, however, need not necessarily be constant, and in the following it will be assumed that the point of partner change may lie at any point between A and B with equal probability.

Let the ratio AP/AB be x. Then the probability that a crossover will lie in region I is $\frac{x}{2}$, similarly for region III, and the corresponding probabilities for regions II and IV are $\frac{1}{2}(1-x)$. With a complete noninterference model, the distribution of i crossovers between regions I, II, III and IV is given by the terms in the multinomial

$$\left(\frac{x}{2} + \frac{1-x}{2} + \frac{x}{2} + \frac{1-x}{2}\right)^i .$$

If all crossovers lie in the same region, say region I, then since there is no chromatid interference the recombination frequency between A and B will be $\frac{1}{4}$. If crossovers lie in two adjacent regions, however, say regions I and II, one-quarter of the A_1B_1 strands will remain unbroken, and likewise one-half of the A_2B_2 and A_4B_4 and all the A_3B_3 strands will be unbroken, giving the overall recombination frequency as

$$\frac{1}{4}\left(\frac{3}{4} + \frac{1}{2} + 0 + \frac{1}{2}\right) = \frac{7}{16} .$$

Other distribution types give recombination frequencies as follows: regions I and III, $\frac{1}{2}$; regions I, II and III, $\frac{5}{8}$; and regions I, II, III and IV, $\frac{3}{4}$.

The probabilities of the different distribution types may now be evaluated from the multinomial, giving the recombination frequency as

$$\frac{1}{4}\left[2\left(\frac{x}{2}\right)^{i} + 2\left(\frac{1-x}{2}\right)^{i} \quad + \frac{7}{16}\quad 4\left(\frac{1}{2}\right)^{i} - 4\left(\frac{x}{2}\right)^{i} - 4\left(\frac{1-x}{2}\right)^{i}\right]$$

$$+ \frac{1}{2}\left[x^{i} - 2\left(\frac{x}{2}\right)^{i} + (1-x)^{i} - 2\left(\frac{1-x}{2}\right)^{i}\right]$$

$$+ \frac{5}{8}\left[2\left(\frac{2-x}{2}\right)^{i} + 2\left(\frac{1+x}{2}\right)^{i} - 8\left(\frac{1}{2}\right)^{i} - 2x^{i} - 2(1-x)^{i} + 6\left(\frac{x}{2}\right)^{i} + 6\left(\frac{1-x}{2}\right)^{i}\right]$$

$$+ \frac{3}{4}\left[1 - 2\left(\frac{2-x}{2}\right)^{i} - 2\left(\frac{1+x}{2}\right) + 4\left(\frac{1}{2}\right)^{i} + x^{i} + (1-x)^{i} - 2\left(\frac{x}{2}\right)^{i} - 2\left(\frac{1-x}{2}\right)^{i}\right].$$

Since x is assumed to be distributed with frequency function dx in the range (0, 1), the mean recombination frequency for i crossovers is obtained by integrating the above expression for x in the range (0, 1), giving on simplification

$$a_i = \frac{3}{4} - \left(\frac{1}{2}\right)^{i+2} - \frac{1}{i+1} + \left(\frac{1}{2}\right)^{i+1}\frac{1}{i+1}.$$

<u>Model 4 - one partner exchange, crossover repulsion.</u>

The same conditions as in model 3 apply, except that crossovers instead of forming independently are now

assumed to repel each other. Thus, if two crossovers are formed between the two loci, they will repel each other into opposite arms of the quadrivalent. Similarly three crossovers will lie on three different arms, and four on four different arms, while the positioning of any subsequent crossovers beyond four is arbitrary. No increase in the frequency of crossovers is postulated, but merely a spreading out in the region AB. (Note however that an increase in the frequency of crossing-over above that occurring with bivalent formation might not be unexpected owing to lowering of chromosome interference across a point of partner change - cf. John and Henderson (1962)). Under this model

$$a_0 = 0, \quad a_1 = \frac{1}{4}, \quad a_2 = \frac{1}{2}, \quad a_3 = \frac{5}{8},$$

$$a_i = \frac{3}{4}, \quad i = 4, 5, 6, \ldots$$

The importance of this model is that it supplies an upper bound to the frequency of recombination which may occur for a given number of crossovers. The maximum recombination frequency of $\frac{3}{4}$ is only attained in the limit with an infinite number of crossovers under models 2 and

3, whereas under this model it is reached with four or more crossovers. In addition it may readily be seen that, in the absence of chromatid interference, for neither one, two or three crossovers can a greater frequency of recombination be given than that predicted by this model.

2. THE UPPER LIMIT OF RECOMBINATION

In the absence of chromatid interference, it is evident that with one or more changes of partner between two loci there is an upper limit of 75 per cent for the recombination frequency. The case where a quadrivalent is formed with all points of partner change lying outside the interval between the two loci must be regarded with respect to the recombination frequency between these loci as equivalent to bivalent formation. (However, when considering the fate of both chromatids in a gamete the formation of a partner change outside the two loci cannot be ignored.) In general, where a point of partner change lies between two loci in a fraction p of cases, the upper limit for the recombination frequency becomes

$$p(0.75) + (1-p)(0.50) = 0.50 + 0.25p.$$

By the argument advanced in the previous section, it appears intuitively correct to say that the upper limit of recombination in an x-ploid organism is $\frac{x-1}{x}$. The attainment of this recombination fraction, however, depends on the occurrence of a sufficiently high frequency of partner changes. With hexaploids for example, the limit $\frac{5}{6}$ is only reached as the number of partner changes becomes infinite, the approach to the limit being oscillatory, and certain combinations of partner changes may cause the limit to be exceeded slightly.

3. THE RECOMBINATION FREQUENCY BETWEEN UNLINKED GENES

The recombination frequency when defined in the usual way has no meaning when applied to two unlinked genes in a tetraploid. However, it may be shown that the amount of recombination given by two unlinked genes is equivalent to that given by two genes linked with a recombination frequency of $\frac{3}{4}$. Consider the gametic array given by two linked genes under the model of random partner exchange (model 2). The properties of the transition matrix defined by this model are such (see e.g. Moran, 1962) that after a sufficient number of crossovers the genes at the two loci will be combined essentially at random, which is equivalent to the array given by unlinked genes. The limiting recombination frequency for the model of random partner exchange has been shown to be $\frac{3}{4}$, thus demonstrating the above assertion.

In practical terms, however, this result cannot be taken to mean that the amount of recombination between unlinked genes is greater in the tetraploid than in the diploid. In this context it is perhaps more relevant to consider both alleles in the gamete of the tetraploid.

For the tetraploid, as well as the diploid, two randomly chosen unlinked genes in the zygote have one-quarter chance of both being present in the gamete, one-half chance of one being present and the other absent and one-quarter chance of both being absent. Double reduction will, in fact, reduce the proportion of the recombinant classes in the tetraploid to a little below one-half.

4. THE RELATIONSHIP BETWEEN DIPLOID AND TETRAPLOID RECOMBINATION FREQUENCIES

In order to specify the recombination frequency for either diploids or tetraploids, it now becomes necessary to give values for q_i in the relation

$$y = a_0 q_0 + a_1 q_1 + \ldots + a_i q_i + \ldots$$

Since no realistic crossover distributional theory exists, it is necessary as a first approximation to calculate y under the assumption of no chromosome interference, and ultimately to consider what effect chromosome interference will have on this calculation.

The assumption of no chromosome interference is equivalent to assuming a Poisson distribution of crossovers. If the mean number of crossovers is m, we have

$$q_i = \frac{e^{-m} m^i}{i!} .$$

The recombination fraction for diploids, y_D, is equal to $\frac{1}{2}q_1 + \frac{1}{2}q_2 + \ldots = \frac{1}{2}(1 - q_0)$, giving the well-known relation, (cf. Haldane, 1919)

$$y_D = \frac{1}{2}(1 - e^{-m}) \ldots \qquad (1)$$

The relationship between y_D and the tetraploid recombination fraction y_T is dependent first on the relative frequencies of crossovers between the two loci in the diploid and tetraploid, and secondly on which of the four tetraploid models is used. The relationships in this section will be derived assuming that the mean frequency of crossing-over per strand is the same in the diploid and tetraploid, i.e. that the mean number of cross-overs in the tetraploid is twice that in the diploid. This particular choice will be discussed briefly in the following section.

Model 1. With no assumptions about the crossover distribution necessary in this case, we have

$$y_T = y_D.$$

Model 2. The value of q_i for this case may be given as $\frac{e^{-m'}(m')^i}{i!}$, where m' is the mean number of crossovers for a quadrivalent. Since we wish to compare the recombination frequencies for diploid and tetraploid when the mean frequencies of crossing-over are the same, we must place $m' = 2m$.

Then $y_T = a_0 q_0 + a_1 q_1 + \ldots$

$$= \frac{3}{4} \sum_{i=0}^{\infty} \left[1 - (\tfrac{2}{3})^i \right] . \frac{e^{-2m}(2m)^i}{i!}$$

$$= \frac{3}{4}(1 - e^{-\frac{2m}{3}}).$$

Then substituting for m from equation (1) we get

$$y_T = \frac{3}{4}\left[1 - (1 - 2y_D)^{\frac{2}{3}}\right] \qquad (2)$$

This is given as curve (2) in Fig. 16.

Models 3 and 4. Once again for both models

$$q_i = \frac{e^{-2m}(2m)^i}{i!}$$

Substituting the values previously found for a_i gives for model 3

$$y_T = \frac{3}{4} - \frac{1}{4}e^{-m} - \frac{1}{2m}\left[1 - e^{-m}\right].$$

Substituting for m from equation (1)

$$y_T = \frac{y_D}{\log(1 - 2y_D)} + \frac{y_D}{2} + \frac{1}{2}. \qquad (3)$$

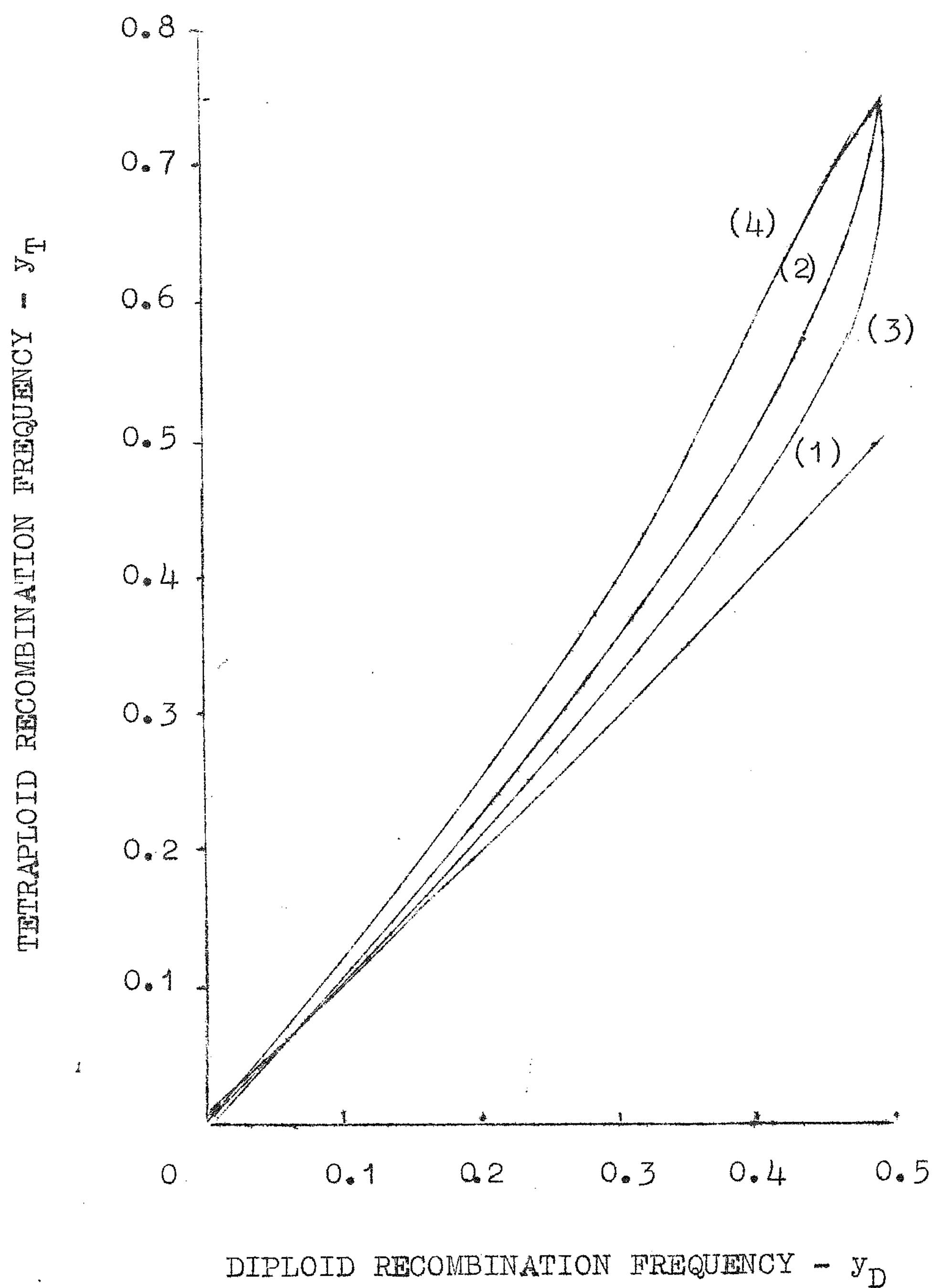


Figure 16. The relationships between diploid and tetraploid recombination frequencies under four tetraploid models

Similarly for model 4 we have

$$y_T = \frac{3}{4} - e^{-2m}\left(\frac{3}{4} + m + \frac{1}{2}m^2 + \frac{1}{6}m^3\right) \qquad (4)$$

which together with equation (1) forms the parametric equations for the relation between y_T and y_D graphed as curve (4).

5. DISCUSSION

Although the above relationships between y_T and y_D have been derived on the assumption of equal frequencies of crossing-over in the tetraploid anddiploid, they could equally well be calculated assuming different rates of crossing-over in the two cases. The relationships given are not those necessarily expected in practice between the two frequencies, but those (for the particular models) expected on the hypothesis that the process of crossing-over is not affected by a doubling of the chromosome set, including such events as changes of partner, etc. Since the frequency of crossing-over rather than the frequency of recombination is the fundamental biological quantity, this appears to be a more appropriate hypothesis to take than the hypothesis of equality of the two recombination frequencies.

It may be seen from the four curves in Fig. 16 that considerably different relationships between y_T and y_D are expected depending upon which of the four tetraploid models is used. In view of the fact that none of the models can be expected to be exactly applicable, it does not seem possible to give a single relationship which will apply in

practice. At best, upper and lower bounds for the value of y_T for a given y_D can be calculated. It will be argued below that under conditions expected to apply in practice, curves (4) and (1) represent such upper and lower bounds.

As pointed out previously the value y_T given by model 4 represents an upper bound to the amount of recombination which may be observed for a given number of cross-overs. The curve (4) is therefore an upper bound for y_T given y_D, and assuming no chromosome or chromatid interference. Since the positive value of the difference $y_T - y_D$ is attributable to multiple crossing-over, it is evident that positive chromosome interference must cause this difference to be reduced. The effect of chromatid interference is more difficult to assess, but it appears that a small amount of positive chromatid interference would tend to lower the difference $y_T - y_D$, and negative interference to raise it. Thus it appears that in the absence of negative chromosome or negative chromatid interference, neither of which is likely in practice, the curve (4) represents an upper bound for y_T for a given y_D when the crossover frequencies are the same in the corresponding diploid and tetraploid segments. The value of y_D could only exceed the value of y_T when there was bivalent

competition, the expected excess even for a strong competition being trivial, so that curve (1) can be given as a lower bound for y_T for a given y_D.

One use of the upper bound may be illustrated as follows. When comparing data on the recombination frequencies for two loci in diploids and tetraploids, two questions may be asked:-

1. Whether the two recombination frequencies differ significantly, and
2. Whether, if the tetraploid recombination frequency is greater than the diploid frequency, the difference falls significantly outside the bounds given by equation (4), i.e. whether the increased recombination frequency necessarily reflects a rise in the frequency of crossing-over.

The data of Oram (1959) may be used in illustrating this analysis. Considering the sugary and glossy loci in maize, Oram finds:-

$$y_D = 0.282 \pm 0.009$$
$$y_T = 0.556 \pm 0.062$$

The difference $y_T - y_D$ may be shown to be significant

at the one per cent level of significance. The second test of significance may be made by plotting the point (0.282, 0.556), and its associated 95 per cent confidence limits in Fig. 16. All points within the confidence interval are found to lie to the left of the curve given by equation (4). Thus an excess in y_T of this order could not be attributed to the effect discussed in this section, but could, however, reflect a rise in the frequency of crossing-over in the tetraploid.

APPENDICES

MARKERS	NUMBER OF CROSSES	TOTAL NUMBER OF PLANTS	χ^2	PROBABILITY
S-Rt	5	388	10.12	.05-.10
Ic	19	745	19.56	.3-.5
Wc	1	99	3.20	.05-.1
Rc	1	88	.00	.95-.98
Oc	19	848	24.35	.1-.2
Ra	12	635	10.23	.5-.7
Rm	3	193	7.51	.05-.1
Hrb	18	1127	19.32	.3-.5
M-Rt	4	269	3.65	.3-.5
Wc	1	16	.29	.8-.9
Rc	1	37	.70	.3-.5
Oc	5	183	9.28	.05-.1
Lf	1	34	5.67	.01-.02
Ra	5	226	5.10	.3-.5
Hrb	8	477	8.02	.3-.5
Rt-Ra	3	113	3.46	.3-.5
Rm	3	84	.42	.9-.95
Hr	1	28	.00	.95-.98
Hrb	2	91	1.99	.3-.5
Ic-Ra	6	305	4.29	.5-.7
Rm	8	339	13.13	.1-.2
Hr	14	793	14.49	.3-.5
Hrb	3	66	3.81	.2-.3
Wc-Ra	1	29	1.45	.2-.3
Hr	2	260	2.25	.3-.5
Hrb	3	202	2.35	.5-.7
Rc-Ra	1	94	.46	.3-.5
Rm	2	115	1.39	.3-.5
Oc-Ra	12	556	18.75	.05-.1
Rm	3	152	11.49	.001-.01
Hr	2	89	3.69	.1-.2
Hrb	6	279	2.72	.8-.9
Lf-Ra	1	25	.24	.5-.7
Ra-Rm	6	244	7.41	.2-.3

Table i. χ^2's for independence of various markers

1960

PLANT NUMBER	PARENTAL CROSS
S60.01 to S60.16	M57.17 X S53.07
S60.13	M58.37 X S58.01
S60.14 S60.15	S58.08 X M57.06
S60.16	M57.18 X S58.07
S60.17	*BP60.06
M60.02 M60.03	M57.17 X S58.07
M60.04 M60.05	S58.06 X L58.54
M60.06	BP60.09
M60.07	L52.33 X M58.27
L60.01	M58.30 X L58.23
L60.02 L60.04 L60.06	L58.52 X S58.08

1961

PLANT NUMBER	PARENTAL CROSS
S61.01 S61.02 S61.03	S60.03 X M60.03
S61.04 S61.05 S61.06	S60.06 X M60.03
S61.07 S61.09 S61.10	S60.08 X M60.06
S61.11 to S61.23	S60.16 X L60.01
S61.30	S60.03 X L60.06
S61.34	S60.15 X M60.04
S61.35	S60.04 X M60.05
S61.37	L60.02 X S60.17
S61.39	S60.15 X L60.01
M61.01 M61.02	S60.03 X M60.03
M61.03	S60.06 X M60.02
M61.04	S60.06 X M60.03
M61.05 to M61.08	S60.03 X M60.06
M61.11 M61.12	S60.16 X L60.01
M61.16	S60.16 X L60.06
M61.23	S60.15 X M60.04
L61.04 L61.05	L60.04 X S60.17
L61.11 to L61.15	S60.16 X L60.01
L61.20	S60.12 X M60.03
L61.23	⌿ R61.02

* From Botanic Park population
⌿ From Roseworthy population

Table ii. Ancestry of plants used in crosses

1962

PLANT NUMBER	PARENTAL CROSS
S62.11	R62.12
S62.12	R62.18
S62.13	S60.15 X L60.01
S62.21	M61.05 X S61.01
M62.10	BP62.02
M62.17	M61.12 X S61.30
M62.22	S61.03 X M61.03
M62.25	M61.05 X S61.01
M62.30	S61.06 X M61.08
L62.07	*R62.02
L62.08	R62.04
L62.12	S61.04 X L61.05
L62.25	*R61.03

* Believed to come from same clone

1963

PLANT NUMBER	PARENTAL CROSS
S63.01 to S63.05	L62.25 X S61.01
S63.06, S63.07, S63.08	S61.02 X L61.14
S63.09, S63.10	S61.06 X L62.25
S63.11 to S63.14	L61.12 X S61.10
S63.12 to S63.29	L61.14 X S61.11
S63.39	L62.07 X S62.12
S63.43, S63.44	S61.37 X S61.39
S63.45	S60.15 X L60.01
M63.06	S61.12 X L61.20
M63.10	L61.14 X S61.11
M63.11, M63.13	S61.11 X M61.11
M63.15, M63.16, M63.17	M61.03 X S61.05
M63.22	M61.05 X S61.03
M63.23, M63.24	M62.10 X L62.08
M63.27, M63.28	M62.25 X S62.21
M63.31 to M63.35	L62.12 X M62.30
L63.01	L61.04 X M61.01
L63.05	L61.02 X M61.07
L63.07, L63.08	S60.15 X L60.01

1963 (cont.)

PLANT NUMBER	PARENTAL CROSS
L63.11	L62.25 X M61.08
L63.22	S61.12 X L61.20
L63.27	M61.16 X L61.23
L63.34	L62.02 X S62.12
L63.38	M60.07 X S62.11
L63.41, L63.42, L63.43, L63.45	M62.17 X S62.13

Table ii (cont.) Ancestry of plants used in crosses

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