GENE CONVERSION AT THE wI LOCUS OF ASCOBOLUS IMMERSUS: CHARACTERISTICS, CONTROLS AND RELATION TO CROSSING-OVER

by

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A B S T R A C T

A method for quantifying the component parts of gene conversion based on the hybrid DNA models, such as that of Meselson and Radding, has been developed. The parameters of gene conversion in Ascobolus immersus have been defined for the conversion control factors (ccfs) acting at the wI locus. The control locus ccf-2, very closely linked to wI, has been found to have effects on initiation of heteroduplex DNA (hDNA), on hDNA symmetry, and on mismatch repair. There is also some evidence that ccf-2 influences which chromatid initiates hDNA formation, and it is likely on the evidence presented that ccf-2 is either the initiation site or is very close to it. The unlinked factors, ccf-3 and ccf-4, which make up the 'super' factor, have a marked effect on the direction of mismatch repair of one specific base-pair mismatch as well as on hDNA initiation frequency. In all data analysed there is evidence that the invading single strand of DNA which begins hDNA formation is always of the same polarity.

Methods of obtaining mutants, both nutrient requiring and drug resistant, were developed. The marker mor-2 maps approximately ten recombination units from w-78 mutant of wI. Analysis of |5:3| octads has indicated that

the occurrence of dissociated crossovers amounts to less than 5 % in this interval.

An auxiliary study was carried out to determine any polygenic control of gene conversion. It was shown that it is possible to select for both higher and lower frequencies of gene conversion without disturbing the spectrum of conversion. Unlike the major control factors, the polygenes are not locus specific and act independently to the known ccf loci.

The present study reviews the current theories and models of recombination and places them in the light of recent results.

C O N T E N T S

		Page
ABSTRACT		1
TABLE OF CONTENTS		3
ACKNOWLEDGEMENTS		6
INTRODUCTION: A LITERATURE SURVEY		7
General Introduction		7
Ascobolus immersus		8
	European Strain 9	
	Pasadena Strain 17	
Sordaria fimicola		22
Sordaria brevicollis		27
Yeasts		31
	Saccharomyces cerevisiae 32	
	Schizosaccharomyces pombe 34	
Escherichia coli		39
	Mismatch Repair 40	
	RecA Protein 49	
Ustilago maydis		55
Other Organisms		60
	Aspergillus nidulans 60	
	Neurospora crassa 61	
	Drosophila melanogaster 65	
Recombination Models		69
	Features 70	
	Models 85	
	Comparisons 95	

GENERAL MATERIALS 123

The Organism 123

The Strains 127

The Chemicals 134

The Equipment 139

GENERAL METHODS 141

Sterilisation and the Organism 141

Culture Techniques 142

Preparing Ascospore Suspensions 144

Germination of Ascospores 144

Scoring Gene Conversion 149

PARAMETERS OF GENE CONVERSION 153

The Equations and Definitions 157

Results 200

Discussion 243

INDUCTION AND ISOLATION OF MUTANTS 249

Spontaneous Mutation Frequency 250

Mutagenesis 250

Selection of Mutations 252

Filtration Enrichment 253

Vitiation Enrichment 266

Indirect Selection 269

Total Isolation 270

Drug Resistance 270

POLYGENIC CONTROL OF GENE CONVERSION 275

Heterogeneity of Results 277

Selection 280

Discussion 288

CROSSOVER STUDIES IN RELATION TO CONVERSION 294

GENERAL DISCUSSION 306

APPENDIX 312

REFERENCES 323

341

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INTRODUCTION:
A LITERATURE REVIEW

In all organisms that undergo meiosis the cells involved are the scene of strange events called recombination. Recombination can be generally defined as the production of new combinations of existing parental characters. In this work, 'recombination' will usually refer to the recombination of linked characters, rather than to recombination of non-syntenic loci by independent assortment in meiosis. On the one hand is the undisputed reciprocal event commonly termed crossing-over and on the other is the non-reciprocal event commonly called gene conversion. It is possible that these two events, each leading to the intra-chromosomal reassortment of genetic information, are both results of a single series of processes directed by the same enzymes at the same time. Alternatively certain evidence suggests that not only are these two events partly or completely separate, but that their apparent relationship is in fact no more than a result of the way in which these two events are measured. Lastly there is a third possibility encompassing the two others as two distinct processes identifiable as two

distinct but related events. The two processes are proposed to occur with different frequencies for different pairs of loci and different sites in various organisms thus explaining why workers who consider only one of these organisms in isolation may hold ideas at variance with those who work on different organisms.

It is the intention of this introduction to set forth the major facts and theories on the subject of recombination, and to review the evidence as a whole. Clearly the conclusions reached might not of themselves suffice to explain the results, but perhaps the survey of a wider field may lead us to think that they contain in germ the solution of the problem.

Because the introduction has been broken down into sections on organisms and recombination models, certain data and interpretations have been arbitrarily divided. It is hoped that the reader will be patient while a survey of the organisms is made.

*

ASCOBOLUS IMMERSUS

A. immersus has large red ascospores. The white ascospore mutants isolated by Lissouba et al. (1962), Yu-Sun (1964), Leblon (1972), Paquette (1978), Helmi (1980) and Howell (1982) to mention but a few, are useful in studying intragenic recombination since mutant and wild

type phenotypes are immediately scorable in the asci. Further, whilst double mutants are not immediately obvious as such, they can be identified by a simple backcross.

The questions which geneticists have set themselves to answer are mainly two:

(1) Are gene conversion and crossing over due to one and the same event ?

(2) Can the molecular processes involved in recombination be identified ?

There are two main strains of A. immersus which have been studied. The Pasadena strain of Emerson, Yu-Sun, and Lamb, and the European strain of Lissouba, Rossignol, and Paszewski. Unfortunately the two geographically isolated strains are infertile when crossed together so that the mutants of one group are not usable by the other. Accordingly the two strains are considered separately.

European Strain:

First isolated by Lissouba in the early 1960's, it was called strain 28. Its appeal came with the ease in selecting spore mutants; by 1981 over 370 mutants had been identified with over 75 given loci and nine linkage groups. In 1987 there are now well over 500 mutants but the linkage groups have not increased in number.

The approach of the French group (Lissouba

et al. 1962) was to analyse the fine structure of the white spore mutant genes. At the time they were limited to the study of intragenic recombination. The problems which they identified were twofold:

(1) the additivity of recombination frequencies is not found, with negative interference predominating, and

(2) intragenic recombinations result from two events, one reciprocal and the other not.

In the latter problem, crossing two white spore mutants gives red spore progeny either from crossing-over or from gene conversion. This was solved by crossing progeny to the parental types and looking for a non-equality of alleles in a single octad as well as the presence or absence of double-mutants. Non-equality of alleles in the products of a single meiosis indicated non-reciprocal recombination, i.e. gene conversion. It was found that for close mutants the recombinants were on the whole more frequently due to conversion than crossing-over. They also showed that since in the same cross some recombinant asci result from one type of recombination and others from the other that it is impossible to define a unit of distance between the sites involved though an order may be given to a series of mutants.

In the closely linked mutants of 'Series 19' it was found that in three mutants studied crossing-over between two of these was more frequent than gene

conversion and that gene conversion between a different two accounted for most of the recombination. The results were accounted for by assuming that two subsegments exist, one containing two mutants, and the other one, the intermediate zone was tentatively termed the 'linkage structure'. In this way it was thought that certain regions of chromosome DNA in some way, more often underwent recombination by gene conversion and others by crossing over.

When a series with more mutants was mapped, using only data from recombinants arising from gene conversion, it was found that the majority parent was always located at the same side of the sequence (to the left in Series 46) thus leading to the term 'polaron'. Polarity in gene conversion is used to describe the situation where conversion frequency of different alleles decreases from one end of a locus to the other. Any interpretations of a polaron must:

a) Account for the fact that all recombinants within it arise from conversion,

b) Be compatible with the expansion of conversion frequencies which is found between very close sites,

c) Explain the conversion frequency which increases from one end of the segment to the other.

It was assumed that the inequality of segregation results from copy choice during chromosome

replication at meiosis and that DNA replication is polarised. It was proposed in addition that the linkage structure where crossing over seems to occur represents a point of possible switch back to the original chromosome after replicating DNA from the 'wrong' chromatid for the length of a segment or polaron.

Rizet and Rossignol (1966) found that whilst flanking markers were unavailable for the gene labelled 75, a certain pair of mutants was available where recombination was predominantly a result of crossing over where wild-type recombinants were involved because both mutants showed little conversion to the wild type (i.e. they both showed disparity to mutant type). Using three mutants with frequent conversion in either direction that mapped between these two sites it was possible to study the association between crossing over and gene conversion. It was shown that some 23% of crossover asci were associated with conversion at the intermediate site. It was proposed that crossing over and gene conversion should be regarded, not as two distinct events, but as representing two different aspects of a single event. Furthermore, since crossing over is known to be a post DNA replication process, gene conversion must also occur after the replication of DNA is complete and so the previous polaron explanation must be wrong.

In these experiments the majority of asci showed

crossover initiation at the end of the polaron which gave a high frequency of gene conversion (as measured by studying the relative frequencies of 3:5 and 5:3 asci with new or original flanking markers).

The b2 spore colour gene has mutations giving gene conversion frequencies varying from 0.1 to 0.3 (Nicolas 1979). Aberrant 4:4 asci can be distinguished from normal 4:4 Mendelian segregations by using a marker that affects spore shape (Paquette 1978)

The pattern or spectrum of a mutation in gene conversion was originally distinguished into one of four major classes (Leblon 1972a), later classes C and D were amalgamated (Whitehouse 1982) and this work follows the latter classification. Class A and class B seem to have little or no post meiotic segregation and show a disparity either to wild-type, as in class A, or to mutant, as in class B. Class C has between 25 and 100 % post meiotic segregation and is characterised as showing little or no disparity between wild type and mutant type. There is a strong correlation between the action of the mutagen used to induce the mutation and its type of spectrum. Class B mutations are guessed to be one GC base pair additions because of the mutagen used (ICR-170). Using intragenic suppressors to revert frameshift mutations to the wild type, a correlation between spectrum class and type of mutation has been found (Leblon and Paquette 1978). It

appears from the results obtained that class B mutations are frameshifts arising from EMS which has been shown to induce base pair deletions or substitutions (Malling and DeSerres 1968) which explains the class C mutations also induced by EMS.

These classifications of class of spectrum and type of mutation are by no means distinct nor without exception. An example is the variety of frameshift class C mutations (Paquette and Rossignol 1978) as opposed to the more frequent base substitutions produced by NMG and EMS (Drake 1970). One point often ignored is that these exceptions may be a result of background spontaneous mutations arising in the organism during the experiment; this of course would be infrequent but of course may not be ignored as in fact the incomplete specific action of a mutagen. Lamb and Ghikas (1979) showed convincingly that spectrum types are not distinct but intergraded on both postmeiotic segregation and percentage conversion to wild-type.

Comparing genes b1 and b2 has revealed that NMG induced mutations are less frequent in the b2 locus (Leblon 1972b). It has been suggested by Rossignol et al. (1984) that this is because base substitutions are often not sufficient to lead to phenotypically detectable mutations in b2: why this should be has not been explained.

Three b2 large deletion mutations show disparity similar to class A or B types having little or no post meiotic segregation (Girard and Rossignol 1974). But in contrast with class A and B mutations, several others show parity in the direction of conversion (Hamza et al. 1987). The conversion spectra of these, when heterozygous, widely differ from each other and from those of point mutations located in the same genetic region. They give lower gene conversion frequencies than point mutations, no postmeiotic segregations, and either parity or disparity that favours the wild type allele. Two effects of G/+ heterozygosity have been observed: a local effect becoming less strong with distance which involves disparity and postmeiotic segregation, and secondly, a long range effect that affects total conversion frequencies and aberrant 4:4s.

The study of interactions between two closely linked mutations of different spectra was reported first by Leblon and Rossignol (1973) and later by Rossignol and Haedens (1978). The closer that two mutants were, the stronger the effect and it is always such that the imposition is of high correction and not of low correction, resulting in a reduction of post meiotic segregation.

Paquette and Rossignol (1978) looked at the conversion frequency of more than a dozen class C mutants.

They found that as the frequency of conversion increased from right to left so the proportion of aberrant 4:4 asci decreased and this increase was most significant where there was a high amount of postmeiotic segregation.

Six class B and 22 class A mutations displayed the same polarity as the C mutations and a similar decline in aberrant 4:4 asci to the left half of the gene (Rossignol and Paquette 1979). In addition however it was discovered that disparity increased from left to right.

A cross involving two class C mutations, one at each end of b2, resulted in 7% of all asci having aberrant 4:4 segregation at either one or at the other site (Rossignol and Haedens 1980). Some 45% of these also had gene conversion occurring at the other site with the most frequent class being 5:3 at one site and aberrant 4:4 for the other. These double conversions were some 10-20 times more frequent than double conversions resulting in the opposite arrangement of ascus types.

Mutants of class C at b2 were mapped on the basis of recombination frequencies and with the help of two overlapping deletions, and were found to fall into seven groups. It was not possible to establish the sequence of the mutant sites within the groups. The numbers of aberrant asci of each kind were counted in several crosses of each mutant with wild-type. A total of nearly three hundred thousand octads was scored (Paquette

and Rossignol 1978). The mutants mapping towards the left gave about 300 aberrants per 1000 asci, while with the remaining mutants aberrants were about half as frequent. There is an increase in aberrant 4:4 frequency towards the right. The inverse relationship between aberrant ascus frequency and aberrant 4:4 frequency is important in later discussions of recombination models.

The significance of these results will be discussed elsewhere, once all results have been presented from other strains and organisms.

*

Pasadena Strain:

Many white spored mutants of this strain have been studied and have revealed a large variation in the frequency and spectrum of gene conversion (Emerson and Yu-Sun 1967; Lamb and Ghikas 1979; Lamb 1984).

All the lines of this strain were originally derived from two standard wild-type stocks of opposite mating types, K,5,+ and P,5,- . It was discovered (Emerson and Yu-Sun 1967; Lamb and Ghikas 1979) that the two original stocks carried different alleles for many loci involved in the control of gene conversion. By altering the genetic background or even the temperature at which crosses were made (Lamb and Wickramaratne 1975; Lamb and Ghikas 1979) it was discovered that the conversion

spectrum types of given mutations could be greatly altered, e.g. from class A to class B, so conversion spectrum types were not fixed for a particular molecular type of mutation.

Post meiotic segregation can be detected using a granular mutant as a second marker (Ghikas and Lamb 1977). This increases the number of classes showing corresponding-site gene conversion. Corresponding-site interference is defined as the interference between the two pairs of non-sister chromatids of a bivalent in hybrid-DNA formation at exactly corresponding sites (Lamb and Wickramaratne 1973). Evidence has been found to support the occurrence of corresponding-site events in what looks like single event octads (Lamb and Wickramaratne 1973). Double events in a single meiosis can occur involving more than one pair of non-sister chromatids. Corresponding-site interference is positive or negative, depending on the cross and also upon the segregation class involved, and on the conversion frequency.

The genetic factors influencing the frequencies and properties of gene conversion at linked and unlinked sites have been identified. At some of these gene conversion control loci it has been shown that there are at least three different alleles which interact and show cis/trans effects (Lamb and Helmi 1978; Lamb and Ghikas

1979; Wickramaratne and Lamb 1978). These loci have been called conversion control factors (ccf's) and there have been as many as five found for a single linkage group (Howell 1982; Howell and Lamb 1984). It is common to number these ccf's but there is some inconsistency in labelling their alleles; for example, at ccf-2 which controls the wI locus the three different alleles are named P, K and 91 after their ancestral origins (Lamb and Helmi 1978) whereas ccf-6 affecting the w-BHj locus has alleles labelled A and B (Howell 1982; Howell and Lamb 1984)

Other conversion control factors have been discovered affecting white ascospore mutants at loci other than at the wI locus (Lamb and Ghikas 1979; Howell and Lamb 1984) and it seems a general property that conversion control factors at different loci interact in a non-additive manner (Helmi and Lamb 1979, 1983). Furthermore, there are strong allelic interactions where these factors are heterozygous, at times showing heterosis for the reduction of conversion frequencies and at other times showing incomplete dominance depending on the cross made.

For the white ascospore locus wI three separate conversion control factors have so far been found. These can give conversion frequencies ranging from less than 1 % up to 33 % . Of ccf-2's three alleles, K and 91 give lower con-version frequencies than P, with P showing incomplete

recessiveness. There also appear to be cis/trans effects of these alleles which are linked to wI (Lamb and Helmi 1978). There is also a combination of unlinked genes known as 'super' which has two interacting factors ccf-3 and ccf-4 which show an approximate doubling compared to non-super, of the conversion frequency dictated by the alleles at ccf-2. The dominance of one ccf-4 allele over the alternative depends on which ccf-2 alleles are present in the cross (Helmi and Lamb 1983).

The cis/trans effects of the P, K and 91 alleles of the ccf-2 locus are expressed in the organism either as total conversion frequency differences or as differences in relative frequencies for the conversion classes arising from two events, and sometimes both.

The specificity of conversion control factors was shown by the studies of ccfs 2, 3 and 4 which only affect wI (Helmi and Lamb 1983). Similarly ccf-5 and ccf-6 affect w-9 and w-BHj respectively but have no effect on any other white spore mutations (Howell and Lamb 1984). The major effect of the latter two factors is expressed in the heterozygous state, when conversion frequency is less than in either homozygous state for both alleles A and B. Both of these factors are linked to the gene which they control unlike ccf-3 and ccf-4 which presumably act by the production of some diffusible product since they are both unlinked to the locus which they affect.

In studies of the w-17 spore-colour mutant, flanking markers have been used to study the relationship between crossing over and gene conversion (Stadler et al. 1970). The flanking markers were a mutant showing colonial growth at 37° C., mapping 10 units to the left of w-17, and a mutant giving resistance to p-fluorophenylalanine, mapping 3 units to the right of w-17. Several different w-17 mutants were crossed individually with wild-type in the presence of the flanking markers and aberrantly segregating asci were scored for flanking marker behaviour. Small numbers of octads were found with two crossovers in the marked interval, one associated with the aberrant segregation and one incidental to it. By the expression 'associated crossovers' is meant those which appear to involve the same chromatids as the aberrant segregation, but with the reservation that there is always some ambiguity about the chromatids of the majority parent. The expression 'incidental crossovers' refers to crossovers that involve the chromatid of the minority parent not involved in the aberrant segregation. The minority parent is mutant in 5:3 and 6:2 asci, and of wild-type in 3:5 and 2:6 asci.

Double crossovers were detected only if each involved a different pair of chromatids. If they share one or both chromatids, one or both of the crossovers will not be detected. Assuming the chromatids involved in the two

crossovers are chosen at random (one being from each parent), the number of undetected crossovers can be estimated (Whitehouse 1982). The percentage of associated crossovers was 38 %. Aberrant 4:4 asci were not included in the investigation.

*

SORDARIA FIMICOLA

Aberrant segregation was discovered in Sordaria fimicola by Olive (1956). The wild type spores are black and the three unlinked ultra-violet-induced mutants obtained were grey, yellow and hyaline. In asci heterozygous for any of these mutants, five black spores and three mutant ones were occasionally present instead of four of each colour. He investigated these aberrant segregations by ascus analysis, using crosses in which at least one other character difference was also segregating (Olive 1959). The second character behaved normally, with a 4:4 ratio in each ascus. Examination of progeny confirmed the genotype for spore colour corresponded to its colour. The crosses studied by Olive also produced asci with 6:2 and 2:6 ratios of wild-type:mutant, and analysis of these asci revealed that the second character difference segregated normally. It was also found that the gray mutant gave aberrant asci of all four kinds, that is, 6:2, 2:6, 5:3 and 3:5.

A fifth kind of aberrant segregation was discovered by Kitani et al. (1961, 1962). In crosses between wild-type and the gray spore-colour mutant, asci were observed with a normal 4:4 colour ratio but with the spores in an abnormal order. These 'aberrant' 4:4 asci were isolated and dissected, and the spores germinated and scored for a second mutant for which the asci were also heterozygous. The second mutant was closely linked to the gray mutant. The centromere is also to the left, but over 50 units away. The results showed that the aberrant segregation of wild-type versus gray resulted from abnormal behaviour at the gray locus and not from events at the level of the whole chromosome or the whole nucleus. Occasionally spindles do overlap at the third division in the ascus after meiosis, producing copies of aberrant asci, but the second marker scoring distinguished true and false aberrants.

The |5:3| and aberrant 4:4 categories show segregation of a character at the first mitosis following meiotic division. This postmeiotic segregation is now referred to as conversion in addition to the other non-Mendelian segregations such as |6:2| (Whitehouse 1982).

A mutant called corona causes a distinctive ring of pigment to form around the site of inoculation. This mutant maps 3.4 units to one side of the gray mutant and mat (a slow growing mutant) maps 0.4 units on the other

side. With such flanking markers for a spore-colour locus the relationship between aberrant segregation and crossing over was investigated (Kitani et al. 1962). The frequency of aberrant asci was about 0.12 % and about 40 % of these showed crossing over. Small numbers of aberrant asci were found with associated and incidental crossovers.

The conclusion reached from this work is that about 40 % of the aberrant asci were associated with crossing over, both as regards its position in the chromosome and as regards the chromatids involved in both events. Any hypothesis to account for the relationship between aberrant segregation and crossing over has to account for aberrant segregation in the absence of crossing over.

Evidence for new mutants arising in aberrant asci was found for the gray spore-colour locus in S. fimicola (Kitani and Olive 1967). In crosses heterozygous either in trans or in cis for alleles 1 and 4, about 10 % of the recombinant asci resulted from crossing over between the sites of the mutants. A derivative of mutant 4 called 4b showed no crossing over when crossed with mutant 1. Mutant 4b was of spontaneous origin and was derived from the odd mutant spore in a 3:5 ascus from a cross between mutant 4 and wild type. Mutant 4 had hyaline spores and mutant 4b had yellow spores. Similarly mutant 2a arose in 3:5 asci from crosses of mutant 2 with wild-

type, 2a having a blue tint to the spores. (Kitani and Olive 1967; Kitani and Whitehouse 1974). Both of the secondary mutants showed marked differences from their parent mutation and so there is contrary evidence for accurate transfer of nucleotide sequences in postmeiotic segregation.

Co-conversion was observed at the gray spore-colour locus directly by Kitani and Olive (1969). Mutant number 1 has grey spores and number 2 has colourless spores. In a trans cross of these alleles, co-conversion asci had six grey:two colourless, or two grey:six colourless, depending on the direction of co-conversion. It was also possible to observe linked postmeiotic segregation by exploiting allelic colour differences. This was revealed as 5:3, 3:5, or aberrant 4:4 segregation for the two spore colours. In ascus analyses (Kitani and Whitehouse 1974), a total of ten crosses involved pairs of mutants differing in colour: grey (1 and 6), light grey(5), slightly tinted(2), and colourless (3, 4 and 4b). These crosses involved recombination frequencies ranging from 8.0 to 22.0 percent asci with wild-type spores, and enabled the frequencies of linked postmeiotic segregation and co-conversion, out of total aberrant asci, to be plotted against site separation (Whitehouse 1974). The results show a decline, both in linked postmeiotic segregation and in other types of co-conversion, with

increasing site separation. The results for gray also indicate that linked postmeiotic segregation usually extends only for a fraction of the length of the gene, although its extent outside the gene is unknown.

In 5:3 and 3:5 asci with non-crossover of flanking markers two genotypes are possible for the octads at the tetrad stage. One has two of the products of meiosis identical and is called the 'tritype', while the other has all four products of meiosis differrent and is called 'tetratype'. If the parental genotype is

$$\frac{M\ b\ N}{m\ +\ n} \quad ,$$

where b represents the mutant, and M/m and N/n are the flanking markers (b/+ representing heteroduplex), then the 'tetratypes' and 'tritypes' can be represented as:-

+:b	Tritype	Tetratype
5:3	M b N M b/+ N m + n m + n	M b N M + N m b/+ n m + n
3:5	M b N M b N m b/+ n m + n	M b N M b/+ N m b n m + n

In S. fimicola, 'tetratypes' account for about one-third of the asci (Kitani and Olive 1967; Kitani and Whitehouse 1974). The percentages of 'tetratypes' for the gray locus are 35 % in 5:3 asci and 24 % in 3:5 asci.

Asci showing aberrant segregation at one site and normal 4:4 segregation at an allelic site provide information about the position of crossovers associated with the aberrant segregation. The information is not exact because conversion itself results in the loss of a marker. In the gray locus about 30 % of the crossovers associated with a 5:3 segregation at a distal site occur on the proximal side of the gene. These crossovers are not incidental to the aberrant segregation as they involve the same chromatids.

*

SORDARIA BREVICOLLIS

In S. brevicollis a lower frequency of associated crossovers was found than in S. fimicola or A. immersus (Sang and Whitehouse 1979). Postmeiotic segregation was associated with a significantly lower frequency of crossing over than conversion with no postmeiotic segregation. The spore-colour locus investigated was buff, and the flanking markers were a methionine auxotroph mapping 5 units to the left and a nicotinamide auxotroph mapping 2 units to the right. The

frequency of associated crossovers was 16 % in postmeiotic segregation asci and 27 % in other conversion asci.

Mutants at three spore-colour loci: grey-3, grey-4 and grey-5, were investigated in an attempt to find a link between mutagen and the class of the mutant (A, B, or C; comparable to those in A. immersus found by Leblon 1972a). The results showed aberrant ascus frequencies between 1 and 4 % (Yu-Sun, Wickramaratne and Whitehouse 1977). The same three classes of mutants as in Ascobolus are recognisable, with characteristic relative frequencies of the different kinds of aberrants, irrespective of absolute frequencies. The A and B classes were distinct, having no intermediates. ICR170 gave rise to two A mutants as well as numerous B mutants. Only C class mutants were obtained from EMS and UV produced C mutants and one B mutant.

The buff spore-colour mutant YS17 shows conversion to wild-type with a much higher frequency than other mutant alleles. In crosses between YS17 and other alleles, aberrant segregation at the allelic sites is increased in frequency compared with crosses lacking YS17, but no examples were found for aberrant 4:4 segregation nor of 'tetratype' non-crossover 3:5 segregation at the allelic site (MacDonald and Whitehouse 1979). The non-crossover postmeiotic segregations at the allelic site

were all 'tritype' and confined to the chromatid which showed conversion to wild type at YS17.

Effectively:

M	+	YS17	N			M	+	YS17	N
M	+	YS17	N			M	+/b	+	N
				gave rise to					
m	b	+	n			m	b	+	n
m	b	+	n			m	b	+	n
				but not to-					
M	+	YS17	N				+	YS17	
M	b	+	N				+/b	+	
				or					
m	b/+	+	n				+/b	+	
m	b	+	n				b	+	

irrespective of flanking marker genotype

where b is a class C buff mutant and M/m and N/n are flanking markers.

The YS17 mutation showed a conversion frequency of about 10 % , compared to nearly thirty other buff mutants which gave a much lower frequency (of 0.5 % and less). Over 98 % of the conversion of YS17 was to wild type. Alleles showed a much greater probability of aberrantly segregating in the presence of YS17, the aberrant segregation involving the same chromatid at each site. YS17 maps near the distal end of buff, about twenty alleles mapping proximal to it and only three distal to it. YS17 imposes its own conversion pattern only on those alleles proximal to it.

Postmeiotic segregation at a distal site of the buff gene is associated with less crossing over than

conversion at YS17 (Sang and Whitehouse 1983). This difference is less evident at proximal sites. Further, random wild-type recombinants from pairwise crosses of buff mutants are associated with about 28 % crossing over if they occur singly (buff x +) and about 45 % if they occur in heteroallelic buff x buff crosses, also that the single wild types have more P2 flanking marker genotypes relative to P1 , than the paired wild types (Bond 1973). This tends to imply an event at the distal site.

P1 and P2 are the flanking marker genotypes of wild-type recombinants from a cross between closely linked mutants, P1 being the parental genotype of the proximal mutant and P2 the parental genotype of the distal mutant. For later reference the flanking marker genotypes R1 and R2 are defined as: R1 corresponding to an exchange between the mutant sites and R2 corresponding to exchanges in all three marked intervals. P1, P2, R1 and R2 are therefore represented as -

M + + N	P1		M 1 + N
m + + n	P2		———
m + + N	R1	from an allelic cross	m + 2 n
M + + n	R2		

where 1 and 2 are the alleles and M/m and N/n are the flanking markers.

*

YEAST (Saccharomyces cerevisiae) and Schizosaccharomyces pombe

In yeasts gene conversion is observed in both vegetative and in meiotically developing cells; however, recombination in the two developmental stages need not be mediated by the same set of genes. Therefore we will concern ourselves with mainly the meiotic events.

Evidence of conversion in Saccharomyces cerevisiae was first discovered in mitotic recombination (Roman 1957) and has also been demonstrated in Schizosaccharomyces pombe (Leupold 1959). The mitotic recombination of a pair of mutants has been demonstrated to increase more than a thousand fold by irradiation with UV (Roman and Jacob 1957). Similarly mitomycin C induces conversion in somatic yeast cells (Holliday 1964).

Later, conversion was found to occur during meiosis (Roman 1958). Post meiotic segregation was first detected in a yeast's four spored asci using adenine auxotrophs of S. pombe, because these mutants accumulate a red pigment as compared to the white wild-type phenotype. Postmeiotic segregation is revealed by sectoring of colonies derived from single spores (Gutz 1971). For example, in a cross between an ade6 mutant and wild type, two of the spores from one ascus gave red colonies, one spore gave a white colony, and the fourth with a white sector and a red sector. Backcrosses confirmed that this

was a 3:5 ascus equivalent to that found in octads. The M26 mutant in the ade6 gene gave wild-type recombinants when crossed with distant alleles with a frequency approximately thirteen times that shown by a closely linked allele, M375, when crossed with them. Out of nearly four hundred other ade6 mutants none behaved like this (Gutz 1971). Tetrad analysis revealed that M26 showed conversion in the order of 5 % in crosses with wild type, conversion to the wild type being over ten times more frequent than to mutant. It is plain from the results that the frequency of conversion of closely linked alleles is greatly increased in the presence of M26. In addition M26 greatly determines the direction of conversion, to mutant when in trans crosses and to wild type in cis crosses.

The effect of heterozygosity/homozygosity of M26 on other alleles such as M210 was studied by Goldman and Smallets (1979). The frequency of aberrant segregation of M210 when M26 was homozygous was double that observed for M210 when M26 was heterozygous, and the direction of conversion lacked the bias imparted to alleles by M26 as a result of co-conversion in the heterozygote.

Information about the relationship between aberrant segregation and crossing over in mitotic recombination was obtained by selecting for wild-type recombinants in diploids heterozygous for pairs of alleles and for flanking markers (Kakar 1963; Hurst and Fogel

1964) in S. cerevisiae. The genotypes of the wild-type recombinants were established by analysis of the progeny from backcrosses. One problem of this analysis is that only one chromatid is revealed. The proportion of the wild-types resulting from crossing over occurring in the interval between the sites of the mutants was 10-20 % . Only a minority of the conversion events was associated with crossing over.

There is a great deal of evidence for the fidelity of gene conversion for different types of mutations including deletions, point mutations, and ochre mutants together with their suppressors (Roman 1957; Zimmermann 1968; Fogel and Mortimer 1970; Fink 1974; Gutz 1971).

Four mutants at the arg4 gene in S. cerevisiae called 1, 2, 4 and 17 map in the sequence

—— 4 — 1 — 2 — 17 ——

on the basis of flanking marker behaviour in pairwise crosses. The frequency of co-conversion declines rapidly with increasing distance between the mutant sites, ranging from 75 % for mutants 2 and 17, to 6 % for mutants 17 and 4. The use of frequency of recombinants induced by irradiation indicated the relative distances between these mutants. When three and four point crosses have been made, numerous co-conversions have been found, involving up to four alleles (Fogel, Hurst and Mortimer 1971; Hurst, Fogel

and Mortimer 1972). There has even been evidence of co-conversion extending from one gene to another (Lawrence et al. 1975) where two adjacent genes cyc1 and rad7 were involved.

The ade7 gene of S. pombe is a classic example of map expansion. Wild-type recombinants for a pair of mutants gave frequencies greatly exceeding the sum of similar frequencies for intervening pairs. Marker effects are the effects of the specific nature of each mutational site on the properties of recombination in its immediate neighbourhood (Stadler 1973; Catcheside 1975; Hastings 1975; Rossignol 1969; Gutz 1971). Map expansion which can be considered as a marker effect is defined as the tendency of recombination observed between two relatively distant sites in a locus to be greater than the sum of values observed over a number of shorter intervening distances.

The mutant classes A, B and C have been found in S. cerevisiae (Fogel et al. 1971; Fogel et al 1979; Lamb 1986). Most but not all deletion mutants have shown parity in direction of conversion (Fink 1974; Fink and Styles 1974; Lamb 1986).

More precise information has been obtained by sequencing the amino acid sequence of iso-1-cytochrome c of S. cerevisiae and determining the molecular basis of a number of mutants in cyc1, its structural gene (Moore and

Sherman 1975, 1977). Mutants 13 and 239 showed an extraordinary high frequency of recombination compared with other combinations of alleles. The deletion frameshift mutant 239 gave a relatively high frequency of recombination compared with transversions 179 and 76. The probable transversion 163 also gave a relatively high frequency of recombination with alleles, in several instances much higher than was found with other base substitutions at the same site. It is not possible to predict how alleles will interact from their behaviour in crosses with other alleles.

In S. pombe two opal suppressors in genes sup3 and sup9 are mutations from TGA to TCA and both enhance the local frequency of meiotic recombination from five to ten fold (Hofer et al. 1979). Tetrad analysis revealed that both mutants gave a high frequency of postmeiotic segregation in one-factor crosses and in the case of one mutant, also in a cross with an allele (Thuriaux et al. 1980). This accounted for the increased recombination because approximately 97 % of the conversion events were in fact co-conversions spanning the whole gene and hence fail to give recombination of alleles. Postmeiotic segregation was normally rare. Another sup3 mutant, r10, increased single-site conversion of nearby sites and reduced co-conversion.

One very interesting difference between mitotic

and meiotic recombination in S. cerevisiae is the existence of polarity of conversion for the ilv1 and his1 genes during meiosis but not in mitosis (Sherman and Roman 1963; Kakar 1963; Fogel and Hurst 1967; Hurst and Fogel 1964).

Aberrant segregation of proximal and distal mutants may be associated with different frequencies of crossing over in S. cerevisiae (Fogel and Hurst 1967). The frequency of crossing over associated with aberrant segregation is about 40 % for the his1 gene at the proximal site and about 60 % at the distal site (Savage and Hastings 1981). These figures are based on large numbers and are reported by Whitehouse (1982) to differ significantly from 50 % . About a quarter of the crossovers associated with conversion at a proximal site are situated distal to the other mutant site.

Recent studies of disparity in S. cerevisiae at the CYC-H3 gene (McKnight et al. 1981) have been followed up with molecular genetic techniques resulting in an altered position of the gene in question in the genome (Pukkila et al. 1986). It seems that the unusual recombinational disparity of the CYC-H3 gene in this organism (loss of CYC-H3 predominating) appears to be retained by cloned copies reintroduced into the genome.

Mutants in S. cerevisiae have been especially selected with the aim of being conversion affecting

factors; more specifically they were isolated because they conferred elevated postmeiotic segregation (Williamson et al. 1985). These mutants have been designated pms1-1 and pms1-2 and both showed a mutator phenotype of up to a fifty-fold increase in mutation rate. Data for pms1-1 shows that the mutant gene is recessive with respect to its effect on post meiotic segregation frequencies. Wild-type and heterozygote data sets represent a statistically homogeneous sample. The pms1-1 data show that the gene affects disparity and ratios of conversion classes.

Three aspects of genetic recombination have been examined in the asci from cells reversibly arrested at pachytene in S. cerevisiae:

1) gene conversion at a dozen heterozygous loci assayed in dissected tetrads,

2) reciprocal recombination between linked genes and in gene-centromere intervals, and

3) postmeiotic segregation as seen by half sectored colonies from single ascospores.

There was a significant increase in postmeiotic segregation and gene conversion in treated cells, averaging over all at almost fifteen fold that of the controls (Davidow and Byers 1984). Reciprocal recombination also increased when cells were temporarily arrested at pachytene with the result that several previously linked pairs of alleles no longer displayed

linkage. Finally three of the four alleles tightly linked to centromeres showed two- to three-fold increases in second division segregation, whereas that of the fourth showed no significant change. Unfortunately it is unknown if heteroduplex is already present at arrest, an answer to this would be of great importance.

As yet there is no evidence of a lack of association between gene conversion and reciprocal exchange except where the conversion is intrachromosomal (Klein, 1984). The association of reciprocal exchange with intrachromosomal gene conversion was examined using an inverted repeat at the his3 locus. Whilst intrachromosomal gene conversions between the duplicated inverted sequences were frequent there was no associated reciprocal exchange in flanking sequences.

Exposure to DNA-damaging agents can greatly enhance recombinational events (Resnick, 1979). Several genes in S. cerevisiae have been found that affect recombination (Game 1983). One of the most interesting of these genes is RAD52. Originally identified as a mutant sensitive to ionising radiation (Resnick 1969), rad52 mutants are deficient in double-strand break repair (Resnick and Martin 1976) and radiation-induced recombination (Saeki et al. 1981).

In the absence of the RAD52 gene product, meiotic recombination is eliminated and mitotic

recombination is reduced up to ten-fold (Prakash et al. 1980; Game et al. 1980).

Another double-strand break repair deficient mutant rad50 has a spontaneous mitotic recombination higher than in RAD^+ (Malone 1983). As with rad52, the rad50 mutants are also defective in meiotic recombination but a difference is clear with multiple mutants of spo13 which lacks the meiosis I reductional division so that products are recoverable even if normally resulting in lethality due to defective recombination (Klapholz and Esposito 1980). Results show that rad50 blocks an early step in recombination resulting in aneuploidy (Malone and Esposito 1981), the RAD52 gene product acting later. The products of RAD52 and RAD50 have been identified as nucleases but their exact functions remain to be determined (Resnick et al. 1984).

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ESCHERICHIA COLI

Recombination at meiosis in eukaryotes has at least two special features which have no parallel in prokaryotes:

(1) It is mediated by a synaptonemal complex (SC) at pachytene of meiosis, and

(2) Crossovers show positive interference (Holliday 1974). The presence of one cross over strongly inhibits

the formation of another nearby, with the degree of interference decreasing with distance. On the other hand cross overs do not interfere with gene conversion (Fogel et.al. 1981).

The study of this prokaryotic organism often includes its viruses T4 and lambda (phages). It is easier to divide this study into two sections: mismatch repair and the recA mutants.

Mismatch Repair:

A mismatch is defined as any non complementary base pair in double stranded DNA. Mismatched base pairs can occur as a result of -

a) errors in DNA replication,

b) hybrid DNA formation during recombination between homologous, but non-identical DNA sequences, and

c) chemical or physical modifications of bases.

DNA molecules arising from the pairing of strands of different parentage are referred to as "hybrid DNA" , "heteroduplex molecules" or more simply "heteroduplexes" (hDNA). If the two original parental DNA molecules differ then replication of the hDNA produces daughter molecules with different sequences at the site where the hDNA had a mismatch or an unpaired base.

The process of recognition and repair of incorrectly paired bases in double-stranded DNA is called

"mismatch repair". The importance of mismatch repair lies in its probable explanation of gene conversion.

The most reliable demonstrations of mismatch repair in E. coli come from transfection experiments using artificially constructed heteroduplexes of phage DNA and single-burst analysis. If a mismatch-containing heteroduplex replicates without being repaired, both parental phage genotypes are found among the progeny derived from that heteroduplex. Repair of the mismatches in a heteroduplex results in the production of phages of a single genotype. The phages will have the parental genotype when the heteroduplex contains a single mismatch or, for more mismatches, if the repair occurs on only one of the two strands. The phages will be of recombinant genotype when the heteroduplex contains two or more mismatches and mismatch repair operates on different strands at the different mismatched sites. Mismatch repair operating on only one of the sites in a heteroduplex with two mismatches results in the production of phages of two genotypes, one recombinant and one parental, or two recombinants if both sites are repaired on opposite strands.

Wildenberg and Meselson (1975) used lambda phage heteroduplexes containing a pair of mismatched amber markers in trans configuration. The frequency of wild-type phage production was not related to the separation of the

mismatches and did not change when a double-mutant helper was used instead of wild-type, indicating that mismatch repair, rather than recombination, was the major source of wild-type phages. The results also imply that repair events often include both mismatches of pairs spaced less than a few thousand base pairs apart.

Evidence that mismatch repair is involved in correcting replication errors comes from mutants deficient in mismatch repair which have mutator phenotypes (Nevers and Spatz 1975; Tiraby and Fox 1979). For mismatch repair to correct replication errors without any interaction between homologous duplexes, it must distinguish newly synthesised strands from parental strands and must act selectively to preserve the sequence of the latter. DNA methylation was proposed as a means for this strand discrimination (Wagner and Meselson 1976). Evidence supporting this idea was that E. coli dam mutants, which are deficient in adenine methylation of GATC sequences, have a mutator phenotype (Marinus and Morris 1973, 1975) and that adenine methylation of newly synthesised strands lags behind replication so that newly synthesised strands are transiently undermethylated (Lyons and Schendel 1984).

In some experiments using heteroduplexes prepared from lambda DNA isolated from phages grown in wild-type E. coli (for methylated strands) or in dam

E. coli (for unmethylated strands), the results indicate that the extent of mismatch repair is similar for heteroduplexes with both strands methylated, one strand methylated, or neither strand methylated (Dohet *et al.* 1986; Pukkila *et al.* 1983; Wagner *et al.* 1984). However doubt has been raised about the efficiency of GATC methylation in preventing mismatch repair (Radman and Wagner 1986). It has been suggested that only some 75 % of the GATC sequences are methylated in the DNA of lambda phages grown in wild-type *E. coli*. When lambda DNA heteroduplexes are fully adenine methylated (*e.g. in vitro*) mismatch repair appears to be completely inhibited. It is the incomplete methylation of wild-type lambda DNA that allowed lambda DNA heteroduplexes to be useful substrates for the initial demonstrations and characterisations of the *E. coli* mismatch repair system.

Mismatch repair appears to occur preferentially on unmethylated strands of hemimethylated heteroduplexes (where one strand only is methylated), a situation that presumably occurs in *E. coli* only in the region immediately behind the replication fork. The speed with which methylation occurs following replication will determine the time in which the mismatch repair system must work.

The results of *in vitro* experiments confirm the characteristics of the mismatch repair system in *E. coli*

discovered by genetics described above (Lu et al. 1983, 1984). Using a circular-DNA substrate with a mismatch in a restriction enzyme-recognition sequence, such that mismatch repair of one strand creates a functional restriction site, mismatch repair activity was detected in extracts of wild-type E. coli cells. As is the case in vivo, the in vitro reaction requires unmethylated GATC sequences and shows methyl direction of repair, i.e. repair occurs efficiently only on unmethylated strands. In addition, the in vitro reaction requires ATP and single-strand DNA-binding protein.

The E. coli mismatch repair system does not recognise and repair all mismatches with equal efficiency. The extent of repair depends on both the mismatch and the neighbouring nucleotide sequence. In general, transition mismatches are better repaired than are transversion mismatches (Kramer et al. 1984; Wagner et al. 1984). For a given mismatch, repair efficiency increases with increasing G-C content in the neighbouring nucleotide sequence (Radman and Wagner 1986). This relationship between G-C content and mismatch repair efficiency is strongest for transversion mismatches and was observed for a region including four base pairs on either side of the mismatch. This relationship is less evident when the region considered is smaller than four base pairs on either side of the mismatch, but is still apparent when

the region is as large as ten base pairs on either side of the mismatch.

The E. coli mismatch repair system can recognise and repair frameshift-wild-type heteroduplexes (Dohet et al. 1986) which do not contain a mismatch, but rather have one strand in which there is an unpaired base. When such heteroduplexes are unmethylated, mismatch repair works equally well on either strand, and the nature of the unpaired base does not influence the extent of repair. The repair of frameshift-wild-type heteroduplexes is methyl directed. Thus, the E. coli mismatch repair system is able to correct both addition and deletion frameshift mutations arising during the course of replication.

Pairwise crosses between genetic markers in the immunity region of bacteriophage lambda indicate that recombination frequency is generally proportional to the number of nucleotides between the markers (Lieb 1981). It was therefore suggested that mismatch repair does not occur in recombining DNA (Gussin et al. 1981). If so, this absence of repair may result from the partial methylation of lambda DNA and the brief existence of recombinational heteroduplexes due to their formation, in general, in replicating DNA. Alternatively the intervals examined may have been small enough that co-repair of both sites on the same strand was much more frequent than independent repair.

A group of markers in the lambda cI gene do not behave normally in pairwise crosses with other markers, but instead appear to be hyper-recombinogenic (Lieb 1983, 1985). A cross between one of these markers and any other marker yields up to thirty fold more recombinants than would be predicted from the number of nucleotides separating them. These "hyper-rec" mutations are C-G to T-A transitions in the sequence CAGG to TAGG. Lieb (1983) hinted that, although these markers are included in hybrid DNA at normal frequency, their hyper-recombinogenicity results from mismatch repair acting on the mismatches formed in hybrid DNA, even in GATC-methylated DNA, and involving a short excision tract that does not cause co-repair of close markers. Markers as close as ten base pairs on either side of one of these hyper-recombinogenic markers are not co-repaired with it. In addition, the repair is apparently unidirectional favouring the preservation of the CAGG sequence regardless of whether the sequence is the wild-type or mutant sequence for a particular marker. Such a repair process could account for high negative interference, the wild-type heteroduplexes formed during recombination will be efficiently and selectively repaired to wild type by the short-patch repair system (Meselson 1986).

Such short-patch repair has been confirmed using a series of heteroduplexes with individual mismatches in

and surrounding a CCAGG sequence in the lambda cI gene (Radman and Wagner 1986). It was found that mismatch repair occurred in fully GATC-methylated DNA only when the mismatch was at the second position from either end of the sequence. It is interesting that this is also the site for methylation by the E. coli cytosine methylase. No repair was observed when the mismatch occurred at the first base pair outside the CCAGG sequence or at the middle base pair of the sequence. The repair acts on the G-T mismatch and not on the A-C mismatch. It replaces the thymine residue and thus restores the G-C base pair. A second site where repair of the thymine of a G-T mismatch gave a mutant CCAGG sequence revealed that the process does not repair only to wild type, but rather acts to restore the CCAGG sequence. Repair events did not extend ten base pairs to either side, but did frequently include a mismatch two base pairs away on the 5' side of the excised thymine.

Shortpatch repair also operates in GATC unmethylated or hemimethylated DNA, but its effects are less noticeable owing to the operation of methyl-directed mismatch repair. As is the case for methyl-directed repair, short-patch repair requires intact mutL and mutS genes. However, short-patch repair is fully operational in mutH or mutU bacteria and requires a functional dcm gene, which codes for the E. coli cytosine methylase (Radman and Wagner 1986). Methyl-directed mismatch repair requires

intact mutH and mutU genes.

Mismatch repair occurs with equal efficiency in hemimethylated DNA heteroduplexes, where it is restricted to the unmethylated strand, and unmethylated DNA heteroduplexes, where repair occurs on either strand (Dohet et al. 1986; Pukkila et al. 1983; Wagner et al. 1984). However, the mutator effect of mutH, mutL, mutS, and mutU mutants and dam mut double mutants, which are deficient in mismatch repair, is about an order of magnitude higher than that of dam mutants (Glickman and Radman 1980), which have undirected mismatch repair. It was suggested that the mechanism of double-strand breaks at or near mismatches in unmethylated DNA is due to the action of the mismatch repair system on both strands. This idea is supported by the results of a series of transfection experiments with unmethylated lambda DNA heteroduplexes. These results indicate that unmethylated heteroduplexes containing one or more repairable mismatches transfect cells with a functioning mismatch repair system less efficiently than they transfect cells deficient in mismatch repair (Doutrioux et al. 1986). No difference is observed when the heteroduplexes either contain a non-repairable mismatch or are fully methylated or hemimethylated.

E. coli mutations reducing the repair of double-strand breaks (recA, recB, polA, lexA, ind, ruv) are

lethal when combined with a dam mutation (Peterson et al. 1985). This lethality can be overcome by the addition of a mut mutation. These results suggest that double-strand break repair may rescue DNA inactivated by the mismatch repair system. Such repair must result in the correction of replication errors in order to account for the lower mutation frequency of dam mutants relative to mut and dam mut mutants.

The three mechanisms of mismatch repair in E. coli namely, methyl-directed repair, short-patch repair and repair via double-strand breaks, have at least one common feature, i.e., all require functional mutL and mutS genes. The requirement for these gene products suggests that they are involved in steps common to all three mechanisms (e.g. recog nition of the mismatch). The results of in vitro experiments demonstrate that purified mutS protein binds to some mismatches (Radman and Wagner 1986).

The recA of E. coli:

Studies on the recA mutant of E. coli provide an insight into homologous genetic recombination and provide a basis for an enzyme system which will carry out recombination from start to finish (for reviews and references see Radding 1982, 1978, 1973; and Whitehouse 1982).

The protein produced by an intact *recA*$^+$ gene promotes synapsis of homologous DNA molecules and the exchange of one strand in duplex DNA for a strand in another molecule thereby creating heteroduplex DNA.

The *recA* gene was discovered by virtue of its effect upon recombination and it has since been found to be pleiotropic on repair mutagenesis and cell division because it produces a specific protease cleaving certain repressors, thereby derepressing an entire set of genes and being a DNA dependent ATPase which promotes homologous pairing of DNA molecules (*i.e.* it has duality).

Current theory is that damage to DNA or inhibition of replication produces a signal that activates constitutive *recA* protein. The nature of the signal/activator resulting from damage to DNA is unknown, but single stranded DNA, certain polynucleotides and oligonucleotides serve as cofactors *in vitro*.

Evidence that *recA* plays a direct role in recombination comes from thermosensitive *recA*$^-$ mutants and from discovery that purified *recA* protein has DNA dependent ATPase activity. The prototype of the reaction catalysed by *recA* protein is the pairing of a linear single strand with duplex DNA to form a D-loop in which some part of the original single strand has become part of the helix forming a heteroduplex region and displacing its homologue. A D-loop is defined as a triple-stranded region

in DNA containing two paired strands and an unpaired strand, the latter covalently linked at both ends to the rest of the duplex DNA.

Observations suggest that single stranded DNA acts as an effector as well as being the substrate. In the presence of recA protein, DNA ligase seals about half of the nicked circular molecules of DNA, and little if any DNA is unwound at the time of closure. However, when single stranded DNA was present all of the closed DNA was extensively unwound. It has therefore been proposed, since binding of duplex DNA and its unwinding are activated by heterologous DNA in the presence of ATPase, the unwinding might be instrumental in promoting the search for homology.

Synapsis can occur in the absence of a free end in any of the DNA strands involved; this implies a structure differing topologically from that of the Watson-Crick DNA model and means that synapsis can occur anywhere along the extent of a single stranded molecule and its complement in duplex DNA.

There is evidence that recA protein promotes strand exchange in a polarised or unidirectional fashion, preferentially displacing the 5' end of the strand by a slow process which makes the formation of joint molecules a pseudoreversible reaction under some conditions, but homologous pairing is non-directional! Exchanges between

single strands and full duplexes directed by E. coli recA protein leads to the production of Holliday structures (see Figure 1, page 86).

The rate of action of recA protein in the presence of single strand binding protein (SSBP) (causing heteroduplex joints to grow) is only four base pairs per second. By comparison the slowest DNA helicase separates strands at about 300 base pairs per second, whilst spontaneous branch migration of D-loops in relaxed DNA is estimated at over 79 000 base pairs per second.

Whilst one may propose that recA protein promotes unidirectional strand exchange merely by biassing slightly the otherwise random two directional 'walk' of branch migration, the observation that recA protein can cause incorporation of as many as 18 mismatched base pairs in a heteroduplex region 1200 base pairs long, indicates the opposite because the incorporation by passive branch migration of even a single mismatch should be energetically unfavourable. The slowness of strand exchange promoted by recA protein means there are other undiscovered factors which are normally involved in vivo.

There is an associated increase in spontaneous and induced mutation frequency with crossing over which indicates a kind of departure from fidelity in recombination when recA$^-$ is present.

Experiments on recA protein have failed to

provide evidence that large discontinuities can be incorporated in heteroduplex DNA. This question of including large insertions or deletions in heteroduplex DNA is a key question in understanding the molecular basis of gene conversion.

SSBP is only one of a series of helix destabilising proteins extracted and these promote pairing of complementary single strands. They stimulate the formation of D-loops by recA protein.

There is an interaction between SSBP and recA protein. Alone both can promote the pairing of complementary nucleotide sequences and each binds cooperatively to single stranded DNA, yet their catalytic mechanisms are quite different and a comparison of their actions shows the uniqueness of each (Radding 1982). SSBP sufficiently stabilises the association of recA protein with single stranded DNA to prevent exchange of recA protein when more single stranded DNA is subsequently added.

The action of recA protein can lead not only to the initial transfer of a strand from one molecule to another but frequently also to a second transfer and the reciprocal exchange of strands.

When duplex DNA with a gap reacts with intact duplex DNA a set of reactions occurs, the formation of a Holliday structure (junction) being impossible for

topological reasons. Instead a pairing takes place in the gap followed by a strand transferring over from the gapped molecule to form a D-loop and transfers back by some form of branch migration. This is supported by the following observations:

(1) Joints are usually located some distance from the gap but the joint molecules are very stable and the integrity of both strands in the fully duplex molecule is unchanged in the course of the reaction.

(2) recA protein does not form joint molecules when the gap is in a region of heterology even when heterology is limited exclusively to the single strand in the gap.

(3) recA protein does not form joint molecules if sequences in the fully duplex molecule do not to some extent overlap the sequences in the gap.

(4) recA protein can pair a single strand with duplex DNA in the absence of any free ends and the location of a gap in the heterologous region of a chimeric molecule reduces but does not eliminate the formation of joint molecules. This suggests that initial pairing may take place both between two fully duplex regions and between the single strand in a gap and homologous duplex DNA.

Experiments have demonstrated the ability of recA protein to patch over a thymine dimer in single stranded DNA with a complementary strand from a duplex molecule. So recA protein polymerises on single stranded

DNA and extends it, as well as binding to duplex DNA and unwinding it by a mechanism stimulated by single strands. In addition recA protein promotes strand exchange in a polar or unidirectional fashion.

It has been observed that recA protein promotes pairing of a circular single strand of DNA at the 'wrong' end of a linear duplex molecule, the end from which the circular single strand promptly dissociates as a result of polar strand exchange; these heteroduplex joints however which form in a direction opposite to the demonstrated polarity of strand exchange are some 300-400 base pairs long (Wu et al. 1982). Wu postulates that synapsis involves a protein-DNA complex where the heteroduplex is held in an unwound position with the complementary strand in a nascent heteroduplex joint. This favours spontaneous heteroduplex formation energy wise but makes recombination energy expensive.

Other rec mutants exist but they merely complicate matters when considering recombination models.

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USTILAGO MAYDIS

Five mutants of this eukaryote, unable to grow on nitrate, were used in recombination studies, and diploids heterozygous for pairs of them were synthesised (Holliday 1966). UV was used to increase mitotic

recombination and a linear map of these nar-1 mutants was obtained. Half tetrad analysis revealed that all of the recombinants had arisen as a result of conversion and not crossing over. Postmeiotic segregation was detected in this organism by isolating mosaic colonies (Holliday and Dickson 1977).

There is evidence that certain mutants working in conjunction in U. maydis have no conversion but crossing over still takes place, though the distribution of mitotic crossovers is abnormal (Badman 1972). These mutants singly cause extracellular DNase deficiency in one (nuc-1) and intracellular DNase deficiency in the other (nuc-2). The intracellular DNase is capable of introducing single-strand breaks into duplex DNA (Holloman 1973). This DNase preferentially inactivates DNA containing mismatched bases (Ahmad et al. 1975). The discovery that mismatched bases provide a substrate for DNase gives strong support to any hypothesis connecting conversion to mismatch correction because inactivity of this nuclease leads to lack of conversion. The nuclease is so sensitive that it demonstrates preference for DNA containing a single purine-pyrimidine mismatch, which presumably produces a very minor distortion.

A protein with similar properties to the recA protein of E. coli has been purified and characterised in Ustilago maydis ; it is absent in rec1 mutants (Kmiec and

Holloman 1984; Kmiec et al. 1983). These mutants have several abnormalities in both mitotic and meiotic recombination. In the absence of the Rec1 protein recombination still ocurs implying Rec1 to be only one of several genes. A rec2 mutant is also defective in mitotic recombination and is blocked in meiosis. The rec1 mutant was isolated as a UV sensitive strain (Holliday 1965). It is also sensitive to other mutagens, and is recessive. In rec1 mutants recombination is defective for crossing over rather than for conversion. Only one recombinant chromosome is formed in crossovers gone wrong. Some five rec1 mutants have been obtained of which no complementation occurs (as expected).

The rec2 mutant of which only one has been found, has an unknown biochemical deficiency. The rec1 mutant lacks an ATP dependent DNA pairing activity (Kmiec and Holloman 1982).

rec-1 shows spontaneous mitotic recombination with a relatively high frequency, but a reduced level of ultraviolet-induced recombination (Holliday et al. 1976).

Like the recA protein in E. coli, the product of the rec-1 gene in U. maydis promotes pairing of DNA molecules, and promotes strand transfer between single strand DNA and duplex DNA in a reaction dependent upon homology and the abundance of ATP (Kmiec and Holloman 1983). It has been shown that strand exchange is polar but

in the opposite direction to that promoted by E. coli recA protein, i.e. junction of molecules begins at the 5' end of the duplex. In a strand transfer reaction between a circular single-stranded molecule and a linear duplex whose ends are not homologous to the circle, the molecules pair but the strands cannot become interwound. The formation of the resulting 'paranemic' joints appears to be coupled to the formation of left-handed Z DNA, as opposed to the right-handed DNA helix of Watson and Crick (Kmiec and Holloman 1984).

There are many other radiation-sensitive mutants in U. maydis defective in mitotic allelic recombination (Holliday, personal communication).

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OTHER ORGANISMS:

Eukaryotes, other than those mentioned above, which have been demonstrated to exhibit aberrant segregation include: Neurospora crassa (Mitchell 1955a), Bombardia lunata (see Whitehouse 1982 for references), Aspergillus nidulans (Strickland 1958a), Drosophila melanogaster (see Whitehouse 1982 for references), and Podospora anserina (Marcou 1969).

Aspergillus nidulans:

Data for Aspergillus nidulans about the relationship between aberrant segregation and crossing over in mitotic recombination have been obtained by selecting for wild-type recombinants in diploids heterozygous in repulsion for pairs of alleles and for flanking markers (Pritchard 1955, 1960). These data for adE alleles 16, 20 and 8 show that the proportion of the wild types resulting from crossing over occurring in the interval between the sites of the mutants was 40-50 % . Only a minority of conversion events was associated with crossing over.

Polarity in recombination has been found in the pabaA gene in A. nidulans (Sidiqi 1962). When pabaA mutants were crossed with one another, wild-type recombinants were selected and scored for flanking markers. There is a well defined distal polarity but it is

less evident in the proximal half of the gene. A distinct feature of all polarity is that mutants show a lower frequency of conversion when they are the left-hand member of a pair than when they are the right hand member. The behaviour of a mutant seems to depend on its position and not to be an intrinsic property of the mutant itself. In other words, the polarity gradient results from a polarity in the recombination process and not because of individual mutants. This is similar to results in A. immersus (Lissouba 1961) and N. crassa (Murray 1960).

The weaker polarity at the proximal end of pabaA in A. nidulans tends to suggest that if more proximally situated mutants were available, the direction of polarity would be reversed. Whitehouse and Hastings (1965) display many examples of this kind of polarity reversal, always with proximal polarity at the proximal end of the gene and with distal polarity at the distal end of the gene (Murray 1969). The classic reversal of polarity is in A. nidulans where the lysF gene with twelve mutants gave a symmetrical polarity pattern (Pees 1967).

Neurospora crassa:

In order to understand polarity in recombination Murray (1968) made use of a structural change in which a long segment of the right arm of linkage group I, in Neurospora crassa, was inserted in inverted sequence into

the right arm of linkage group V. This allowed her to discover whether the polarity is determined locally, or if it is determined from a distance. The met-6 gene and its flanking markers was transferred to the structural change. Recombination at met-6 was studied in a strain homozygous for the inversion but heterozygous for the mutant. She discovered that the polarity was unchanged with respect to the flanking markers, but reversed with respect to the centromere: proximal polarity when structurally normal became distal polarity in the inversion. It seems then that polarity is intrinsic to the local region of the chromosome and not imposed from a distance.

The question whether polarity in recombination is intragenic, or whether it can extend from one gene to the next, has been investigated in N. crassa using the loci met-7 and met-9. These loci are believed to be separate but closely linked (Murray 1970). The polarity pattern within the met-7 gene is strongly distal in the distal part of the gene, becoming weakly proximal at the proximal end. The met-9 is distal to met-7 but the strong distal polarity at the distal end of met-7 does not extend into met-9 . The met-9 mutants show a lower frequency of conversion than distal met-7 mutants. The conversion frequencies are as follows:

— centromere — intermediate — low — high — intermediate —
(met-7) (met-9)

When his-1 mutants of N. crassa are crossed, genetic differences in stocks can be found for determining the frequency of wild-type recombinants (Jessop and Catcheside 1965). The differences have been attributed to genes called rec. One allele for low frequency, rec-1$^+$, is dominant, the recombination frequency from the homozygous rec-1 cross being higher by more than ten-fold. In the rec-1 homozygote there is proximal polarity for recombination of his-1, and in rec-1$^+$ the polarity is distal. Loci of various recombination repressor genes have been found that affect recombination at other unlinked sites in addition to those where their effect was discovered (Smith 1966; Catcheside 1966; Angel et al. 1970). Polarity is affected in the neighbourhood of the sites of action of the repressors.

The rec-2$^+$ recombination repressor in N. crassa reduces the frequency of recombination of mutants at the his-3 locus and reverses the polarity of this gene from distal to proximal. There is also a genetic factor, called cog, at 1 unit distal to his-3, that affects the sensitivity of his-3 mutants to the repressor. The dominant allele, cog$^+$, produces a higher frequency of recombination of his-3 mutants than the recessive cog by a factor of about seven when recombination is derepressed by

having rec-2 homozygous. In addition polarity is affected, cog giving a weak proximal polarity. When recombination is repressed, by rec-2^+, the cog alleles have no effect (Angel et al. 1970).

The rec-3 locus has three alleles rec-3^+, rec-3^- and rec-3^L (Catcheside 1975). rec-3^+ represses recombination in am-1 and his-2. The relative yields of recombinants with rec-3^+, rec-3^L and rec-3 are 1, 8 and 25, respectively, for am-1 and 6, 6 and 40, respectively, for his-2. i.e. rec-3^L is similar to rec-3^+ in repressing recombination in his-2, but less efficient than rec-3^+ in am-1.

An interesting phenomenon occurs in crosses heterozygous for cog. If the his-3 mutant associated with cog$^+$ is the distal member of the pair, the polarity is distal and in those crosses in which the his-3 mutant is proximal, the polarity is proximal. i.e. cog$^+$ promotes conversion in the his-3 gene in the chromatid in which it occurs, irrespective of the position of the his-3 mutant involved (Angel et al. 1970).

In the mutant TM429 the distal quarter of his-3 together with the cog site are transferred to another linkage group. When TM429 is crossed with distal alleles in a cog$^+$ homozygote a rec-2 derepression raises the level of his-3 recombination about twenty-fold, i.e. the result is normal. If the structurally normal chromosome is cog

the response to derepression is less and the fraction of the recombinants with recombinant flanking markers is increased (Catcheside and Angel 1974). When TM429 is crossed with proximal alleles derepression raises the level of recombination to the normal extent, when cog$^+$ is homozygous, despite the site of the structural change lying between cog and the region where recombination is being promoted. When the structurally normal chromosome has the cog genotype there is no promotion of recombination when derepression occurs.

In N. crassa the am-1 gene codes for NADP-specific glutamate dehydrogenase (Fincham 1974). In pairwise crosses of am-1 mutants in the presence of flanking markers, one of the non-crossover flanking marker genotypes (P1) is more frequent among random recombinant wild-types than the other flanking marker genotypes (P2) in almost every cross.

Drosophila melanogaster:

Aberrant segregation at garnet, an eye-pigment locus, in Drosophila melanogaster has been found in females heterozygous for pairs of mutants which map about 22 units from the centromere of the X-chromosome. Six garnet mutants have been used in these pairwise crosses in the presence of flanking markers (Chovnick 1958, Hexter 1958). Half tetrad analysis was permitted by using strains

with the pair of X-chromosomes linked at the centromeres. It is evident from the results that much, if not all, recombination between the mutants originally studied arose from conversion, but later studies proved that recombination could also occur as a result of crossing over (Chovnick 1961).

Another mutant closely linked to the X-chromosome centromere of D. melanogaster is ma-1. Mutants of this gene have brown eyes and are deficient for xanthine dehydrogenase (XDH). Selection for wild-type recombinants is possible because aqueous purine solution selectively kills the mutants. About 40 % of these recombinants showed flanking marker crossing over even though the markers were only 4.2 units apart on the linkage map (Finnerty et al. 1970). This technique has been applied to X-attached strains and two complementary ma-1 were used to give normal eye colour but low XDH activity. From several million progeny only twenty nine wild type recombinants were recovered. Conversion to wild type at the site of the proximal mutant with respect to the centromere was responsible for at least half of the recombinants, and conversion to wild type at the site distal mutant for the remainder. None of the recombinants arose as a result of crossing over between the sites because there was an absence of double mutant chromosomes.

The structural gene for XDH is rosy and is

situated at about 5 units from the centromere on right arm of chromosome III of D. melanogaster. Using strains with two right arms of chromosome III permitted tetrad analysis (Chovnick et al. 1970). At least twelve of the twenty three recombinants from over two million progeny arose through conversion and at least one through crossing over between the sites of the alleles involved in the pairwise cross. With increasing site separation the events at the rosy locus, that gave wild-type recombinants, were more often associated with flanking marker crossing over Chovnick et al. 1971).

Similar observations have been obtained for other loci and also there has been evidence of postmeiotic segregation because of mosaic individuals. In addition strong proximal polarity has been found at the r locus giving rudimentary wings (Carlson 1971).

Co-conversion was discovered in D. melanogaster at the rosy locus using electrophoresis to determine the position in the gene of the sites of difference between the wild-type, i.e. there was co-conversion of the mutants and the electrophoretic site (Gelbart et al. 1974).

Conversion-defective mutants are difficult to detect as are exchange defective mutants, especially in Drosophila where the low chromosome number insures that even mutants that abolish exchange, and hence chiasmata, produce a relatively high proportion of euploid gametes

from random segregation of homologues.

Mutants mei9 and mei218, appear to uncouple the two processes of gene conversion and crossing over (Carpenter 1982). Crossovers for both are decreased tenfold throughout the genome, whereas conversion recombinants are not decreased. Experiments have been performed to see the effect on co-conversion tract lengths. In mei9 there seems no difference in co-conversion tract but in mei218 the tract is shorter by a factor of two (Carpenter 1984). Whilst co-conversion distances are shorter in mei218, the absolute frequency of conversion events measured as intragenic events without exchange of flanking markers is two-fold higher. This implies that the frequency of initiation of events is increased relative to the controls, i.e., the function of the mei218^{+} product is necessary for crossing over but not for gene conversion. Unfortunately these results are not significant statistically because of the small numbers of convertants recovered.

Carpenter (1975) discovered structures, which she called recombination nodules, associated with the synaptonemal complex in D. melanogaster. Approximately spherical, about 100 nm in diameter, their positions on chromasomes corresponded with crossover numbers and distribution. There is confirmation of the existence of recombination nodules in other organisms (Carpenter 1979b;

Rasmussen and Holm 1980).

Four years after first being found, a second type of recombination nodule was reported which was ellipsoid and did not display interference like the spherical type as expected if it corresponded to crossovers (Carpenter 1979a). It has been postulated that these ellipsoid nodules are the precursors of the spherical ones which comprise the enzymes and DNA-binding protein required for recombionation (Carpenter 1982).

The similarities between Drosophila and fungi include: conversion of either mutant, with or without associated crossing over; postmeiotic segregation; crossing over between the sites of the alleles; co-conversion; polarity; and the occurrence of all these events within a group of alleles.

*

RECOMBINATION MODELS

By looking at the genetic composition of spores of eight-spored fungi or by analysing other organisms for sectoring/mosaicism, one can determine the genetic constitution of each DNA strand of each chromatid present immediately following meiosis (Leslie and Watt 1984). In this way one can determine the events during meiosis and come to some conclusions regarding the progress of events and thus form some opinions about the actual process of

recombination. Not until some means of actually studying the event visually (or otherwise directly) will there be only one proposed and accepted model, and even then there may be some arguments and discrepancies as predicted by one of the leading workers on recombination, Radding (1973),

> "The complex interactions among recombination, replication and repair, make it unlikely that any single model of recombination will ever suffice."

Recombination Features:

Aberrant segregations fall into three classes:

(1) The information present on one chromatid is lost and replaced with the corresponding information from another chromatid. Such a nonreciprocal transfer of information is termed gene conversion and yields 6:2 and 2:6 segregations in eight-spored asci.

(2) The second is where information present on a single strand of one DNA duplex is replaced by information from one strand of another chromatid. This event produces a DNA duplex in which the two strands contain different information for the segregating marker. Heteroduplex DNA with a mismatch is not detected genetically until a round of DNA replication produces two new duplexes, each effectively copied from a single strand. Such segregations

are called postmeiotic segregation and produce 5:3 and 3:5 classes of segregation.

(3) Aberrant 4:4 segregation is a double postmeiotic segregation due to heteroduplex DNA in two chromatids following meiosis.

Heteroduplex DNA can be of two distinct types, depending on whether it occurs on two chromatids for the same site at the same time, when it is termed symmetric, or whether it only occurs on one chromatid at the site in question, when it is termed asymmetric. There exist models of recombination for both of these types of heteroduplex and also models where only one of these types exist; these models will be discussed later.

Many geneticists insist on distinguishing postmeiotic segregation from gene conversion (e.g. Orr-Weaver and Szostak 1985), but this may lead to dividing a subject which rightly ought to be considered under the more general title of non-reciprocal recombination. In this work the term conversion will be more broadly used to include both postmeiotic segregation and the classical gene conversion which leads to |6:2| type segregation. This is also less confusing when one starts to talk about co-conversion where, under the rejected classification of conversion/postmeiotic segregation, no conversion may be taking place.

One way of distinguishing between symmetric and

asymmetric heteroduplex is that symmetric heteroduplex results in two classes of |5:3| segregation events, 'tritype' and 'tetratype' (see page 26). At buff in Sordaria brevicollis (Sang and Whitehouse 1979), w-17 in A. immersus (Stadler and Towe 1971), and SUP6 in S. cerevisiae (DiCaprio and Hastings 1976) 'tetratype' |5:3| segregations represent less than 5 % of the |5:3| events. But, at the gray locus of Sordaria fimicola, where symmetric heteroduplex is observed, two-thirds of |5:3| segregations are 'tritype' and one-third are 'tetratype' (Kitani and Whitehouse 1974).

Postmeiotic segregation is thought to be a result of either asymmetric heteroduplex DNA or symmetric heteroduplex DNA where one of the two chromatids which has mismatches undergos repair. Several experiments indicate that asymmetric heteroduplex DNA is often formed (see Whitehouse 1982, for references). Aberrant 4:4 segregations are diagnostic of the presence of symmetrical heteroduplex DNA. Aberrant 4:4 asci are quite rare in S. cerevisiae compared with the frequencies predicted from the appearance of 5:3 and 3:5 segregations (Fogel et al. 1979) suggesting that in yeast at this locus symmetric heteroduplex is rare.

Finally if symmetric heteroduplex is formed, the originally wild-type chromatid is corrected to mutant while the originally mutant chromatid is corrected to wild

type, and if no associated crossovers occur, then the resultant progeny look like double crossovers involving two chromatids. In the absence of symmetric heteroduplex, double crossovers involving two, three, or four chromatids are predicted, in the absence of chromatid interference, to occur in a ratio of 1:2:1. No excess of two strand double crossovers within short intervals is observed in S. cerevisiae and so symmetric heteroduplex is thought to be rare in this yeast (Fogel et al. 1979).

By these criteria little symmetric heteroduplex DNA is found in S. cerevisiae for all loci tested (Orr-Weaver and Szostak 1985). The buff locus of S. brevicollis and the w-17 locus of A. immersus also have little symmetric heteroduplex DNA. In contrast, symmetric heteroduplex DNA is common in the b2 locus of A. immersus and the gray locus of S. fimicola. Any accepted single model of recombination will therefore have to account for the existence of both asymmetric and symmetric heteroduplex DNA.

The existence of co-conversion, discussed separately under the individual organisms above, suggests that heteroduplex not only occurs as long tracts of DNA but also with increasing distance the chance of the heteroduplex continuing decreases.

Frequencies of gene conversion within a gene tend to reflect the position of an allele in that gene.

For genes in which a large number of alleles have been studied, frequencies tend to be higher for alleles at one end, decrease to the other end and sometimes even begin to rise again at the other end (polarity reversal). The existence of polarity is used to postulate the existence of fixed sites of initiation of recombination. The probability of an allele being involved in a recombination event is thought to decline as a function of its distance from the initiation site (Holliday 1964). In the case of polarity reversal (e.g. as found in the lysF gene of A. nidulans, Pees 1968) it is postulated that there is an initiation site at each end of the gene.

In studying the polarity of the b2 locus of A. immersus it has been found that the locus shows polarity both in overall conversion frequencies and in the relative frequency of aberrant 4:4 to |5:3| segregations (Paquette and Rossignol 1978; Rossignol and Paquette 1979; Rossignol et al. 1978). This has been suggested to indicate that the low conversion end of the gene contains symmetric heteroduplex DNA more frequently than the high conversion end . It follows from this argument that the heteroduplex DNA at the initiation site is asymmetric and the further it moves the more chance it has of becoming symmetric.

Whether this tract of symmetric/asymmetric heteroduplex is continuous was determined by Rossignol and

Haedens (1980). They used crosses in which one chromosome contained two mutations, one from each end of the b2 gene, and the other chromosome was wild type. The two alleles both showed high postmeiotic segregation frequencies. Aberrant segregation of the allele at the high conversion end was most often a single event, not accompanied by aberrant segregation of the allele at the low conversion end of the gene. On the other hand, aberrant segregation of the allele at the low conversion end was frequently accompanied by aberrant segregation of the other allele. Of the aberrant 4:4 events detected, the vast majority were at the low conversion end, and 50 % of these occurred with simultaneous |5:3| postmeiotic segregation at the high conversion end. From the low correction frequencies of both alleles, both are expected to give frequent aberrant 4:4 segregation if they are covered by symmetric heteroduplex DNA. The preponderance of |5:3| segregation at the high conversion end suggests that, when asymmetric heteroduplex DNA is initiated it somehow switches to symmetric heteroduplex DNA in moving down the gene. This switching from asymmetric to symmetric heteroduplex has been supported by other work on the b2 locus (Hamza et al. 1981).

The frequency of crossing over associated with aberrant segregation varies widely even between alleles of the same gene (Fogel et al. 1979). In addition there is

evidence that crossover frequencies can differ between the types of segregation event (Sang and Whitehouse 1983; Nicolas 1982). Analysis of the position of a crossover relative to an aberrant segregation event in a locus with polarity provides information on the relationship between the two. If heteroduplex is propagated from an external initiation site, into the locus, and a crossover occurs at the endpoint of the heteroduplex tract, then the polarity of postmeiotic segregation events is expected to be associated with crossovers. However, the majority of data seems to show quite the opposite, in that the high postmeiotic segregation end of a gene is more often associated with crossing over than the other (Sang and Whitehouse 1983; Theivendirarajah and Whitehouse 1983). Thus a model for recombination must explain the positions of associated crossovers as well as explain their existence.

Questions asked about recombination initiation sites (Orr-Weaver and Szostak 1985) include:

(i) do the initiating lesions occur on the strand that is the donor or the recipient of genetic information ? and

(ii) do the initiating lesions occur at specific initiation sites or merely in the vicinity of such sites ?

Mutations which appear to generate recombination initiation sites provide opportunities for answering these

questions. The M26 mutation of S. pombe and YS17 of S. brevicollis are thought to be such mutations.

M26 gave a greatly enhanced conversion of linked alleles. It has been suggested that the DNA at the M26 site preferentially undergoes single-strand breakage, thereby initiating recombination at this site (Gutz 1971). cis/trans and homozygous crosses for M26 have indicated that in the homozygote M26 acts independently as an initiation site in chromatids of each parentage to give an additive result, like cis and trans crosses superimposed (Goldman and Smallets 1979). It is therefore obvious that the initiation of recombination of M26 is a direct result of its nucleotide sequence and not a result of the interaction between the sequence and the wild type sequence. YS17 acts in all things like M26 (MacDonald and Whitehouse 1979) and so is also considered to be an initiation site for recombination.

If M26 and YS17 are mutations that act as recombination initiators, as the evidence suggests, they must act as a recipient of a nucleotide chain from the other parent, and not a donor to it. This is explained because they both show high frequency conversion to wild type rather than to mutant.

A naturally occurring mutation, rec1, is observed to depress the frequency of YS17 conversion. The gene is recessive, unlinked and is postulated to encode an

endonuclease that acts on the YS17 nucleotide sequence to initiate recombination.

The mutant *cog*$^+$ in *N. crassa* behaves analogously to M26 and YS17 in being the recipient rather than the donor of genetic information.

cog$^+$ is a dominant promoter of recombination. It has been suggested that the site is a recognition site for an enzyme that initiates recombination. It has further been postulated (Angel *et al*. 1970) that the repressor, the product of *rec-2*$^+$, acts on a control site situated either -

(1) alongside the structural gene for a nuclease postulated to initiate recombination at *cog*, or

(2) near *his-3*.

The repressor would then block either -

(a) the synthesis, or

(b) the action of the nuclease, respectively.

The idea that the repressor acts directly on *cog* is not likely because of the lack of the *cog* difference in a *rec-2*$^+$ background.

A distinction between these hypotheses has been made with the *rec-3*^{L} allele (see above under *Neurospora*). It has been suggested that the repressor blocks the action rather than the synthesis of the nuclease, the control sites near *his-2* and *am-1*, to which the repressor is presumed to bind, differing slightly in nucleotide sequence

(Catcheside 1975). Evidently the recombination operators, to which the repressor binds, are in the chromosome regions where recombination is affected and not at the structural gene for a nuclease. Applying this to rec-2, there is a recombination operator near his-3 to which the rec-2$^+$ repressor bound, thereby blocking the action of the nuclease at cog. It has further been implied that a single nuclease might be responsible for all the initiation of recombination, at least in Neurospora (Whitehouse 1982).

The results relating to crosses heterozygous for cog$^+$ and cog indicate that the molecule of DNA which is recognised initially becomes the recipient of a strand from the other parent rather than the donor of a strand to it. Results with TM429 suggest that cog$^+$ is very strongly competitive over cog as the site for initiating recombination.

Behaviour of cog$^+$ induced recombination in strains containing a translocation of the distal segment of his-3 and the cog site is important. When a his-3 allele on the same side of the break point as cog was crossed to the his-3 translocation strain, cog$^+$ exerted a normal stimulation of recombination when homozygous. However, a derepressed level was observed if the strain was heterozygous for cog/cog$^+$, with the normal chromosome being cog. This is consistent with initiation at cog$^+$ but not at cog. If the his-3 allele is proximal, cog$^+$ still

stimulates recombination, but the production of recombinants requires that *cog*$^+$ be on the normal chromosome. Therefore, in the recombination event mediated by *cog*$^+$, the information transferred is able to skip across a translocation breakpoint. This excludes a mechanism in which the heteroduplex DNA is initiated at *cog*$^+$.

To account for recombination events initiated at *cog*$^+$ not being blocked by a chromosomal discontinuity, it has been suggested (Catcheside and Angel 1974), that an endonuclease nicked one strand of the duplex at *cog*$^+$ and that nick migration then took place, that is, the site of the nick moved along the chromatid as a result of exonucleolytic breakdown ahead of it and DNA synthesis behind it, using the other strand of the duplex as template. If the initial single-strand break was at *cog*$^+$ in the structurally normal chromosome and if the nick migration was in the direction of *his-3*, once the nick had passed the site of the TM429 discontinuity in the other chromosome, heteroduplex formation could take place and lead to recombination of the *his-3* mutants. The fact that this promotion of recombination did not occur when the structurally normal chromosome was of *cog* genotype confirmed that *cog*$^+$ was strongly competitive over *cog* as an initiation site, and showed that initiation was not taking place elsewhere in the *his-3* region when *rec-2*

derepression occurred.

A different explanation of the TM429 data is migration of the endonuclease enzyme without nicking (Markham and Whitehouse 1982). This hypothesis also explains why cog$^+$, though favoured over cog for recognition, promotes conversion in its own chromatid (as though it were a recipient and not a donor of a strand). If the endonuclease which recognises cog$^+$ nicks the homologous molecule, that is, of cog genotype, the latter would become the donor of a strand. But if this nicking was delayed until the endonuclease was beyond the TM429 site, the failure of this mutant to block derepression of recombination with proximal alleles could be accounted for.

The migration of an enzyme along the DNA from a recognition site offers an explanation of several otherwise unexplainable features of recombination in fungi. It can explain the occurrence of crossing over between the sites of alleles without either site apparently being included in hybrid DNA. Such crossing over is frequent in some genes (Sang and Whitehouse 1983; Savage and Hastings 1981; Whitehouse 1982). To account for polarity it is necessary to postulate fixed sites of initiation of recombination outside the genes: enzyme migration solves this paradox by allowing initiation outside the gene manifesting itself only once it is within

the gene.

There is evidence of extragenic initiation of recombination for intragenic crossing over in gene 46 of A. immersus (Lissouba et al. 1962). Mutant 277 showed crossing over in the interval between its site and the mutant 63 to its left, only if mutant 138, mapping to the right of 277, was not present. For crossing over to occur to the left of 277 it seems to be necessary for 277 to be the right-hand member of those crossed. It is necessary for 63 and 138 to be right-hand members in order to show conversion, and for 277 to be a right-hand member in order to show postmeiotic segregation. The inference is that recombination has to be initiated at the right-hand end of the gene, not only to account for this polarity in conversion but also to account for crossing over to the left of mutant 277.

Enzyme migration before heteroduplex formation may also explain mutant specificity of crossing over between alleles. As there is no hybrid DNA at either mutant site with such crossing over, it is not obvious how the mutant specificity comes into being. If a mutant has a molecular structure that interferes with heteroduplex formation, selection for recombinants might imply selection for events where enzyme migration moved heteroduplex initiation beyond the site of the mutant. There is some evidence in A. immersus (Whitehouse 1967)

that mutants with a high specificity for crossing over are deletions and it is in such large regions of heterology that heteroduplex formation may be expected to be unfavourable.

A third reason for assuming enzyme migration before heteroduplex formation is that recombination of distal origin may bring about conversion at a proximal site, and similarly, a recombination of proximal origin allows conversion at a distal site. This obviously makes it necessary to reconsider the reasons for polarity reversal. Rather than polarity reversal being a result of two different initiation sites at either end of the gene, a single initiation site may be responsible and the migration of the enzyme then specifies where the heteroduplex is formed.

The conversion control factors in A. immersus, K, P and 91, which are closely linked to wI have been proposed to be initiation sites for recombination. It has been suggested that if the initial step in recombination were the asymmetric invasion of one chromatid by a nucleotide chain from the other, part of the variations which have been observed in conversion patterns might be an indirect consequence of variations in the invasion frequencies of each nucleotide chain of each chromatid (Lamb and Helmi 1978). It is also necessary to postulate that the control factors also affect mismatch repair. It

has been suggested that the K, P, 91 site (ccf-2) acts in the same way as proposed for the cog site in Neurospora, an endonuclease recognising different nucleotide sequences at the site with different efficiencies (Whitehouse 1982). Two unlinked genes, ccf-3 and ccf-4 are able to enhance or reduce conversion frequencies at wI (Helmi and Lamb 1983). The alleles at ccf-3 are E and e (for enhancer, enhancement being dominant) and at ccf-4 the alleles are R and r (for reducer, reduction being dominant). The probable explanations of these unlinked genes are that they are similar to rec1 in S. brevicollis which is also unlinked and observed to depress the frequency of YS17 conversion. The gene rec1 is recessive and is postulated to encode an endonuclease that acts on the YS17 nucleotide sequence to initiate recombination. Why there should be two such genes in Ascobolus is not known nor why one should be recessive and the other dominant for the character; perhaps this is a clue.

Mitotic and meiotic recombination differ so much that it would not be wrong to conclude that they are differently controlled events, being different even in their mechanics. Such differences include: the yeast mutants which are specific to only one of the cell division types (Fogel and Roth 1974), and the same gene having a different polarity and conversion spectrum in the two types of division (Hurst and Fogel 1964).

Recombination Models:

In this introduction the Holliday (1964) and Meselson-Radding (1975) models of recombination will be discussed in relation to observations from mitotic and meiotic recombination. In addition the latest model, adapted from yeast plasmid recombination, will be described and commented on.

The Holliday Model

Holliday (1964) proposed that two homologous chromatids are nicked at the same place on strands having the same polarity (3'-5' or 5'-3'). The strands were then supposed to separate over an unspecified distance and pair with the unbroken strands of the other unwound chromatid to form a heteroduplex DNA (see Figure 1,a). If the resulting symmetric heteroduplex DNA formed by this strand exchange has a mismatch which remains unrepaired, aberrant 4:4 segregation occurs. Repair of one mismatched chromatid DNA molecule results in |5:3| segregation, and unidirectional repair of both heteroduplexes results in |6:2| segregation. Repair of both chromatids in opposite directions results in normal 4:4 inheritance. In this way classical conversion occurs by mismatch repair and postmeiotic segregation occurs as a result of at least one mismatch remaining unrepaired.

The Holliday model accounted for crossing over

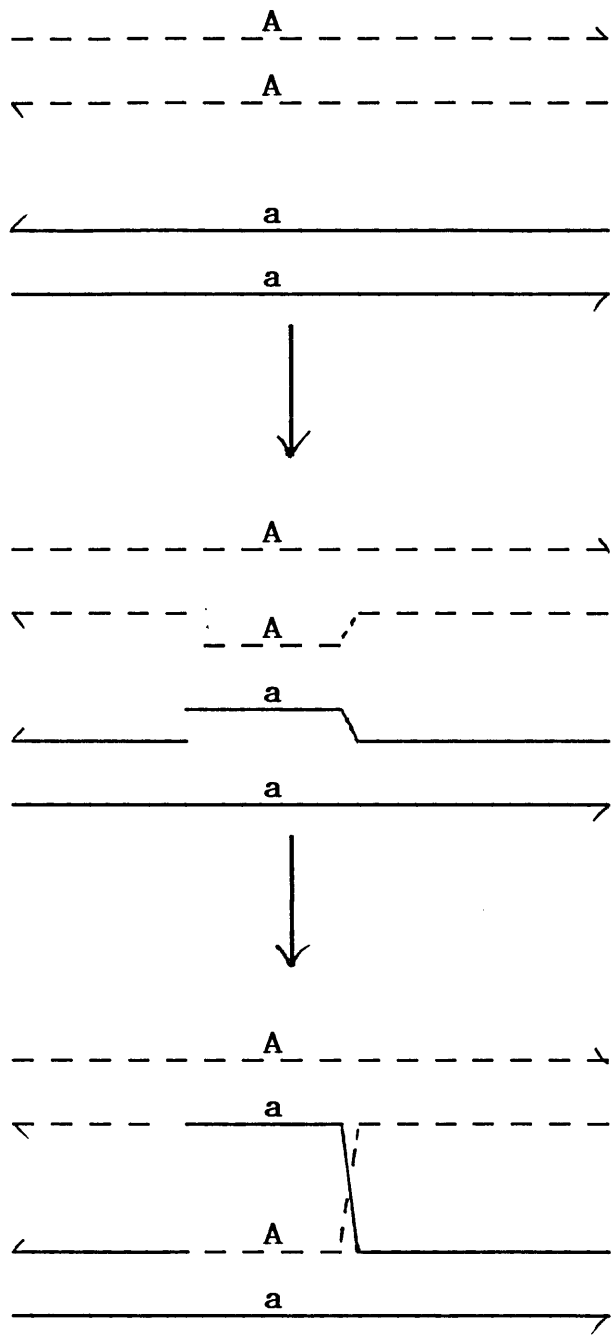


Figure 1,a.

Holliday model for recombination. Strands of the same polarity are nicked at homologous sites and are then exchanged to produce symmetric hDNA. The crossed strand, or Holliday junction, can be resolved either with or without exchange of flanking markers.

by the formation and resolution of a Holliday structure, i.e. a cross-strand or half chromatid chiasmata. The resolution of the Holliday structure was postulated to occur by cutting and resealing of the non-crossed strands or by resealing of the crossed strands to give an associated crossover. Cleavage and re-ligation of the 'outer' strands causes a reciprocal exchange of flanking markers (see Figure 1,b).

Molecular modelling showed that such junctions could be formed with all bases of both duplexes remaining paired (Sigal and Alberts 1972). The crossed and uncrossed strands of the junction are equivalent, being interconvertible by isomerisation without the need to break bonds. If this is truly the case then the cleavage of the Holliday junctions is predicted to yield crossover and non-crossover products at equal frequencies. Because the cross connection was supposed to diffuse along the helices, with bases of one chromatid being exchanged with the other, it was realised that branch-migration would generate tracts of heteroduplex, the length of which could vary with certain dependants (Meselson 1972).

The Meselson-Radding Model

This model proposed in 1975 takes the Holliday model one step further in that strand exchange is initiated on only one chromatid, and strand transfer is

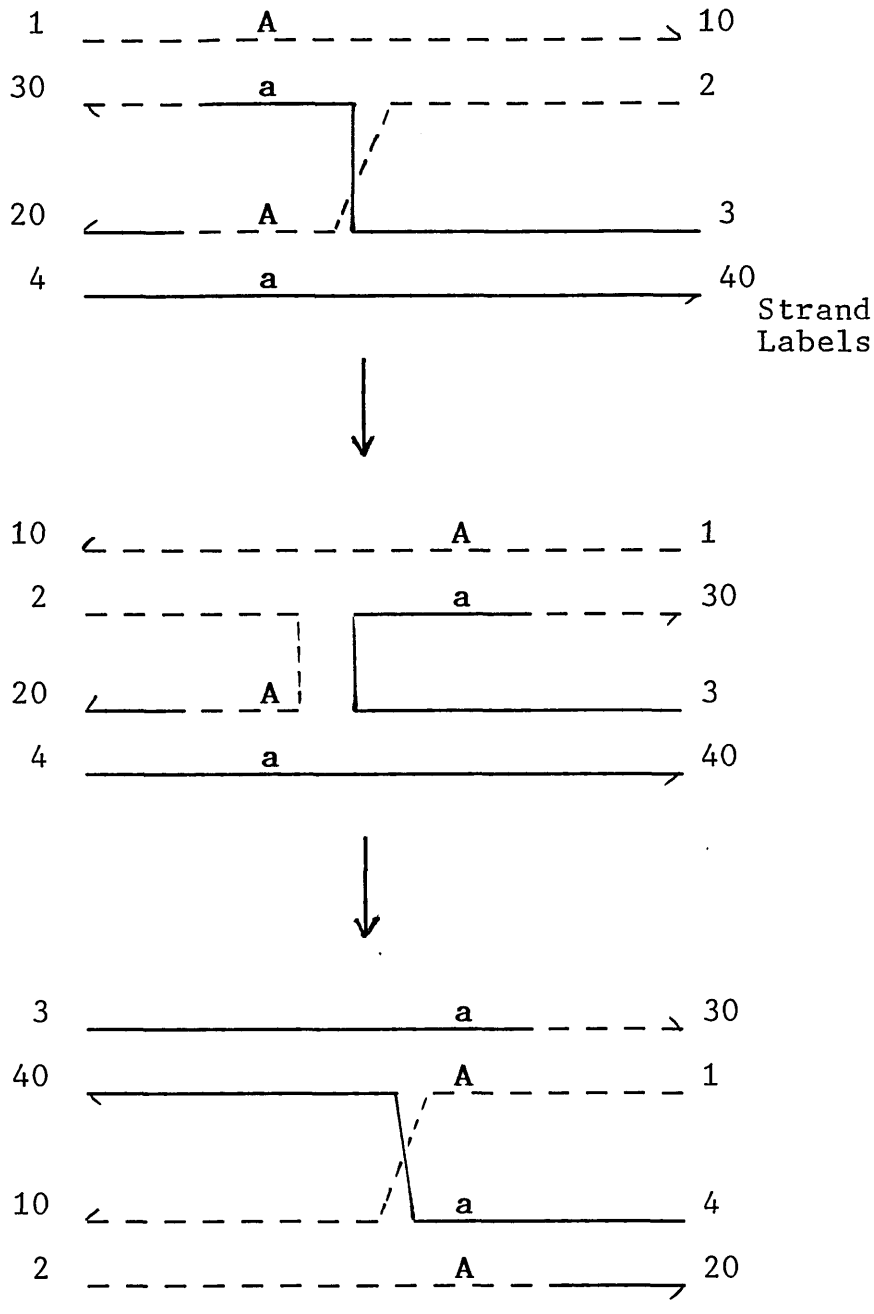


Figure 1,b.

Isomerisation of a Holliday junction. The Holliday junction can isomerise through a symmetrical intermediate without bond breakage. Therefore, resolution can occur by cutting either the originally crossed strands or the originally non-crossed strands. The numbers to the sides act as labels to the strand ends.

driven by DNA synthesis. Asymmetric heteroduplex DNA, symmetric heteroduplex DNA and crossing over all follow from one initiation event.

Recombination is initiated by a nick on one chromatid. One end of the cut strand (considered to be the 3' end) now acts as a primer for DNA synthesis, displacing a single strand which then invades the homologous region of the other chromatid. The resulting D-loop is degraded and the invading strand is ligated in place. Asymmetric heteroduplex DNA can then be expanded by concerted DNA synthesis on the donor duplex and the exonucleolytic degradation of the recipient duplex (see Figure 2,a). After the enzymatically driven production of asymmetric heteroduplex DNA stops, either branch-migration or isomerisation can bring the single stranded ends into 'apposition' so they can be ligated. The resulting Holliday structure/junction can branch-migrate to form symmetric heteroduplex DNA. Resolution can yield either the crossover or non-crossover configuration just like in the original Holliday model (see Figure 2,b).

In summary, the Meselson-Radding model has two phases, one enzymatically driven asymmetric phase and the second a symmetric phase generated by branch-migration of a Holliday junction. As in the Holliday model, gene conversion may occur both as a result of mismatch repair and of non-repair.

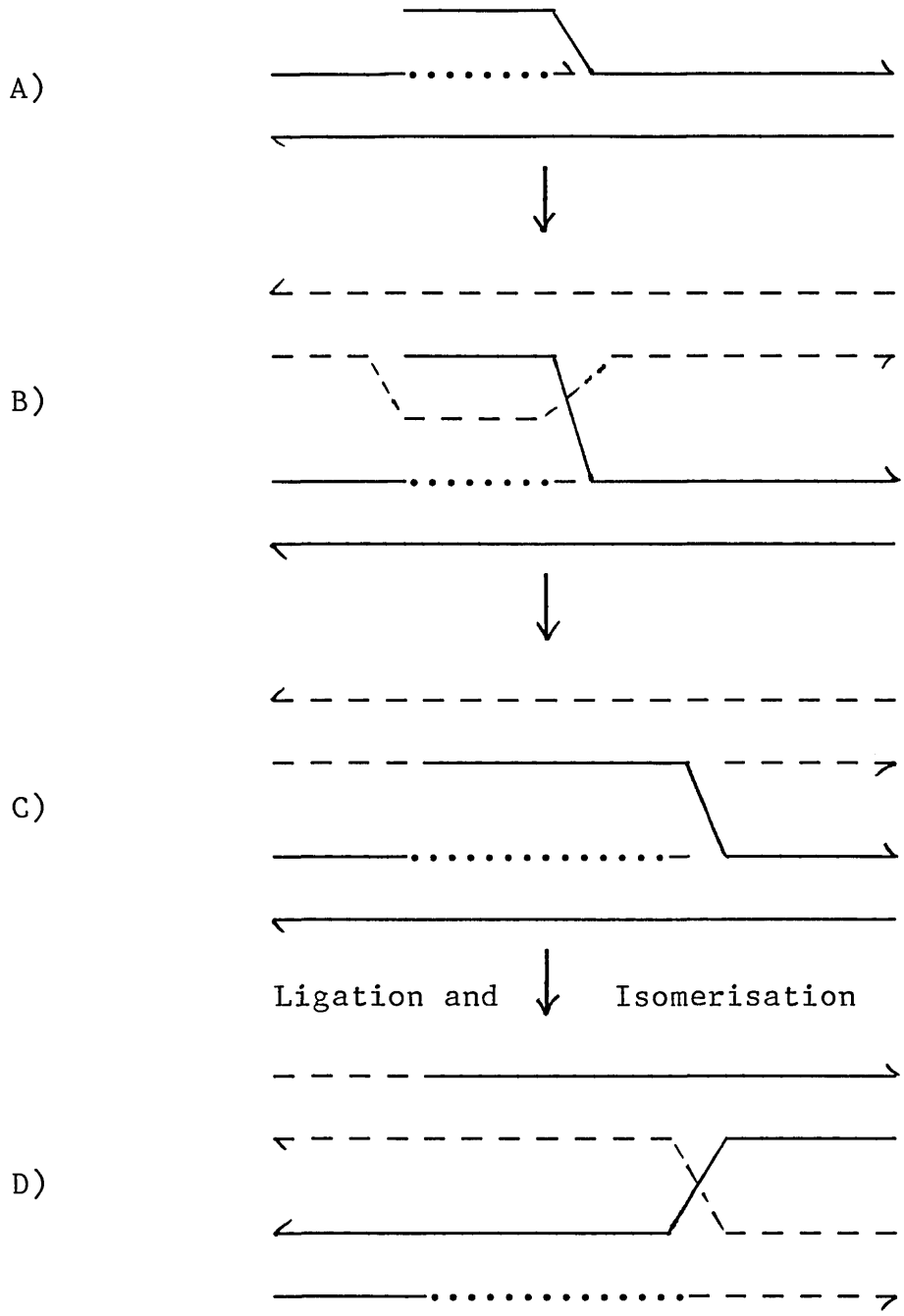


Figure 2,a. Meselson-Radding model. (A) Recombination is initiated by a single-strand nick which serves as a primer for DNA repair synthesis. (B) This displaces a single strand that can then pair with a homologous region on the other chromatid. (C) The resulting D-loop is degraded, and the asymmetric hDNA is enlarged by DNA synthesis on the donor chromatid coupled with degredation on the recipient duplex. (D) Branch migration and ligation of the nicks produces a Holliday junction which can be isomerised.

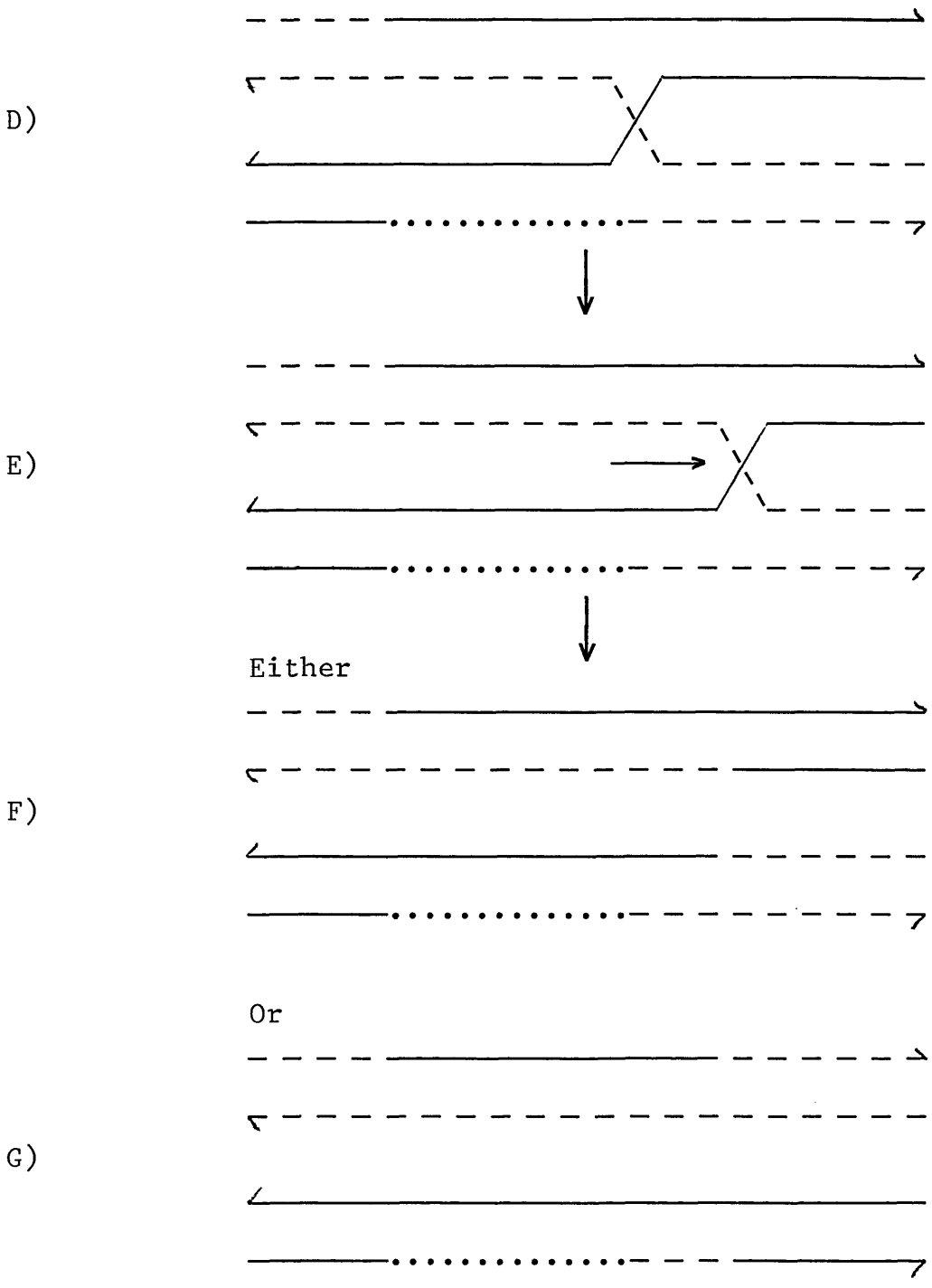


Figure 2,b. Resolution of the Meselson-Radding model intermediate. (D) Branch migration and ligation of the nicks has produced a Holliday junction. (E) Symmetric hDNA can be formed by branch migration (horizontal arrow) of the Holliday junction. Resolution with nicking of crossed strands (F) can yield either the crossover or with isomerisation the non-crossover (G) configuration.

The Double-Strand Break Repair Model

This model, first proposed in 1983 by Szostak et al., is based upon studies of yeast transformation with E. coli plasmids containing fragments of yeast DNA.

The double-strand break repair model (DSBR) differs from the Meselson-Radding model in four important ways and in some ways goes back to the antiquated copy-choice hypothesis of recombination:

(1) the recombination event is initiated by a double-strand break;

(2) the initiation chromatid is the recipient rather than the donor of information;

(3) gene conversion can result from transfer of two strands of information to a gapped chromatid without a heteroduplex intermediate (except in the proposed D-loop) or from mismatch in heteroduplex DNA adjacent to the region of gap repair; and

(4) the recombination mechanism produces a region of gene conversion flanked by two Holliday junctions.

The basic model is shown in Figure 3. Recombination is initiated by a double-strand endonucleolytic break. This is enlarged by exonucleases to a gap with 3' single-stranded ends. One of the free 3' ends then invades a homologous region on the intact chromatid, displacing a small D-loop. This D-loop is enlarged by repair synthesis primed by the invading 3' end. The enlarging D-loop, now

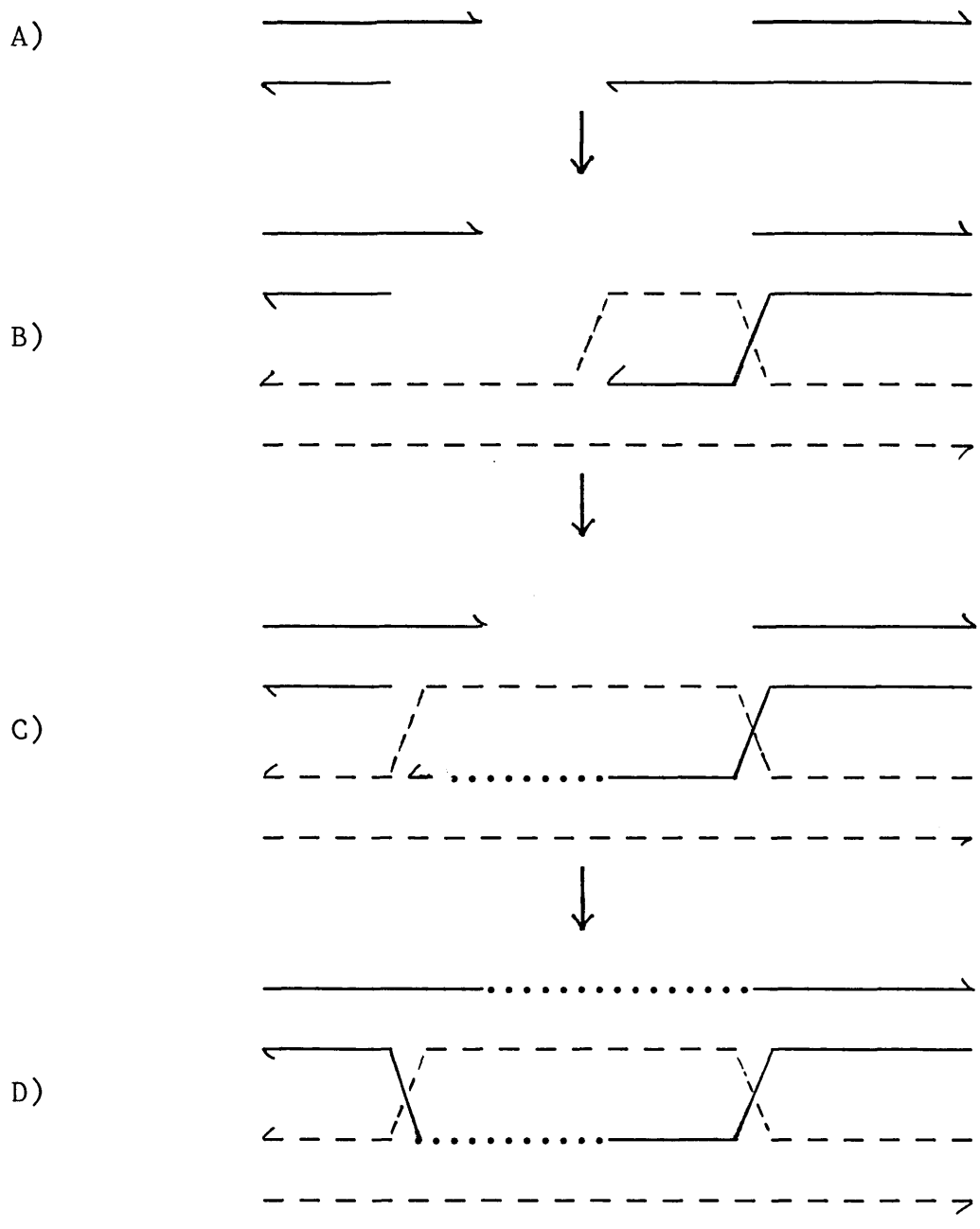


Figure 3. The DSBR model. (A) A double-strand cut is made in one duplex, and a gap flanked by 3' single strands is formed by the action of exonucleases. (B) One 3' end invades a homologous duplex, displacing a D-loop. (C) The D-loop is enlarged by repair synthesis until the other 3' end can anneal to complementary single-stranded sequences. (D) Repair synthesis from the second 3' end completes the process of gap repair, and branch migration results in the formation of two Holliday junctions.

effectively single-stranded, then pairs and serves as a primer for repair synthesis on the other strand. Thus the gap is repaired by two rounds of repair synthesis. There are two regions of asymmetric heteroduplex DNA formed by the pairing of the 3' ends of the gap, and two cross strand junctions flank the gap repair region. In the diagram the left-hand junction is identical to a junction in a Meselson-Radding model, so asymmetric heteroduplex on this side may be extended by concerted repair synthesis and exonucleolytic degradation. Branch-migration of the two Holliday junctions can form symmetric heteroduplex DNA.

Gene conversion can arise if a marker falls within a double-strand gap; in this case the marker is converted by a double-strand transfer of information. Alternatively, if the marker falls within either of the flanking regions of the asymmetric heteroduplex, mismatch correction will produce a |6:2| or correction 4:4 segregation. Heteroduplex DNA in which a mismatch is not repaired yields a postmeiotic segregation.

Gene conversion by double-strand gap repair results in an associated crossover when the two Holliday junctions are resolved in the opposite sense (i.e. cutting the inner strands of one junction and the outer strands of the other). Resolution in the same sense gives the non-crossover configuration of flanking markers.

Comparing Models:

In various guises the Meselson-Radding and the DSBR models can account for the vast majority of data obtained. The asymmetric heteroduplex DNA in S. cerevisiae can be explained by assuming that branch-migration of the Holliday junctions does not occur in those loci in yeast which fail to show symmetric heteroduplex DNA. The data available from A. immersus agrees well with the predictions made by the Meselson-Radding model. If in the DSBR model the double-strand gap is always small, perhaps only one or two base pairs wide, an event initiated by a double-strand break would be indistinguishable from a Meselson-Radding type of event (Whitehouse 1983).

The phenomenon of co-conversion can occur by one of three methods:

(1) Both alleles are included in a double-strand gap and thus are co-repaired together, or,

(2) A mismatch repair event occurs in which the excision extends over both mutations. This is the only explanation for one mutation being able to influence the segregation pattern of another. An allele showing predominantly |6:2| segregation for example, is recognised efficiently by the mismatch repair machinery and therefore is responsible for an excision event extending across adjacent alleles that normally show |5:3| segregation. Alleles with few |6:2|s and many |5:3|s are presumed not

to be efficiently recognised. The allele that normally gives |6:2| segregation will affect the segregation of an allele normally showing |5:3| segregation by altering the probability of mismatch repair. It has also been shown that alleles displaying |6:2| segregation and located on either the high or the low conversion side of an allele normally displaying |5:3| segregation can reduce the amount of postmeiotic segregation of that allele (Fogel et al. 1979). If recombination is initiated by an enzyme that recognises a site and then moves and initiates recombination at various distances from that site, the gap could be on either the high or the low conversion side of an allele. Thus an allele on either side could be included in heteroduplex DNA along with the site itself. Lastly,

(3) Asymmetric co-hybrid DNA can give co-conversion without co-excision and without repair and a possible neighbouring site effect exists in the influence on asymmetric heteroduplex DNA propagation.

Orr-Weaver and Szostak (1985) have argued that if conversion in A. immersus and S. cerevisiae occurs by the Meselson-Radding model then the mismatch repair systems must be quite different. This is hotly disputed by Lamb (1987). It is possible to argue that if conversion in both these organisms is a result of the DSBR model then the activities of exonucleases in A. immersus are low or strand invasion and annealing occur rapidly.

Many mutations thought to be base substitutions show mostly postmeiotic segregation in A. immersus, but this is rarer in yeasts. The observation that most types of mutations show approximate parity in yeasts, in contrast to the disparities often observed for insertions and deletions in A. immersus, would suggest that only the DSBR model functions in yeast (Szostak et al. 1983; Orr-Weaver and Szostak 1985) but, it is evident that there are exceptions in each of the two organisms discussed and therefore such an argument is not valid (Lamb 1987).

The primary result of double-strand gap repair leads to no postmeiotic segregation and parity is explained as an equal probability of initiation on either chromatid. Heteroduplex DNA resulting in conversion can lead to disparity without any complicated postulations, however, the restoration-substitution experiments performed (Hastings and Savage 1984; Savage and Hastings 1981) indicate that if conversion in yeasts occurs via heteroduplex DNA then the mismatch correction system has to recognise and repair in favour of the strand that is invading.

The DSBR model predicts equal and opposite disparity in restoration as in substitution. For the b2 locus the E1, E2 pair of closely linked point mutations found in A. immersus, it has been shown that substitution and restoration are equal (Hastings 1984; Hastings and

Savage 1984; Savage and Hastings 1981). In yeast a less exact method was used. Different alleles showed different substitution/restoration disparities with no apparent relationship between the disparity and the map position of the alleles but instead it was allele specific. Restoration is less common than substitution and casts doubt on the application to yeast of models of conversion by excision correction of mismatches in heteroduplexes.

There have been proposed two explanations for why restoration could be lower than substitution:

(1) There is the process of excision correction of heteroduplexes that exercises a preference as to which chain is excised according to its origin.

(2) The DSBR model involves short lengths of heteroduplex flanking the excised gap. Gap filling will lead only to substitution, whereas excision repair in the heteroduplex length can lead to substitution or restoration, resulting on average more substitution than restoration.

Szostak and his co-workers (1983) explain the alleles in yeast showing high postmeiotic segregation as exonuclease pause sites, which increase the probability of the gap ending near the mutant allele. However this hypothesis would predict that highly postmeiotic alleles would impose a high frequency of postmeiotic segregation on alleles with normally low postmeiotic segregation. The

opposite is the truth.

One may also question what is the allele specific nature of the excess of substitution over restoration, which implies that the decision as to substitution or restoration is made on the basis of the nature of the mismatch and not on the basis of the marker within the event. Hastings (1984) suggests that a mismatch in heteroduplex yeast may incite the cutting of one or both DNA chains. If one chain is cut, conventional excision repair leads equally to substitution and restoration. If both chains are cut, the resulting double-strand gap is filled by substitution, taking information from the homologue as has been demonstrated for the integration of a gapped plasmid (Orr-Weaver et al. 1981).

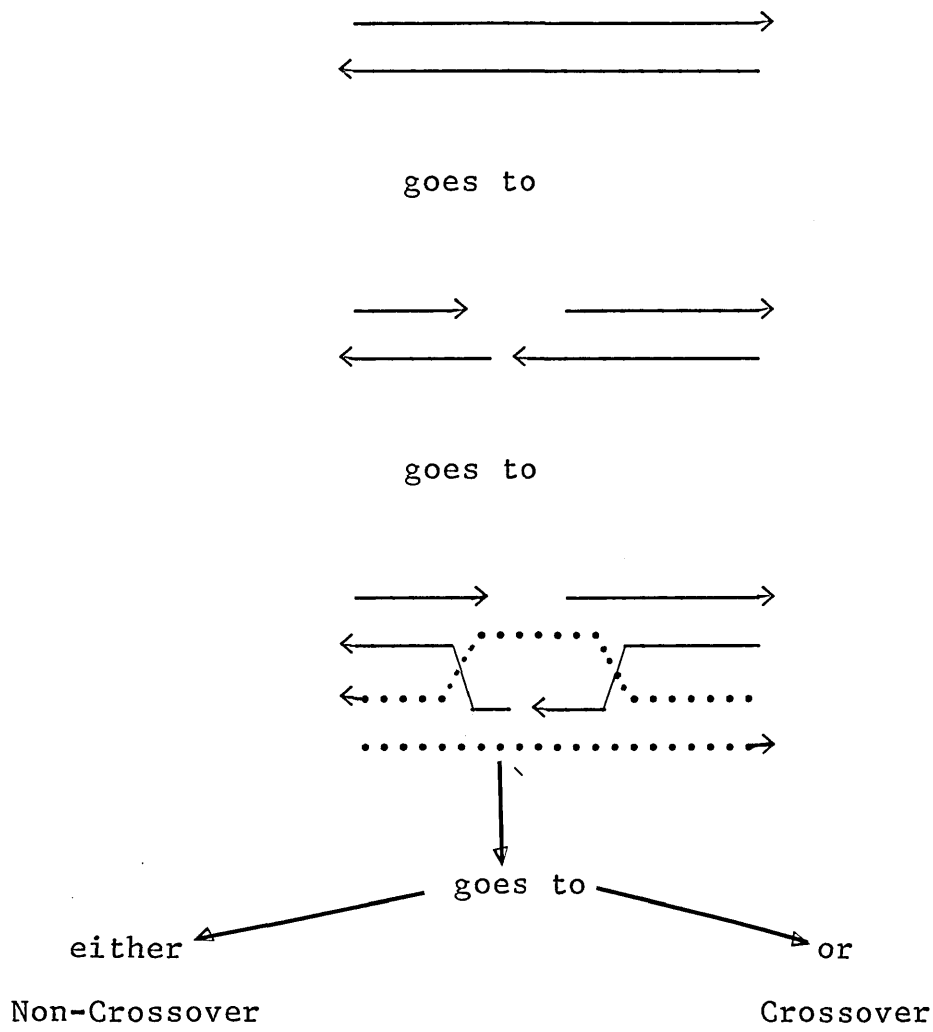
Nuclease alpha from U. maydis has been shown to cut DNA at a mismatch making a mixture of one- and two-stranded cuts (Holliday et al. 1979). Similar enzyme activity has been shown in Neurospora (Chow and Frazer 1983) and in yeast (Chow and Resnick 1983) although their activity on DNA containing a mismatch has not been reported. Many of the biochemical characteristics of some of the enzymes involved in recombination have been defined (Holliday et al. 1984).

In vitro analysis of bacteriophage T4 endonuclease VII has shown the enzyme to be capable of specifically resolving Holliday structures (Kemper et al.

1984). The enzyme acts by introducing a pair of cuts placed diagonally across the junction, as postulated *in vivo*, and depending upon the cuts, either gives reciprocal outside marker exchange or not. Cruciform structures, formed by Kemper *et al*. (1984), using artificially made inverted repeats, are similar to Holliday structures because of the presence of four arms branching from a common point. However, the torsional strain is not the same because the cruciforms used were supercoiled. It was assumed that endonuclease VII recognises crossing strands and cleaves these symmetrically at a distance from the point of crossover corresponding to one or two base pairs the distance being fixed by thermodynamic considerations. The enzyme's involvement in recombination seems to be evident in the deficiency caused in its absence (*mut49*$^-$). It is possible that topoisomerases are also important (Kmiec *et al*. 1983).

Rothstein (1984) has proposed that the initiation event of meiotic recombination is a double-strand break that is enlarged to a gap on one of the chromatids that interact during a gene conversion event. The two free ends invade the homologous region of one of the two non-sister chromatids and creates two Holliday structures on one on each side of the gap. The ends are shown to invade asymmetrically. Another version depicts

symmetric invasion with rapid branch migration of the double Holliday structure.



It is attempted to show how DSBR model can generate sectored colonies of ade6, an observation analogous to postmeiotic segregation.

Contrary to the report that in both A. immersus and S. cerevisiae large deletions show parity of conversion, Lamb (1986) has re-examined the Ascobolus and yeast data and shown that large deletions have shown significant disparity in both directions where sample sizes have been large. Care, however, must be taken in selecting data and it must be kept in mind that whereas one can detect disparity, that using statistics one will, with a selection of random deletions, on average find 5% significant disparity even where none exists. Even so the significant disparity was for many more than 5% of the deletions studied.

Because deletions are claimed to show parity in direction of conversion it has been proposed by Radding (1978) that repair synthesis of the single-stranded loop always occurs and that when the Holliday junction is resolved, conversion is equally likely to wild type or mutant. Parity would be explained where the heteroduplex DNA includes a large heterology. The double-strand break repair model can also explain parity of deletion conversions. If a gap is generated on the deletion chromatid and covers the position of the deletion it must lead to conversion to the deletion. This is because the

wild-type chromosome containing the gap cannot interact with the other chromatid until the wild-type sequences have been removed by exonucleolytic enlargement of the gap, so that sequences with homology to the other chromatid are exposed. In other words, the non-deletion chromatid section has nothing to pair with, having no homologous sequence in the deletion chromatid.

Both the Meselson-Radding model and the DSBR model can explain the frequency of associated crossing over. The position of this crossover has been explained by the DSBR model without the need for enzyme migration. It is important that crossovers in polar genes occur at either the high or the low conversion side of an allele that displays postmeiotic segregation. Because theoretically the two Holliday junctions formed by gap repair flank the site of segregation, the crossover may appear genetically to lie on either side of the site showing aberrant segregation. Unfortunately it has been overlooked in the past that such a hypothesis argues against itself in that such behaviour would also yield a very high proportion of double crossovers on either side of the heteroduplex DNA, and there is strong evidence against this.

In the Meselson-Radding model crossovers are predicted to occur only on the low conversion side of the gene. The data can be explained by the Meselson-Radding

model if one assumes that the Holliday junctions branch-migrate back towards the initiation site. In most yeast genes studied, this explanation would require the Holliday junction to branch-migrate in a unidirectional manner. Branch-migration away from the initiation site is assumed not to occur because symmetric heteroduplex DNA would be observed.

Initiation of recombination might occur as a result of an enzyme binding at a specific site and then moving along the DNA for a distance before nicking (Catcheside and Angel 1974). If asymmetric heteroduplex DNA can form to either side of the nick, the crossover can appear to lie on either side of the region of heteroduplex DNA.

Up to thirty five percent of associated crossover events have the crossover separated from a converted allele by an allele that shows normal 4:4 segregation. This is extremely difficult to explain by the Meselson-Radding model. As early as 1978 it was proposed that two Holliday junctions are produced (Holliday 1978) so that the converted allele lies in between the two junctions, just like in the DSBR model, and the unconverted allele is outside of the junctions. Branch-migration of the two junctions in the same direction until both have crossed over the second allele followed by resolution of the junctions, will produce the observed

pattern of inheritance. No satisfactory driving mechanism for this unidirectional branch-migration has been explained and any plausible hypothesis would argue against the presence of polarity reversal.

Because when duplex DNA reacts with intact duplex DNA the formation of a Holliday junction is impossible for topological reasons, one Holliday structure may initiate a single-strand break nearby favouring a second Holliday junction for topological reasons. This could explain why two Holliday junctions would arise from the Meselson-Radding model.

To explain this class of crossovers in terms of the Meselson-Radding model, an independent, though associated, second strand transfer must be initiated on the other side of the second allele, between the same two chromatids, and be resolved with a crossover. If such multiple, independent events occur there is also a need to explain why the expected high frequency of dual events (recombination at the same loci in the same cell but on different chromatids) does not occur. In other words this hypothesis suggests high negative interference which has not been found.

The low frequency of 'tetratype' |5:3| segregation cannot be explained by the DSBR model as originally presented. If asymmetric heteroduplex DNA is present on both chromatids, in the absence of mismatch

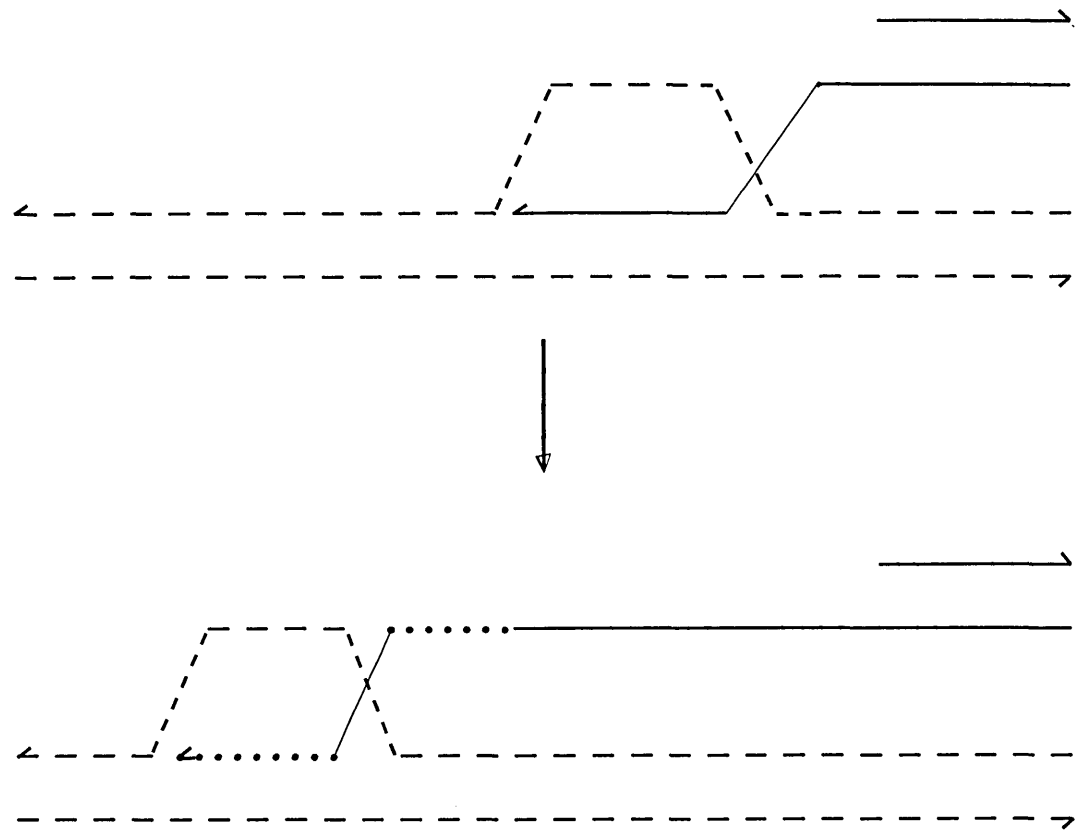


Figure 4,a.

Asymmetric hDNA is formed only on one chromatid. hDNA is created on the right-hand side of the gap by strand invasion and on the left-hand side of the gap by strand annealing. Single-stranded branch-migration during the first round of repair synthesis might remove the right-hand hDNA.

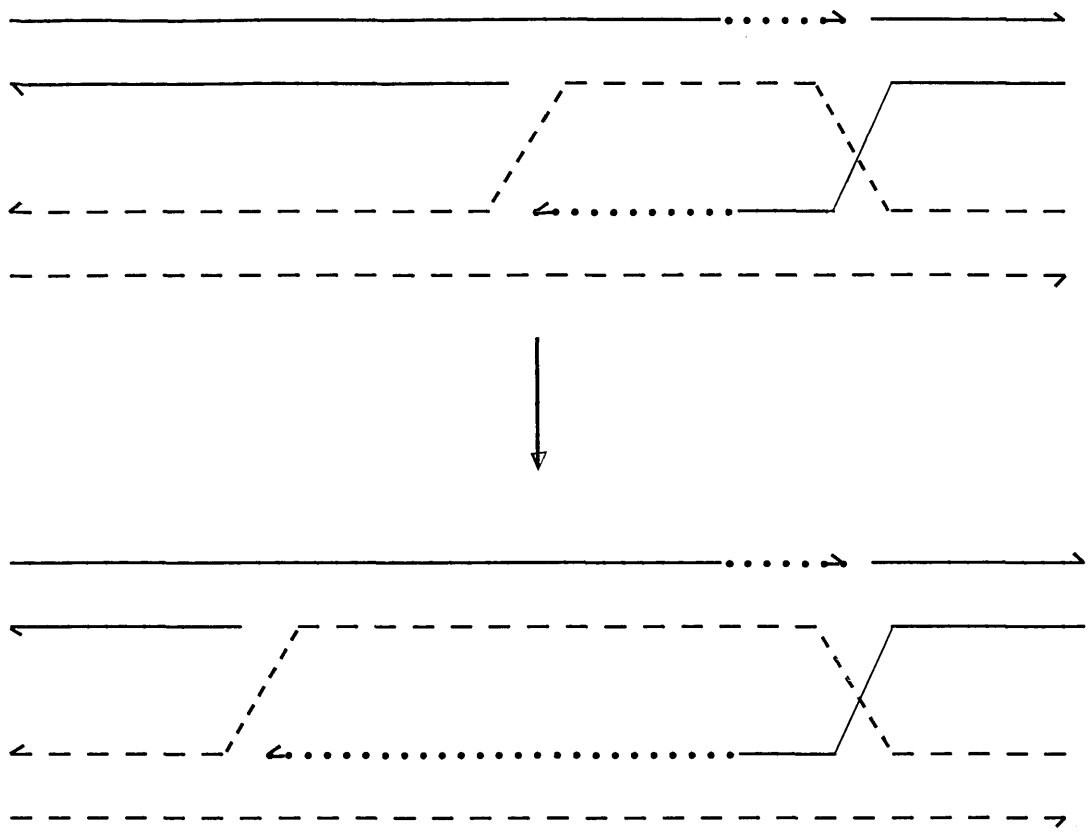


Figure 4,b.

If the right-hand region of hDNA is quite short, and if the left-hand region is extended enzymatically asymmetric hDNA will only form on one chromatid.

repair a 'tetratype' |5:3| segregation may occur. To exclude this possibility two constraints have been imposed upon this model (Orr-Weaver and Szostak 1985):

(i) only one chromatid may carry hDNA; and

(ii) there must be some restriction on the resolution of the Holliday junctions .

The first constraint can be met if, after formation of the initial heteroduplex DNA and the initiation of repair synthesis, branch-migration occurs (Figure 4a, based on that of Orr-Weaver and Szostak 1985). Alternatively, if the heteroduplex formed by the initial strand invasion is quite small, and the heteroduplex on the other side of the gap is extended enzymatically, it is probable that any observed postmeiotic segregation will be derived from the longer region of heteroduplex DNA (Figure 4,b). It is possible that the initiation of recombination occurs by single stranded gap formation which then initiates a double-strand gap. The resulting 5' and 3' ends could invade the homologous duplex so as to form a chromatid with two stretches of heteroduplex (Figure 5, based on that of Orr-Weaver and Szostak 1985). Figure 4 shows heteroduplex DNA to be present only on the recipient chromatid. In order to avoid an aberrant |5:3| segregation at least one Holliday junction must be resolved by cutting the crossed strands. If two closely spaced Holliday junctions interfere, and therefore prevent

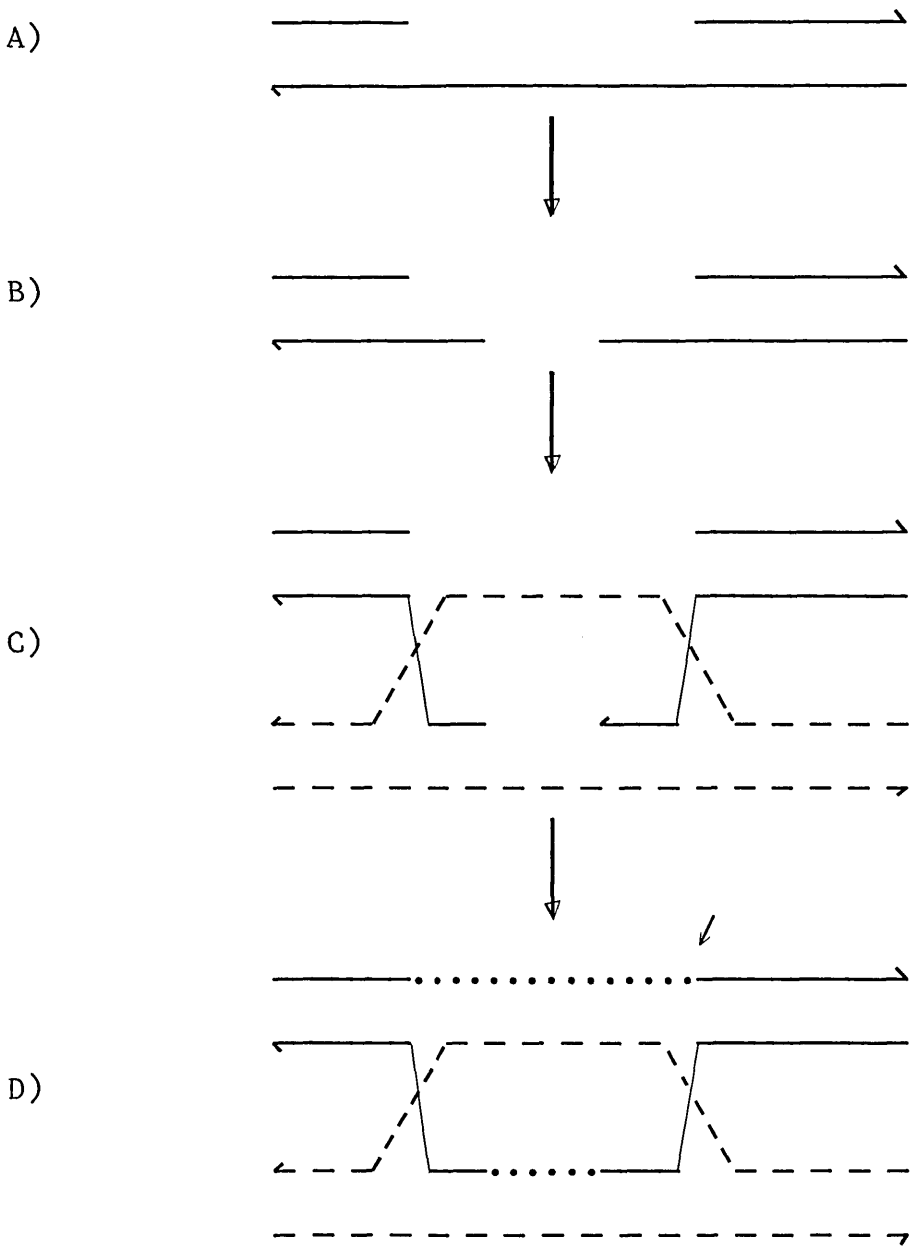


Figure 5,a. Overhanging 5' and 3' ends initiate recombination. Initiation occurs first by the formation of a single-stranded gap (A), the other strand is then cleaved by nucleases to produce 5' and 3' ends which overhang and act 'sticky' (B). These invade the homologous duplex (C), and the double-strand gap is repaired by two rounds of single-stranded repair synthesis (D).

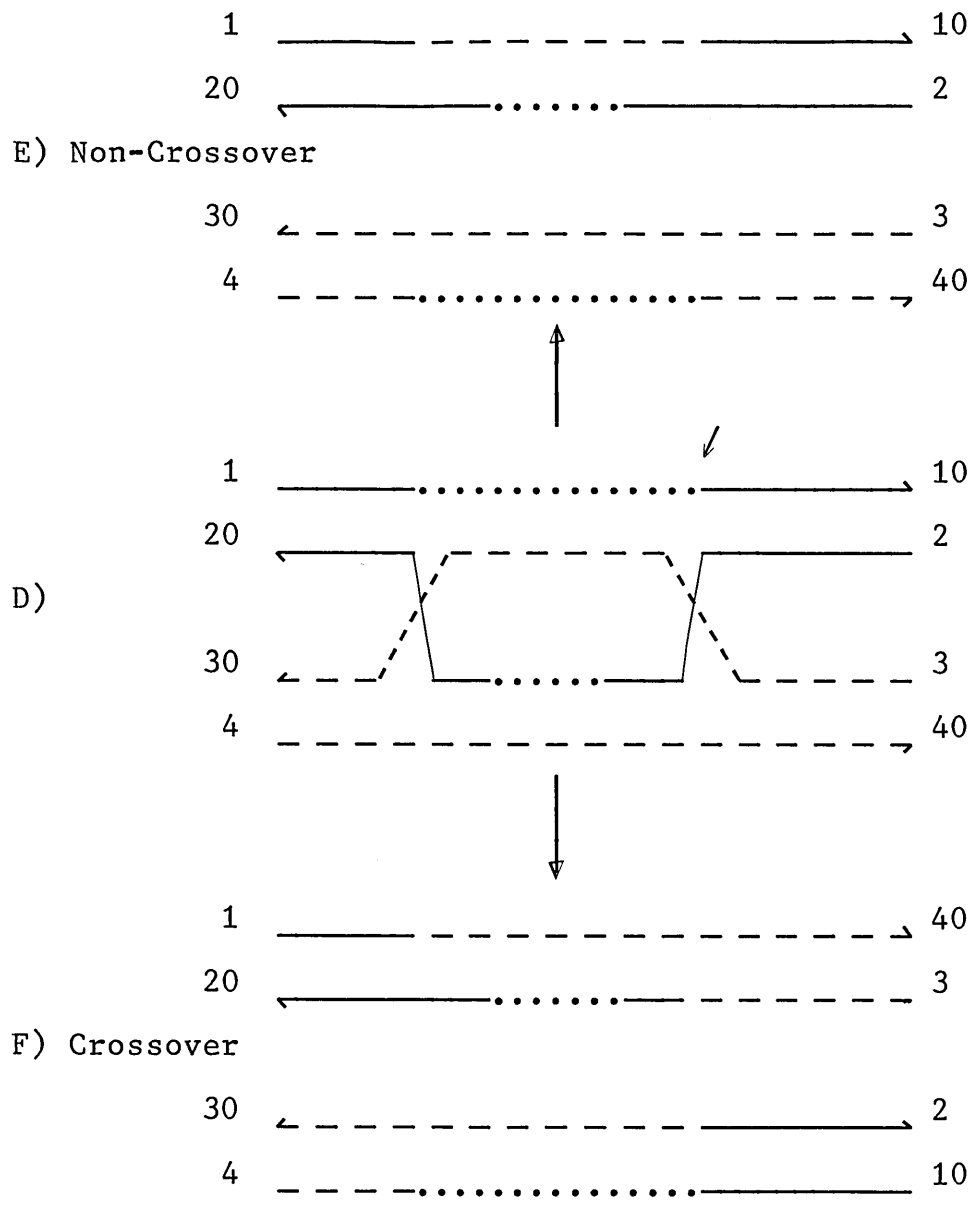


Figure 5,b. Holliday junction resolution. To avoid the generation of 5:3 asci one Holliday junction is resolved by cutting the outer strands. Resolution of the left-hand junction can result in either a non-crossover (E) or crossover (F) configuration.

isomerisation, the first junction may be resolved by a cleaving enzyme leaving the second free to isomerise. In an alternative model (Figure 5) resolution of one junction must be by cleavage of the outer strands. Note that after the second round of repair synthesis (Figure 5b, D), the strand labelled 1-10 is already cleaved / . This nick in the strand may sufficiently bias how the Holliday junction is resolved so that the outer strands, as drawn, are the ones that are cut.

The genetic variations which are the best candidates for recombination initiation sites, M26, YS17 and cog$^+$, are all similar in that the initiating chromatid is the chromatid that gets invaded. The behaviours of M26 and YS17, showing strong disparity to the wild type, is predicted by the DSBR model but contradicts the Meselson-Radding model. Radding (1982) has proposed that nicking of DNA is followed by enzymic action to produce a single-strand gap. The formation of asymmetric hDNA on the initiating chromatid is caused by strand invasion. Alternatively, an initiation enzyme somehow recognises the site of initiation and introduces a nick at the homologous site on the other chromatid (Markham and Whitehouse 1982). It is hDNA at the initiation site that is a common feature of these hypotheses. Data obtained on YS17 shows postmeiotic segregation in rec1$^-$ strains, but one seldom finds postmeiotic segregation when YS17 acts as an

initiation site. This suggests the absence of hDNA at the YS17 initiation site (MacDonald and Whitehouse 1982).

If initiation is restricted to specific sites, then polarity can be explained by the variation in extent of gap repair and formation of heteroduplex DNA. There is a decreasing probability of falling within a gap or within heteroduplex DNA as the distance increases away from the initiation site. If initiation requires an enzyme binding to a specific site, but allows for the movement of that enzyme along the DNA before cutting and initiating recombination, then polarity arises as a function of distance from the 'central binding site'. The initiators, M26 and YS17, seem to behave as initiation sites that are fixed because they themselves are converted at high frequencies and mutations adjacent to M26 and on either side are co-converted with M26. This is in sharp contrast to the behaviour of $\underline{cog}^+$ which acts as a recognition site. $\underline{cog}^+$ is able to have an influence on non adjacent sites. The $\underline{cog}^+$ gene is even able to have an influence across breaks in chromatids and so it seems probable that both types of mechanisms for recombination initiation are functioning. This ability of $\underline{cog}^+$ to affect events across breaks is unexplainable by the DSBR model.

Physical observations on DNA using electron microscopy are consistent with multiple Holliday junctions (Bell and Byers 1982, Kobayashi and Ikeda 1983). It has

been found that most DNA molecules joined by cross-strands are joined by a fused region or by two junctions separated by 10^2 to 10^3 base pairs.

It has been proposed that mutations with a raised postmeiotic segregation frequency are defective in mismatch repair (Orr-Weaver and Szostak 1985). The DSBR model proposes that these mutations affect the activity of an exonuclease that creates double-strand gaps so that a defective single-strand specific exonuclease increases the size of the single-stranded tails. The same exonucleases must be involved in mitotic mismatch repair in order to explain the mitotic mutator phenotype of these mutants.

Support for the DSBR model being capable of the mismatch repair required for the data in fungi comes from E. coli where methyl-directed repair appears to be the primary mechanism for the correction of replication errors. Short-patch repair operates only on specific mismatches in specific sequences. Recombinational repair via double-strand breaks occurs only in unmethylated DNA and requires all of those genes involved in double-strand-break repair. The methyl-directed repair system acting on unmethylated DNA may introduce double-strand breaks or gaps in the DNA in the vicinity of the mismatch (Doutrioux et al. 1986). These breaks or gaps would then be substrates for the E. coli recombinational repair system. Recombination with the sister duplex would regenerate the

parental sequence in the region of the mismatch.

The conversion spectra of large heterologous combinations in b2 of Ascobolus have been demonstrated to differ from those of point mutations located in the same genetic region (Hamza et al. 1987). When heterozygous deletions impose their own spectrum onto close mutations these effects occur on both sides of the heterologies. The effects upon postmeiotic segregation and disparity of linked point mutations gradually decline with distance. The effects on conversion frequencies and on aberrant 4:4 segregations are polar, persisting for all mutations located downstream from the high conversion end of the gene. This last effect may reflect a blockage in symmetric heteroduplex DNA formation by large heterologies, whereas the epistasis of the conversion spectrum of large heterologies over that of closely linked point mutations has been argued to suggest that large heterologies and point mutations undergo conversion by means of distinct pathways (Hamza et al. 1987). it is more likely that repair and hDNA formation/elongation being affected, different types of spectra are observed.

The variety of conversion spectra displayed by nonpoint mutations probably reflects the different location of these mutations with respect to the recombinational signals operating in the b2 region and/or differences in sizes of the respective mutations. The

facts that G234 (and G40) are epistatic to G0 and that 10 is in turn epistatic to them suggest the existence of complex interactions and/or hierarchies.

All previous studies in Ascobolus strongly supported the Meselson-Radding model, in which gene conversion occurs by two steps: heteroduplex DNA formation involving either one (asymmetric) or two (symmetric) interacting duplexes and mismatch correction.

The simplest hypothesis to account for the long range effect of mutations on conversion in the same gene is that G/+ heterologies block branch-migration of symmetric heteroduplex DNA propagating from the left end of b2 to the right. This hypothesis explains why G40 gives about one-third less conversion than closely linked point mutations and why G234 and G40 also reduce by about one third the conversion frequencies of point mutations in the middle region and in the right b2 portion. A calculation by Paquette and Rossignol (1978), that about one-third of heteroduplex DNA formed in the middle region of b2 was in the symmetric phase, also nicely fits the conversion frequency decrease and gives support to the proposed hypothesis. This effect on symmetric heteroduplex is not specific to large heterologies. Two one-point mutations in the same region also show a polar effect, but the effect is quantitatively less drastic, with aberrant 4:4 at one locus reduced by approximately 50 %.

The polar effect of G/+ heterologies upon heteroduplex formation does not seem to involve asymmetric heteroduplex DNA, since |5:3| segregations for distant right end class C (and D) mutations are not affected by the presence of G/+ heterologies. The same result was also found for double point mutations. This suggests that asymmetric heteroduplex DNA is not blocked by these heterologies and leaves the possibility that the conversions of G, like that of point mutations, occur by mismatch correction.

Class B mutations are guessed to be one G-C base pair additions because of the mutagen used (ICR-170). In crosses with wild type, the preferential conversion to mutant thus would indicate that it is the shorter strand which is excised and further corrected by copying the longer mutant strand. The excision of the shorter strand can also account for the preferential conversion to wild type of type A mutations that are complementary to class B and should thus correspond to one base pair deletions (Leblon 1972a; 1972b)

A mismatch correction process could account for the local effect of G/+ heterologies upon closely linked class C mutations: the postmeiotic segregation decrease may reflect mismatch correction triggered at G/+ and spanning the class C mutations. The weaker decrease of postmeiotic segregation with increasing distance would

reflect the limited extension of the correction tracts. The same type of effect of class A and B mutations upon class C mutations was used to argue that |6:2| segregations of class A and B mutations result from heteroduplex formation followed by a single strand mismatch correction.

One simple hypothesis that explains the hierarchical conversion between large and smaller heterologies is that larger loops are more likely to trigger mismatch correction. In the models of conversion proposed by Radding (1978) and by Hastings (1984) parity is predicted of large heterologies, but large deletions can show disparity (Lamb 1986). Both models retain the assumption that large heterologies become part of a heteroduplex region but they differ by its processing: Radding supposes that DNA synthesis makes the loop double stranded and that a resolution event similar to the resolution of Holliday structures eliminates or retains the double stranded loop with equal likelihood; Hastings proposes that mismatch repair begins by cutting both strands, generating a double strand gap which is repaired by double strand gap repair. Hastings' model also can explain the influence of G conversion upon closely linked mutations by variable extension of the double stranded gap region.

The two hypotheses make different predictions

about the fate of the G heterology when heteroduplex spans it. Radding's model predicts that conversion to the invader genotype will equal restoration to the resident one (if replication and isomerisation are independent of which strand is the invader). Hastings' model predicts that all events will lead to conversion to the invader genotype if the double strand gap is always repaired from one of the two homologous chromatids, never from the sister. These two hypotheses were tested in experiments using G234 (Rossignol et al. 1984; Hamza et al. 1986). There are significantly more than 50% (between 72 and 89%) of the events leading to conversion. This is more in agreement with the prediction of Hastings' model than with that of Radding.

Alternatively, the conversion of G heterologies may not involve a heteroduplex intermediate at all. It could occur de novo via a double strand gap repair event initiated in its vicinity. Since closely linked class C mutations exhibit no postmeiotic segregation, this model requires that G/+ heterologies prevent heteroduplex formation in their vicinity as well as at the site of heterology itself. Since it is supposed that heteroduplex DNA begins at the left end of b2 and propagates rightward (Paquette and Rossignol 1978; Hamza et al. 1981), we would expect that G/+ heterologies would abolish all heteroduplex DNA (asymmetrical as well as symmetrical) to

the right. They do not: asymmetric heteroduplex DNA formation is not affected. For this reason, preference is given to the former hypothesis of heteroduplex DNA being a prerequisite for G conversion.

If, as seems the most likely, G/+ heterologies prevent the rightward propagation of symmetric heteroduplex DNA, the results presented suggest that this effect begins at a distance, to the left of the deficiency heterozygosity, rather than at its very border. If the effect began at the border, then symmetrical heteroduplex DNA should span the point mutations nearby, an hypothesis which leads to the following three predictions:

(1) The drop in conversion frequencies should not be as strong for the nearby point mutations as for mutations farther on the right,

(2) Frequent arrest of heteroduplex DNA between the nearby point mutations and G should lead to frequent recombination via single site conversion involving the point mutations, and

(3) Mismatch correction of the nearby point mutations within the symmetrical heteroduplex DNA fraction should not be under the influence of the heterology and thus retain partial disparity.

None of these predictions is met: with G/+, nearby point mutations have their conversion frequencies as strongly reduced as mutations on their right, reassociate

extremely rarely with G ($< 10^{-4}$) and have a disparity value that drops to 1.0 (1.0 represents no disparity on their formula). If true, this might suggest that either the blockage of the Holliday junction, or the strand cutting mediating its resolution, is not adjacent to the start of the heterologous region.

Two features of the double-strand break repair model have been tested recently in phage lambda and a plasmid (Thaler et al. 1987) and in S. cerevisiae (Kolodkin et al. 1986). In these studies, in vitro derived restriction enzymes were used to make a double-strand-cut at a defined location. The two features of the DSBR model which were supported by their results are:

(1) a double-strand-cut delivered to the phage, within the region of homology shared with the plasmid, stimulates recombination between the phage and plasmid. A cut delivered outside the region of shared homology has no effect.

(2) Among recombinants that have spliced-in the plasmid there is a loss of whichever allele was in cis to the cut site.

The second feature agrees with the prediction that a recombinator sacrifices itself in the act of stimulating recombination. According to the DSBR model the loss of alleles in cis to a cut site is a consequence of exonuclease action starting at the cut site. Alternatively

it has been suggested that the loss of alleles cis to the cut site can be accounted for by supposing an asymmetry of mismatch correction which is biased against the chromosome that initiated the recombination (Szostak et al. 1983).

In the DSBR model proposed only the strand that ends 3' can act to prime repair synthesis. The 3' tips of the new strands are thought to pass each other as they are being synthesised in opposite directions. The result is the complete synthesis of both strands by double-strand-gap filling. It has been postulated that an invading duplex can initiate a full replication fork by synthesis on both the leading and the lagging strand (Thaler et al. 1987). Initiation of full replication forks primed from the tips of invading duplexes is the major mode of late T4 DNA replication (Luder and Mosig 1982).

Lamb (1987) has devised two tests of double-strand gap repair as a source of gene conversion. In identifying the different predictions of DSBR and hDNA models, he has been able to demonstrate that most data from Ascobolus, Sordaria and yeast are consistent with meiotic conversion arising predominantly from hDNA. DSBR does not produce |3:5|s, or aberrant and correction 4:4s; only |6:2|s are produced. Within a length of this type of gap repair, there is no expected effect of mutational type on conversion spectra, and one heterozygous mutation should not influence neighbouring markers. This renders

the DSBR open to testing by comparing conversion frequencies for neighbouring sites within a locus. It was found in the analysis that the large differences within loci were inconsistent with the DSBR model accounting for the majority of conversion in the organisms considered.

The other test is based on the relationship between disparity and postmeiotic segregation which reflects the predicted explanation that postmeiotic segregation differences are due to dissimilarities in hDNA length/gap length ratios in the DSBR model. Lamb (1987) found no significant correlation between 6:2/2:6 disparity and the relative frequency of postmeiotic segregations in the Ascobolus or yeast data studied suggesting that most if not all conversions arise from hDNA.

One possible way of resolving the very different models of recombination is to use recombinant DNA technology in an attempt to identify and characterise recombination initiation sites. The identification of such sites has been suggested to allow the identification of proteins that act at the initiation site (Orr-Weaver and Szostak 1985).

G E N E R A L M A T E R I A L S

The Organism

The euascomycete fungus Ascobolus immersus was used throughout this work. All strains used were from the Pasadena strain and derived from the two wild type stocks of opposite mating type collected in 1963 from dung in California (Yu-Sun 1964). Because of fertility problems the original strains were not used, instead strains isolated by previous workers have been utilised as the basic progenitors for the work (Ghikas 1978; Helmi 1980; Howell 1982). The following classification and description is according to Minter (1978).

Family Ascobolaceae: characterised by relatively broad asci which protrude above the level of the hymenium when they ripen. Hence the ascospores commonly lie in two or three irregular rows in the ascus instead of in the single vertical row usual in most operculate asci. The apothecia are sessile, pulvinate, usually small to very small and the majority of species occur on dung, a few on burnt ground, soil or plant debris. This family has two tribes; Ascoboleae and Pseudoascoboleae.

Tribe Ascoboleae: the ascospores have a double wall with a hyaline, persistent, inner layer and a dark

brown to purple layer, the epispore, which commonly cracks into a characteristic pattern. The epispore swells up and disappears in potassium hydroxide solution or ammonia.

Genus Ascobolus: the apothecia are cup-shaped, smooth or scurfy, often yellowish to olive in colour, sometimes with a slightly greenish hue, dotted with the almost black protruding tips of ripe asci. Each ascus contains four or eight ascospores, purplish, eliptical, the epispore either verrucose or cracking into striae which are usually more or less inter-connected, rather variable in size, shape and ornament. Most species occur on dung.

Species A. immersus: the apothecia are subcylindrical or hemispherical, immersed in dung up to the margin, the disc being up to one millimetre across, flat or convex, yellowish or green, with only a few protruding asci. There are from one to forty asci per apothecium, up to 700 by 130 μm , each containing eight spores which are oblong/ellipsoid, mostly 50-75 by 25-40 μm in size, violaceous to brown in colour. These ascospores are smooth and surrounded by a thick colourless gelatinous envelope easily digested by a pronase solution. The paraphyses, which are sterile hyphae to which the asci are attached, are slender, colourless, up to 3 μm thick and are embedded in yellow mucus. In nature this organism

is found in the dung of cows and many other animals, being most easily obtained in autumn.

*

Below are listed some of the advantages of working with A. immersus:

(1) The life cycle is relatively short, taking about two weeks depending upon the strains crossed and the environment.

(2) The average ascospores measure 60 by 32 μm and therefore are easily separated and handled.

(3) The production of octads (though unordered) allows the identification of hybrid DNA formation more easily than in organisms such as Drosophila where post meiotic segregation is detected as a mosaic in the tissues (Leslie and Watt 1984).

(4) When the asci are mature their contents are launched upwards by dehiscence which makes it unnecessary to dissect apothecia, especially since the octads are usually dehisced whole.

(5) The heterothallic mating system of plus/minus mating types allows one to know that the progeny are from the cross and not a result of self fertilization. This removes the need for genetic markers to identify the

origin of asci.

(6) There is a ready abundance of ascospore mutants and they are easily added to both by spontaneous mutations and by mutagenic induction.

(7) This organism can be cultured on a defined medium (Yu-Sun 1964) with the exception of certain germination requirements of the ascospores. This allows selection for biochemical mutants.

(8) The existence of known genetic factors affecting gene conversion of ascospore mutants (Emerson and Yu-Sun 1967; Lamb and Helmi 1978) and the potential reinforcement of these factors to achieve relatively high frequencies of gene conversion reduce the size of samples needed.

(9) In today's high economic perspective in university research, the organism is relatively cheap to grow and manipulate.

Below are listed some of the disadvantages of working with A. immersus:

(1) There is the lack of asexual spores, hindering selection of mutants by conventional methods.

(2) The octads are unordered and are therefore of no use in identifying second division segregation and postmeiotic segregation in aberrant 4:4s etc. at one site without further genetic markers.

(3) The strains used have a tendency to lose fertility with time which means that the worker has to re-

isolate from crosses which may lead to alterations in genotype as there are no isogenic lines of opposite mating-types.

(4) Because of the heterothallic nature of the mating system there are no isogenic lines of opposite mating types, and of course the organism is self-incompatible rendering it more difficult, if not impossible, to reduce background genetic variation to an imperceptible level.

The Strains

All stocks were derived from those held by Dr B. C. Lamb at Imperial College, London.

The strains used in this work are shown in Table 1; ancestry can be traced by the reference to the workers mentioned. WT refers to the wild-type spore colour referred to as red though in truth they are more violaceous; w refers to the mutant ascospore strains referred to as white whereas they are in fact colourless.

The pedigrees of strains derived in this work are shown in Figures 6 and 7.

All mutagenesis was carried out on crosses of sk,7,R,7,- with 9,r,+ and the mutant-carrying strains were isolated and purified by backcrossing to one of these parents, depending on mating type.

In this work the labelling of new strains has been consistent with the rules set out in Figure 8.

Strain	Spore-colour genotype	Octad type isolated from	Parental strains	Reference
3,r,-	WT	unknown	''	Ghikas 1978
9,r,+	WT	unknown	''	''
25,5,R,+	WT	6:2	w78,- x 9,r,+	''
w3c1,+	w3c1	4:4	92,- x 9,r,+	''
wBHj,-	BHj	4:4	BH,R,1,- x 91,2,R,+	''
BB,9,w9,+	w9	4:4	BB,R,I,- x 9,r,+	''
7,R,7,-	WT	8:0	BH,R,2,- x 1D,2,+	Helmi 1980
3w1,DEC,-	w78	4:4	Ec,11,w78,7,- x 2,D,2,+	''
2,D,2,+	WT	8:0	PA,8,dm,- x 23,+	''
3,w78,3,-	w78	4:4	92,- x 1,w3,KEC,+	''
42,(91),-	WT	8:0	BH,R,2,- x 91,2,R,+	''
fpr1,-	WT	unknown	unknown	''

Table 1. Pedigrees of strains used

Strain	Spore-colour genotype	Octad type isolated from	Parental strains	Reference
w78,+	w78	unknown	(P),5,- x (K),5,+	Emerson & Yu-Sun 1967
w78,(K),-	w78	unknown	''	''
w10,+	w10	unknown	''	''
w10,-	w10	unknown	''	''
BH,R,2,-	WT	2:6	Ec11,w78,7,- x w10,+	Personal communication from Dr. Lamb
91,2,R,+	WT	unknown	isolated from wild	Personal communication from Dr. Lamb
(P),5,-	WT	unknown	''	Yu-Sun 1964
(K),5,+	WT	unknown	''	''
EAa,1,w10,1,+	w10	2:6	92,w10,- x 1,WT,3,+	Wickramaratne 1974

Strain	Spore-colour genotype	Octad type isolated from	Parental strains	Reference
31,NG,99,-*	WT	unknown	unknown	Personal communication from Dr. Helmi
25,NG,99,-**	WT	unknown	unknown	''
KIV,(K),+	WT	1:7	w78,- x w10,+	Helmi 1980
11,R,-	WT	8:0	7,R,7,- x 25,5,R,+	Howell 1982
1,BB,9,w9,-	w9	4:4	7,R,7,- x BB,9,w9,+	''

* originally labelled lys31,-

** originally labelled lys25,-

Figure 6.

Strain	Spore-colour genotype	Octad type isolated from	Parental strains	Reference
sk,7,R,7,-	WT	8:0	7,R,7,- x 9,r,+	This Work
sk,7,R,3,-	WT	8:0	''	''
sk,9,R,1,+	WT	8:0	''	''
etc.				
sk,1,w78,4,+	w78	4:4	3,w78,3,- x 9,r,+	This Work
sk,1,w78,5,-	w78	4:4	''	''
sk,1,w78,7,+	w78	4:4	''	''
sk,2,w78,4,-	w78	4:4	''	''
sk,2,w78,7,-	w78	4:4	''	''
sk,3,w78,7,-	w78	4:4	''	''
sk,4,w78,7,+	w78	4:4	''	''
sk,4,w78,8,+	w78	4:4	''	''
sk,42,w78,1,+	w78	4:4	''	''

Strain	Spore-colour genotype	Octad type isolated from	Parental strains	Reference
Sup,1,w78,3,+	w78	4:4	3,w78,3,- x 2,D,2,+	This Work
Sup,2,w78,2,+	w78	4:4	''	''
Sup,2,w78,3,-	w78	4:4	''	''
Sup,2,w78,6,-	w78	4:4	''	''
Sup,2,R,1,+	w78	4:4	''	''
Sup,2,R,5,-	w78	4:4	''	''
etc.				
st,12,fpr1,1,+	WT	4:4	fpr1,- x sk,1,w78,4,+	This Work
st,4,fpr1,6,-	WT	4:4	''	''
st,4,w78,fpr1,7,-	WT	4:4	''	''
st,4,w78,fpr1,8,+	WT	4:4	''	''
etc.				

Figure 7.

Strain	Spore-colour genotype	Octad type isolated from	Parental strains	Reference
sy,3,w78,(91),1,+	w78	2:6	42,(91),- x sk,1,w78,4,+	This Work
sy,3,w78,(91),3,-	w78	2:6	''	''
sy,3,R,(91),6,+	WT	2:6	''	''
sy,5,R,(91),8,-	WT	4:4	''	''
etc.				
sz,6,w78,(K),2,-	w78	2:6	sk,1,w78,5,- x KIV+	This Work
etc.				

For example-

sk,5,grn-20,K,2,mor2,+

is a strain derived from cross sk; it is from the fifth ascus dissected, of wild type (because w/R/r unspecified) for the spore colour, carrying a granular mutant (grn-20) as one marker and a morphological mutant (mor-2) as a second marker: it has allele K at ccf-2 and is of a plus mating type. Finally it is of non-super (because it does not contain the combination Sup) genotype with regard to ccf-3 and ccf-4. The cross sk signifies the cross using 9,r,+ as one of the parents.

*

The Chemicals

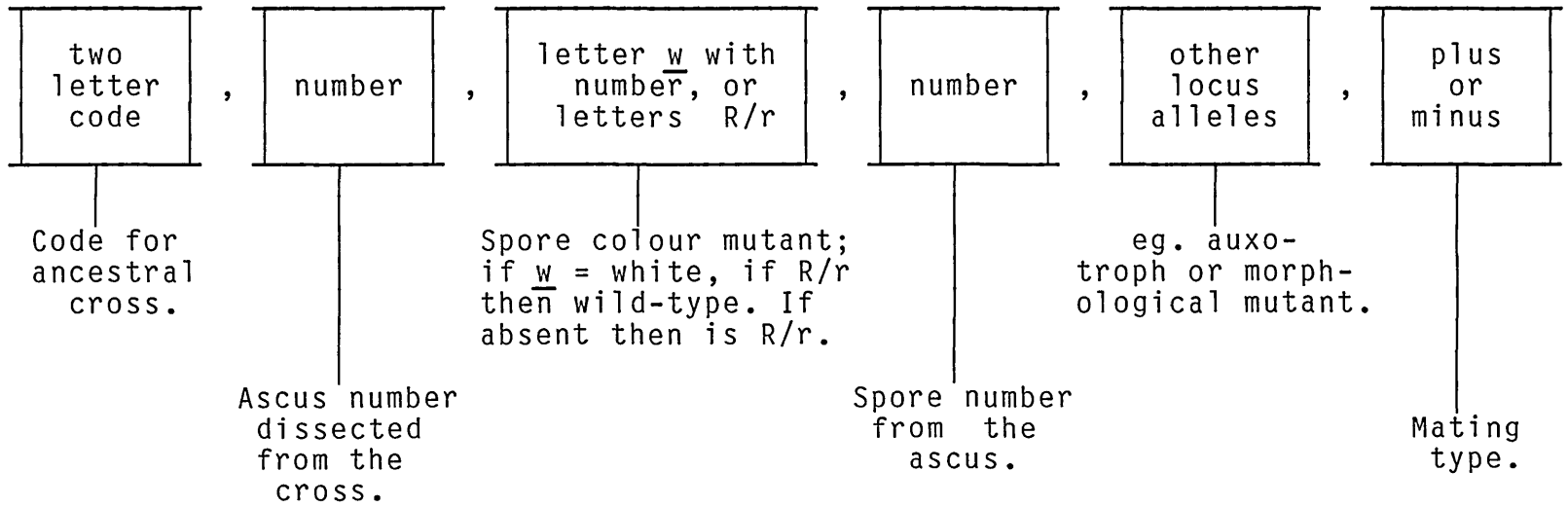
All drugs and resistance chemicals were supplied by Sigma. Other supplies were obtained as follows-

Aldrich Chemicals Co: N-Methyl-N'-Nitro-N-Nitroso Guanidine (NMG).

BDH. Analar: Trace Elements for Vogel's Medium; Sucrose and D-Glucose.

BDH. Chemicals: Biotin; Pronase; all Amino Acids and Vitamins; Monopotassium Phosphate; di-Sodium Phosphate and Pepsin.

Difco: Bacto-Agar; Bacto-Casamino Acids and Bacto-Yeast Extract.



There is no difference between the labels r and R, these inconsistencies arising from earlier works dating back to the very beginning of Ascobolus Pasadena strain research.

The letters/numbers; P, K, 91, or Sup indicate the type of alleles carried at the ccf sites. Where unspecified the strain is P at ccf-2 and of non-super genotype.

Figure 8.
Explanation of the strain labelling used in this work.

Hopkin and Williams: Methyl 4-Hydroxybenzoate.

Koch-Light Labs. Ltd. : all Nucleic Acids.

Whatman: Fibrous Cellulose Powder.

Unless otherwise stated, media and solutions were autoclaved for fifteen minutes at 15 p.s.i.

MINIMAL MEDIUM RECIPE: [Modified from Yu-Sun (1964)]

10	ml	Phosphate and Biotin Solution,
5	ml	Magnesium Sulphate Solution,
0.1	ml	Vogel's Trace Elements Solution,
10	g	Glucose,
17	g	Bacto-Agar,
5	g	Asparagine,
0.1	mg	Thiamine Hydrochloride,
980	ml	Distilled Water, and
2.5	mg	Riboflavin (to quicken growth).

[Other media Yu-Sun 1964 unless otherwise stated]

PHOSPHATE AND BIOTIN SOLUTION:

25	g	KH_2PO_4,
30	g	K_2HPO_4,
1	mg	Biotin, and
480	ml	Distilled Water.

MAGNESIUM SULPHATE SOLUTION:

25	g	$MgSO_4.7H_2O$, and
250	ml	Distilled Water.

VOGEL'S TRACE ELEMENTS SOLUTION:

[See Vogel 1956]

STOCK MEDIUM:

10	ml	Phosphate and Biotin Solution,
5	ml	Magnesium Sulphate Solution,
0.1	ml	Vogel's Trace Elements Solution,
3	g	Yeast Extract,
10	g	Glucose,
17	g	Bacto-Agar, and
980	ml	Distilled Water.

COMPLETE MEDIUM:

As Stock Medium,

plus	0.5	g	Casamino Acids.

CROSSING MEDIUM:

As Stock Medium,

plus	0.5	g	Casamino Acids, and
	2	g	Cellulose Powder.

PHOSPHATE BUFFER FOR NMG:

Set to pH 7.0 -

41.3 % (v/v) of $1/15^{th}$ Molar K_2PO_4, and

58.7 % (v/v) of $1/15^{th}$ Molar $Na_2HPO_4.2H_2O$.

PLAIN AGAR:

20 g Bacto-Agar, and

Distilled Water to make up 1 litre.

SPORE COLLECTING MEDIUM: [Lamb, personal communication]

17 g Bacto-Agar,

7 ml Mould Inhibiting Stock Solution, and

Distilled Water to make up 1 litre.

MOULD INHIBITING STOCK SOLUTION:

10 % by weight Methyl-4-Hydroxybenzoate,

in 96 % Ethanol (not autoclaved!).

PRONASE SOLUTION:

0.1 % by weight Pronase,

in Sterile Distilled Water; filter sterilised,

not autoclaved.

DUNG GERMINATION STOCK SOLUTION:

135 g Frozen Horse Dung, and

1 l Distilled Water; for method see later.

GERMINATION MEDIUM (LIQUID):

100 ml Dung Germination Stock Solution, and

200 ml Distilled Water.

GERMINATION MEDIUM (SOLID):

As above,

plus 6 g Bacto-Agar.

*

The Equipment

Whatman: No. 1 Filter Papers, 25 mm diameter.

Cellophane: British Sidac Ltd.

Incubators: see Wickramaratne 1974.

Pyrex Test Tubes: Large- 15 cm x 2 cm,

Small- 7½ cm x 12 cm.

Glass Petri Dishes: Large- lid, 10 cm diameter,

Small- lid, 5 cm diameter.

Filters: Henry Simon Ltd.

305 μ pore size,
501 μ ,
700 μ ,
1 000 μ ,
1 600 μ ,
2 000 μ ,

Sterilising Filters: Gelman Hawksley, Ltd.

0.2 μ .

Filter Holders: Gelman Filtration Products.

G E N E R A L M E T H O D S

Sterilisation And The Organism:

Glassware was sterilised in one of two ways: petri dishes were kept at 150° C. for four hours, whilst flasks and test tubes, etc. were autoclaved for fifteen minutes at 15 p.s.i. For auxotroph selection, before being autoclaved equipment was first well rinsed in distilled water and kept at 150° C. for two hours in order to 'burn off' organic matter.

Water and media were sterilised by autoclaving for fifteen minutes at 15 p.s.i. Chemicals were similarly sterilised unless they were either very toxic or unstable at such temperatures (e.g. Nystatin), in which case they were either filtered through 0.2 μm filters (membrane type) if soluble, or suspended in 95 % ethanol prior to use.

Standard microbial techniques were used in handling the organism. Procedures which were very susceptible to contamination were carried out in a fume cupboard kept sterile with 80 % ethanol on solid surfaces and a U.V. lamp when not in actual use (the fan was kept switched off). General conditions in the laboratory were

good on account of the filtering air conditioner and by keeping windows closed to the dirty London City air.

Culture Techniques:

These were basically as described elsewhere (Wickramaratne 1974; Helmi 1980). Stock cultures of A. immersus stored for long periods were first grown on stock medium in large test tubes at 25° C. and stored in a fridge. Short term cultures were grown in large or small test tubes on stock medium and refrigerated at just below 10° C.

Crosses were made by inoculating both parents onto distant parts of a petri dish containing crossing medium, and thereafter transferred to illuminated incubators set at 17.5° C. The most asci are produced when the two parents meet at about the centre of the dish and because of the different growth rates of the various strains experience teaches which parent to inoculate first and how long to incubate for (usually 12-24 hrs.) before adding the faster growing parent. For example 9,r,+ is a considerably faster grower than sk,1,w78,5- .

It was found unnecessary to clear mycelium away from the apothecia and the freeing of apothecia by the removal of pieces of agar was never carried out. In selecting strains for this research preference was given to types giving good prolific, unobstructed ascus

dehiscence.

There seemed a great variation in fertility as measured by the number of octads dehisced, even from replicate crosses made at the same time. One factor was the amount by which the crossing medium dried out. Once spores began to be dehisced, or shortly before, as forewarned by the darkening spores, plates of spore-collecting medium were used as lids. To prevent incubator vibrations from dislodging the collecting plates, sellotape was used to stick the lids to the plates; this also had the added effect of prolonging the dehiscence period of the crosses by delaying the drying out of the crossing media.

Normally, collecting lids were removed before distinct spore octads became indiscernible because of overcrowding, which occurs at about 500 - 1 000 octads for large plates. Depending upon the parental strains, each crossing plate would normally suffice for from one to three collecting lids.

The covered collecting plates were stored either on the open bench or in large (biscuit type) tins with little apparent deterioration for up to two months, after which spores tended not to germinate and scoring was made more difficult because of discolouration or striation of the collecting media.

Preparing Spore Suspensions

Spores were collected on collecting medium in petri dish bases acting as lids for crossing plates. If required, octads could be removed individually, but in selection experiments the spores were required separately and in suspension. The collecting plates were treated with approximately 5 ml of 0.1 % (by weight) pronase solution for 15-20 minutes, during which time the plates were agitated regularly to ensure an even spread of the enzyme as well as assisting to physically release the spores from the ascal gelatinous envelope. If at the end of 20 minutes the enzyme solution had been soaked up by the agar medium then sterile distilled water was added to facilitate the Pasteur pipetting of the spores into McCartney or Universal bottles ready for use. The spores were then allowed to concentrate on the bottom by settling under the action of gravity. This spore suspension was now ready to be added to the germination medium, and was never stored in this manner for more than half an hour.

Germination Of Spores

Because of the high percentage of germination required for complete octad analysis, the existing germination medium (Helmi 1980) was adapted in this work.

Stock solutions of germination media were prepared by mixing 135 g of frozen horse dung with a litre

of distilled water and leaving the suspension in flasks on a bench shaker for forty-five minutes to an hour. The dung suspension was then strained through a double layer of muslin gauze to which physical pressure was applied so that the remaining solids weighed between 60 % and 75 % of the original frozen dung. The liquid collected was centrifuged at 17 000 r.p.m. for ten minutes at 10° C. The centrifuge tubes were carefully handled to prevent the resuspension of settled matter. The suspension was subsequently autoclaved at 15 p.s.i. for fifteen minutes. The sterile stock was stored below 10° C. in the dark for up to three months. The efficiency of the medium deteriorated with time so that after three months the germination was about forty percent of that when the medium was fresh.

When germination medium was required the stock solution was diluted threefold with distilled water. If liquid medium was required then the diluted stock solution was separated into 50 ml parts into 500 ml screw-top conical flasks, and allowed to cool after autoclaving at 15 p.s.i. for fifteen minutes. Then between 10 and 20 ml of spore suspension, containing 3-5 thousand spores, were added. Alternatively, solid medium required for plating out was prepared by adding 20 g of agar to each litre of diluted stock germination solution and autoclaved. Petri dishes were filled to about half depth and allowed to

cool. Ascospores were then transferred to the agar medium with a sterile needle and separated as required, under a dissecting microscope (X20 magnification).

With the knowledge that the active germination-promoting ingredient in the dung medium was photo-sensitive (Helmi 1980) the stock germinating solution and prepared medium were protected from light at every possible stage, especially from sunlight, by drawing blinds in the laboratory and by covering storage bottles and flasks with aluminium foil. Once prepared the germination medium was kept in the dark as much as possible and below 10° C. until required. Prepared dung medium was never stored for more than forty-eight hours and where possible was used as soon as possible, if not immediately.

Once the spores were in contact with the germinating medium the dish, or flask container, was placed in a fanned incubator, warmed to 50° C., for two hours, after which the incubator was allowed to cool to ambient room temperature overnight. After cooling to room temperature the spores, together with the germination medium, were transferred to an incubator set at 37° C. and stored there for up to eight hours. Then the spores, most of which had germinated, were incubated at 25° C. Spores in liquid medium needed to be placed on flask shakers after the two hour heat shock to prevent 'matting' of

hyphae from different spores. Allowing spores to cool overnight after the heat shock appeared to give higher germination than if the spores were transferred immediately to a 37° C. incubator. Because of the bulk of flask shakers, spores in liquid germination medium often had to be agitated at room temperature after the heat shock treatment.

As soon as spores germinate on solid medium, they can be individually transferred to normal culture media by needle. In liquid germination medium, after the 37° C. incubation, the spores can be trapped on muslin gauze and washed with sterile distilled water to remove the undefined dung medium.

Whilst there is experimental evidence showing that the type of horse dung used is not important for germination (Helmi 1980), throughout this work horse dung was collected from the South-West corner of Hyde Park on overcast days. The dung was collected whilst still warm and care was taken to choose dung with as little extraneous matter (sand and hay) as possible. The dung was used or frozen within an hour of collection and stored for less than six months, or until it was found to have deteriorated. When dung was prepared for weighing it was found that in its frozen state it was extremely difficult to break up into convenient pieces. To get around this problem, the dung was transferred to a refrigerator about

twelve hours before use, after which it was easily broken apart with a spatula or similar instrument.

Using the methods described a very high percentage of germination was achieved, reducing the number of octads requiring dissection for complete octad analysis. In forty-nine octads fully dissected from a cross between wild-type and w-78, twenty-one octads completely germinated, another fifteen had seven spores germinate, three with six and the remaining had only four or less spores germinate. As a percentage of spores germinating, 78.06 % was obtained in this preliminary study (306 out of 392). However, if one ignores the octads with less than five spores germinating, the percentage was increased to 93.27 (291 out of 312).

It is noted that white spores, of the genotype w-78, germinated about 1.2 % more than the wild-type in the same crosses under the same germination conditions of dung medium and two hours heat shock at 50° C. This is contrast to the recommendation by Dr. Helmi (1980) that white spores should be germinated on plain agar. There appeared no need to vary the germination medium for different spore genotypes and this also applied to most granular mutants (including grn-20).

Attempts were made to germinate spores on a variety of different media: plain agar, Bactopeptone and even pH adjusted media (to pH 7 using the phosphate

buffers) and whilst w-78 spores had little difficulty in germinating anywhere, even on dry glass, wild-type spores completely failed to germinate except on a dung supplemented medium. Even the original medium devised by Yu-Sun (1966) was of no use, not a single red spore being found to germinate out of the thousands tested.

With the aim of hastening hyphal growth immediately following germination, it was found that the addition of 3 g of yeast extract per litre of germination medium caused no observable deterioration in the percentage of spores germinating. This yeast extract supplement was not used over-much and only used to reduce the time needed to store bulky glassware and so effectively created more incubator space, which was often the limiting factor in an experiment.

Scoring Gene Conversion

Octads were scored for gene conversion for spore colour markers by scanning with a dissection microscope at X20 magnification. Octads were only scored if they were discrete and had a full complement of eight spores. With an average of nearly a thousand spores per collecting plate (10 cm size) it took between fifteen and twenty minutes to record all the scorable asci into the segregation classes 8:0 through to 0:8 . With the addition of a granular mutant to the red/white system, in order to

score aberrant 4:4s, and other post meiotic segregation classes hidden in a one marker cross, scoring slowed down to more than half an hour per plate.

It was previously known that the environment (such as temperature) can affect gene conversion in Ascobolus (Wickramaratne and Lamb 1978; Ghikas 1978) and there was some concern about using different shelves in the same incubator and combining the results. An experiment was devised to test variation arising from keeping crossing plates in different places and the results presented in Tables 2 and 3 show that there are no statistically significant differences between shelves or incubators. The shelves were classified as: uppermost (U), middle (M) and lowermost (L). For the statistical analysis see Steel and Torrie (1981).

Incubator and Shelf effects on Conversion

Dish Number	Incubator					
	in Corridor			in Laboratory		
	Incubator Shelf			Incubator Shelf		
	L	M	U	L	M	U
1	36	44	47	49	54	47
2	39	38	48	53	46	62
3	47	39	45	56	52	62
4	58	55	57	47	50	29
5	54	50	50	44	38	58
Shelf Totals	234	226	247	249	240	258
Incubator Totals	707			747		
Incubator Means	235.7			249.0		

Chi-Square for Incubator means = 0.365

which is not significant.

Table 2.

Number of aberrant Octads out of 500 scored per plate.

Analysis of Variance of Incubator Data

Source of Variation	df	SS	MS
Among Shelves	5	130.67	
Incubators	1	53.33	53.93
Among Shelves within Incubators = experimental error	4	77.34	19.33
Among Dishes within Shelves = sampling error	24	1690.78	70.45
Total	29	1821.45	62.81

Test for Incubator differences given by :-

$$F = \frac{\text{Incubator MS}}{\text{Experimental Error MS}}$$

$$= 2.79 \quad \text{Not Significant.}$$

Test for Experimental Error is given by :-

$$F = \frac{\text{Experimental Error MS}}{\text{Sampling Error MS}}$$

$$= 0.27 \quad \text{Not Significant.}$$

Table 3.
Variation in gene conversion frequency between shelves and incubators for the cross sk,7,R,7- x sk,1,w78,4,+

P A R A M E T E R S O F
G E N E C O N V E R S I O N

We have seen that many models, or variations of them, have been proposed to explain the observations seen as a result of recombination. The interpretations which have been made of the Meselson-Radding model and the double-strand break repair (DSBR) model have several features in common which have allowed the break down of the mechanism of recombination into its constituent parts, referred to here as parameters.

The similarities of the Meselson-Radding model and the DSBR model include:

(1) The formation of either asymmetric or symmetric heteroduplex DNA (hDNA) at the site under investigation is a result of a single initiation.

(2) The recipient chromatid of genetic information can be either of wild-type or of mutant genotype at the site considered.

(3) The heteroduplex DNA with mismatches may or may not be repaired, leading to classical conversion or postmeiotic segregation respectively.

(4) Repair of mismatches may be to wild-type or to mutant. Wild-type and mutant are considered here but the

same principles apply if the two alleles at a locus are for example A and a.

Taking the basic characters of proposed recombination models at the time, the amenability of the hDNA model to an algebraic analysis has been discussed by various workers (Whitehouse 1965; Emerson 1966; Paquette and Rossignol 1978; Fincham et al. 1980; Kalogeropoulos and Thuriaux 1982, 1985; Lamb and Helmi, personal communication).

An attempt at statistical testing of algebraic modelling was by Kalogeropoulos and Thuriaux (1982). They recognised enough classes of recombinants to allow up to six parameters in their equations and so distinguished the two different types of mismatches that can occur when heteroduplex DNA is formed at the same base pair. In previous attempts to this, all statistical analysis was made making certain arguable assumptions and involved setting certain parameters to estimated values rather than solving the equations. Unfortunately one of the assumptions of the algebraic model is that all aberrant segregation is a result of hDNA formation, not true of the DSBR model unless we assume that the double-strand gap is small and merely acts as an initiation site for hDNA formation.

Because the algebra in this work is based on that of Kalogeropoulos and Thuriaux (1982), their basic

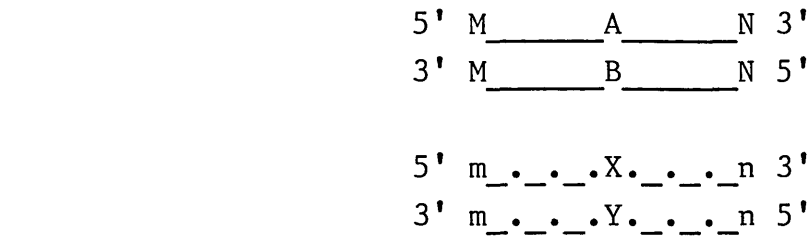
methods are outlined below.

In the original homoduplex DNA of the parental chromatids let us assume that the simple difference between the wild-type and the mutant is the result of a point mutation, i.e. it is a transition or a transversion mutation. This is not totally necessary, because the point that is being put across is also true of other mutations such as deletions and additions. Let there be base pair X-Y in the mutant chromatid and A-B in the wild-type chromatid at the heterozygous site. It is clear to see that a mispair of anti-polar strands must be one of TWO kinds, A-Y and X-B. These two mispairs must be different and there is some evidence in Ascobolus that they will be mismatch repaired differently: this evidence being that different base pair additions have different conversion spectra where other marker effects are supposed to be insignificant (Dohet et al. 1986).

If we label the flanking markers as M/m and N/n then we can diagrammatically represent symmetric and asymmetric heteroduplexes as in Fig.9 (large case M and N being originally linked to the wild-type allele) where the heteroduplex does not extend to the flanking markers.

In this work the top strand (as drawn) is presumed to have a 5'-3' polarity and the bottom strand of a chromatid to have a 3'-5' polarity. i.e., nucleotide bases A and X are carried on the 5'-3' strands.

Figure 9. The original two homoduplex chromatids



ASYMMETRIC hDNA		SYMMETRIC hDNA
wild type is recipient	mutant is recipient	mutual exchange
(a)	(c)	(e)
M A N M . . Y . . N m . . . X . . . n m . . . Y . . . n	M A N M B N m . . . X . . . n m . . B . . . n	M A N M . . Y . . N m . . . X . . . n m . . B . . . n
or	or	or
(b)	(d)	(f)
M . . X . . N M B N m . . . X . . . n m . . . Y . . . n	M A N M B N m . . A . . . n m . . . Y . . . n	M . . X . . N M B N m . . A . . . n m . . . Y . . . n

Four parameters have been defined for the formation of hDNA: α , β , γ and δ.

(γ) is the probability of forming hDNA at the site in question.

(α) is the probability that the hDNA formed will be asymmetric. The probability of the hDNA being symmetric is $(1-\alpha)$.

(β) is the probability that the recipient chromatid in asymmetric hDNA formation is the one carrying the mutant (X-Y) allele.

(δ) is the probability that the invading single strand of DNA has a 3'-5' polarity. The probability that it is 5'-3' is then $(1-\delta)$.

So in Fig.9 , the first two columns would show single meioses with α having a value of 1.0, and the third column α has a value of 0.0 . Diagrams a and b have a β value of 0.0 , and c and d have β values of 1.0 . Diagrams a, c and e have a δ value of 1.0 and b, d and f of 0.0 .

Four parameters have been proposed for the repair of base pair mismatches: p , q , r , and s .

(p) is the probability of repairing a base-pair mismatch A-Y, with a probability of not being repaired of (1-p).

(r) is the probability of the mismatch A-Y being repaired to wild type (A-B), given that it is destined (by

p) to be repaired. The probability of A-Y being repaired to mutant (X-Y) is then (1-r).

(q) is the probability that mismatch X-B will be repaired. The probability that X-B will not be repaired is (1-q).

(s) is the probability that mismatch X-B will be repaired to wild-type (A-B), given that it is destined (by q) to be repaired. The probability of X-B being repaired to mutant (X-Y) is (1-s).

These parameter definitions differ from those used by some other workers, as shown below. The parameters of other workers are given below.

Fincham et al. (1980)	This work
p	pr
q	p(1-r)
r	qs
s	q(1-s)

The seven equations which result for the seven recognisable segregation classes are based on the following premises (Kalogeropoulos and Thuriaux 1982) -

(1) Formation of symmetrical hDNA requires the

exchange of co-polar DNA strands (as proposed by Holliday 1964).

(2) Aberrant segregations are entirely due to the formation of hDNA.

(3) Formation and repair of hDNA is entirely determined by the eight parameters ν, α, β, δ, $\underline{p}$, $\underline{q}$, $\underline{r}$ and $\underline{s}$.

(4) The parameters are independent of one another.

(5) Where symmetric hDNA is repaired, the repair of one mismatch does not affect the repair of the other.

(6) The relationship between hDNA formation and crossing over of the flanking markers is independent of the symmetry of hDNA formation and of mismatch repair. In the analysis performed later in this work this premise and the next one are not required because there is no outside marker.

(7) There is no clustering of crossovers around the region of hDNA.

In Table 4 the symbol '+' is used for a wild-type spore and 'o' is used to represent a mutant spore in a hypothetical organism that produces octads. The table only shows symmetric hDNA and the exchanged strand in this example is of 3'-5' polarity. The probability of this stage having been reached is

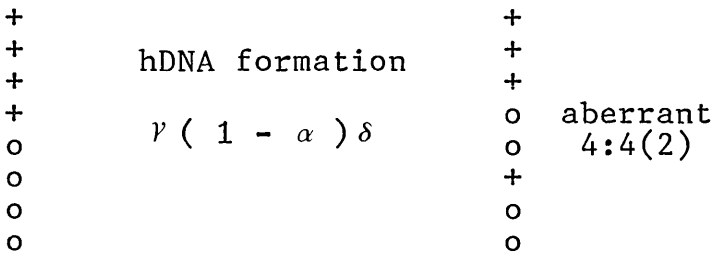
$$\nu(1-\alpha)\delta \quad .$$

Mismatch A-Y	Mismatch X-B: Correction (q) (s)		Mismatch X-B: Correction (q) (1-s)		Mismatch X-B: No Correction (1-q)	
(r) Correction (p)	+ + + + + + o o	6:2	+ + + + o o o o	4:4	+ + + + o + o o	5:3
(1-r)	+ + o o + + o o	4:4	+ + o o o o o o	2:6	+ + o o o + o o	3:5
No Correction (1-p)	+ + + o + + o o	5:3	+ + + o o o o o	3:5	+ + + o o + o o	aberr 4:4

TABLE 4.

Octad types as a result of possible repair / non-repair of base mispairs.'+' are wild type- , and 'o' are mutant - spores. Other orders are possible for the spore pairs.

The original chromatid pair, before and after hDNA formation, is represented below , left and right respectively -



The equations which follow show the seven segregation classes of asci distinguishable in a three factor cross.

The frequency of a class of segregation will be represented as f(G:H), where G:H is the ratio of wild-type:mutant. Therefore, f(6:2), refers to the frequency of octads with six wild-type spores and two mutant spores.

EQUATION ONE:

$$f(6{:}2) = \gamma(1-\alpha)\ \underline{prqs} + \gamma\alpha\beta\,[\,\delta\ \underline{qs} + (1-\delta)\ \underline{pr}\,] .$$

EQUATION TWO:

$$f(2{:}6) = \gamma(1-\alpha)\ \underline{p}(1-r)\underline{q}(1-\underline{s}) + \gamma\alpha(1-\beta)\ [\,\delta\ \underline{p}(1-\underline{r}) + (1-\delta)\ \underline{q}(1-\underline{s})\,] .$$

EQUATION THREE:

$$f(5:3) = \gamma(1-\alpha)\,[\,\delta\, \underline{qs}(1-\underline{p}) + (1-\delta)\, \underline{pr}(1-\underline{q})\,] \,.$$

[substitution]

EQUATION FOUR:

$$f(5:3) = \gamma(1-\alpha)\,[\,\delta\, \underline{pr}(1-\underline{q}) + (1-\delta)\, \underline{qs}(1-\underline{p})\,] + \gamma\alpha\beta\,[\,\delta\,(1-\underline{q}) + (1-\delta)\,(1-\underline{p})\,] \,.$$

[restoration]

EQUATION FIVE:

$$f(3:5) = \gamma(1-\alpha)\,[\,\delta\, \underline{p}(1-\underline{r})(1-\underline{q}) + (1-\delta)\, \underline{q}(1-\underline{s})(1-\underline{p})\,] \,.$$

[substitution]

EQUATION SIX:

$$f(3:5) = \gamma(1-\alpha)\,[\,\delta\, \underline{q}(1-\underline{s})(1-\underline{p}) + (1-\delta)\, \underline{p}(1-\underline{r})(1-\underline{q})\,] + \gamma\alpha(1-\beta)\,[\,\delta\,(1-\underline{p}) + (1-\delta)\,(1-\underline{q})\,] \,.$$

[restoration]

EQUATION SEVEN:

$$f(ab4:4) = \nu(1-\alpha)\ (1-\underline{p})(1-\underline{q})\ .$$

Equations 1-7, as already mentioned, can only be used for data from three factor crosses. In one factor crosses, as mostly used in the present work, there is no distinction between equations 3 and 4 nor between 5 and 6.

In a one factor cross, the f(5:3) and f(3:5) are represented by equations 8 and 9 below.

EQUATION EIGHT:

$$f(5:3) = \nu(1-\alpha)\ [\underline{pr}(1-\underline{q}) + \underline{qs}(1-\underline{p})] + \nu\alpha\beta[\ \delta\ (1-\underline{q}) + (1-\delta)\ (1-\underline{p})]\ .$$

EQUATION NINE:

$$f(3:5) = \nu(1-\alpha)\ [\underline{p}(1-\underline{r})(1-\underline{q}) + \underline{q}(1-\underline{s})(1-\underline{p})] + \nu\alpha(1-\beta)\ [\ \delta\ (1-\underline{p}) + (1-\delta)\ (1-\underline{q})]\ .$$

The order of the repair parameters in this work is such that repair precedes non-repair and associated direction of repair is placed immediately after the parameter with which it is associated.

Kalogeropoulos and Thuriaux (1982, 1985) have had success at fitting these algebraic models to S. fimicola data at the gray locus. However, one of the drawbacks of the system is that insufficient classes exist

for a full test of the model.

It is proposed here that to increase the number of classes of recombinants, not only two chromatid events be taken into account, but also four chromatid events at a site. With two chromatid events only the narrower ratio segregation classes are obtainable (i.e. 6:2, 5:3, aberrant 4:4, 3:5 and 2:6). By considering four chromatid events one can add the four wider ratio segregation classes (i.e. 8:0, 7:1, 1:7 and 0:8). With the ten classes (including normal 4:4) available one can now have up to nine parameters and still be able to test with a significance of fit test such as χ^2 .

In fact, one inaccuracy of the above equations is that they ignore the possibility of narrower ratio segregation asci resulting from four chromatid events. This is rectified in the equations presented here.

New assumptions required to expand the model are :-

(8) hDNA formation at a site between two chromatids precludes hDNA formation of these chromatids with either or both of the other two chromatids at the same site. In other words, after conversion at a site on a chromatid this chromatid cannot form hDNA again at that site.

(9) Once hDNA is formed in more than one pair chromatids at the site in question, repair or non-repair on one chromatid does not affect repair or non-repair on

any other chromatid.

The equations below are for one factor crosses, though it would be simple to alter them for three factor crosses. As an example of deriving the equations the derivation of f(7:1) is shown in detail; for other classes see the diagrams in Appendix I.

Class 7:1 asci may arise only from four chromatid events. A four chromatid event implies the involvement of both pairs of chromatids in hDNA formation at a site, but hDNA may be formed on one or both members of a pair (asymmetric or symmetric hDNA respectively), so that hDNA may be on 2, 3 or 4 chromatids at that site. The probability of hDNA occurring between both pairs of chromatids is represented as $\nu_1\ \nu_2$, where ν_1 is the probability that hDNA will be formed between any two homologous non-sister chromatids at the site concerned, and ν_2 is the probability of hDNA being formed in the remaining pair of homologous non-sister chromatids at the site, given that hDNA is already formed, or being formed, between two chromatids. It is unimportant to the model whether the two events are simultaneous or temporally distinct.

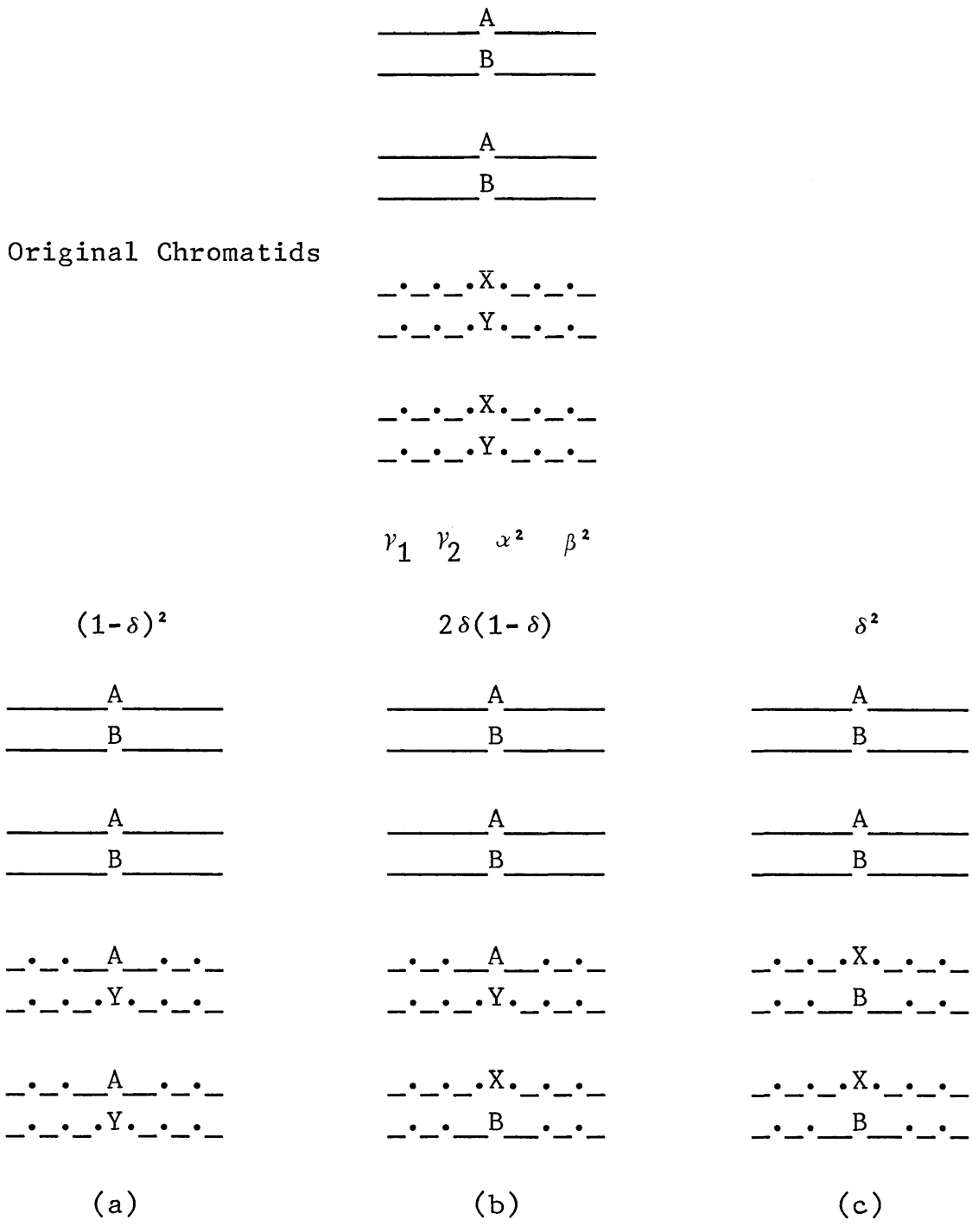


Figure 10.
Asymmetric hDNAs leading to 7:1, shown before any repairs.

If both heteroduplexes have asymmetrical hDNA at that site, then in order to get a 7:1 ascus class, both the recipient chromatids must be wild-type.

Therefore so far we have

$$\gamma_1 \; \gamma_2 \; \alpha^2 \; \beta^2 \; .$$

The invading strands may:

both be 5'-3' $(1-\delta)^2$,

or

both be 3'-5' δ^2 ,

or

one 3'-5' and one 5'-3' $\delta(1-\delta)$,

Because either one of the strands may be 3'-5' in the last case, this class should be multiplied by a factor of two. This argument is valid because it can be seen that the sum of these alternate invasions must add up to one.

i.e.,

$$\delta^2 + (1-\delta)^2 + 2\,\delta\,(1-\delta) = 1 \; .$$

Figure 10 shows the three possible forms of asymmetric hDNA which may lead to a 7:1 class before any correction takes place. For each of the three alternative forms only one of the two mismatches repairs in each four-chromatid set. The algebra for the repairs is given below with a brief explanation.

(a)

$(1-\delta)^2$ One of the two A-Y mismatches is repaired

($\underline{p}$) to wild-type ($\underline{r}$) and the other is not repaired ($1-\underline{p}$). Because there is the identical alternative one must multiply the result by two to give:

$$2\ (1-\delta)^2\ \underline{pr}(1-\underline{p})\ .$$

(b)

$2\delta(1-\delta)$ Either, the A-Y mispair is repaired ($\underline{p}$) to wild-type ($\underline{r}$) and the X-B mispair is unrepaired ($1-\underline{q}$), or the X-B mispair is repaired ($\underline{q}$) to wild-type ($\underline{s}$) and the A-Y is left unrepaired ($1-\underline{p}$):

$$2\delta(1-\delta)\ [\underline{pr}(1-\underline{q}) + \underline{qs}(1-\underline{p})]\ .$$

(c)

δ^2 Only one of the X-B mispairs is repaired ($\underline{q}$) to wild-type ($\underline{s}$) and the other remains unrepaired ($1-\underline{q}$). Again there are two alternatives and so the result must be multiplied by two:

$$2\delta^2\ \underline{qs}(1-\underline{q})\ .$$

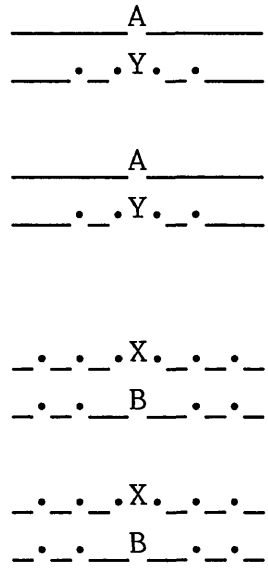
The final equation for the asymmetric formation of 7:1 asci is given as :

$$\nu_1\ \nu_2\ \alpha^2\ \beta^2\ (\ 2(1-\delta)^2\ \underline{pr}(1-\underline{p}) + 2\delta(1-\delta)\ [\underline{pr}(1-\underline{q}) + \underline{qs}(1-\underline{p})] + 2\delta^2\ \underline{qs}(1-q)\ ).$$

If both heteroduplexes have symmetrical hDNA at that site, $(1-\alpha)^2$, then in order to get a 7:1 ascus class, three of the resulting four mismatches must repair to

wild-type and any one of the four may remain unrepaired.

The diagrammatic representation below of the four chromatid strands shows the heteroduplexes before any repair.



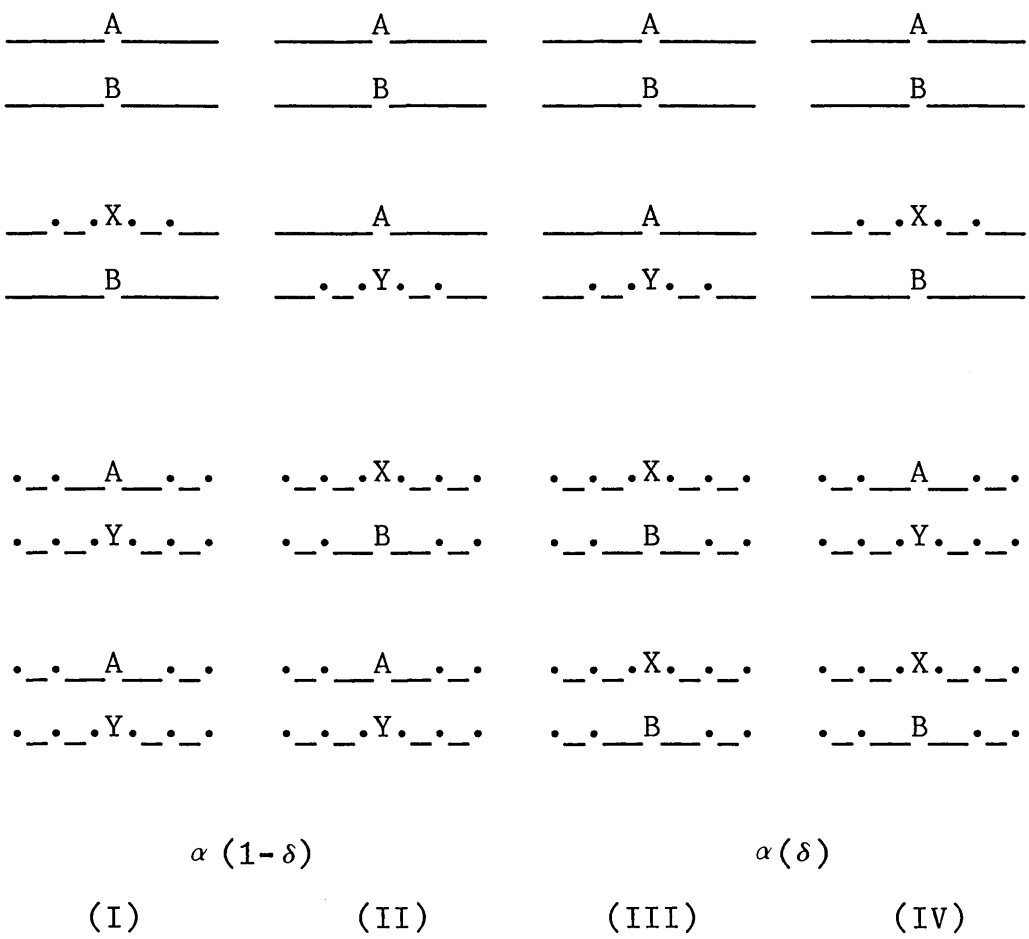
$\nu_1 \nu_2 (1-\alpha)^2$

If it is one of the A-Y mispairs which is not repaired, $(1-\underline{p})$, then the other A-Y must repair, $(\underline{p})$, to wild-type, $(\underline{r})$, as must both the X-B mispairs, $(\underline{q}^2\underline{s}^2)$. Because there is a choice of which of each of the types of mispair is repaired one must multiply each sub-equation by two. The f(7:1) from this origin is therefore:-

$$\nu_1 \nu_2 (1-\alpha)^2 [2\underline{p}^2\underline{r}^2 \underline{q}\underline{s}(1-\underline{q}) + 2\underline{q}^2\underline{s}^2 \underline{p}\underline{r}(1-\underline{p})].$$

Where one of the heteroduplexes is asymmetric and the other symmetric, the invaded chromatid of the

asymmetric hDNA must be mutant (β). This gives what appears to be four possible alternatives before repair.



(I) This type is formed by asymmetric, (α), invasion by the 5'-3', ($1-\delta$), from the wild-type chromatid, (β), and symmetric, ($1-\alpha$), hDNA formation in the other chromatid pair by exchange of the 5'-3' strands ($1-\delta$). A 7:1 can be obtained either by repair of both A-Y mismatches, ($\underline{p}^2$), and non-repair of the X-B, ($1-\underline{q}$), or by

repair of only one A-Y, (p), and the X-B mispair, (q) with two ways in which that could happen: repair would always have to be to wild-type, (r or s). A 7:1 of this origin would thus have a frequency :-

$$\nu_1 \ \nu_2 \ 2\alpha(1-\alpha) \ \beta \ (1-\delta) \ [p^2r^2(1-q) + 2prqs(1-p)].$$

The multiplying factor in front of the 'asymmetric' / 'symmetric' part of the equation is there because chromatid pair one could be symmetric and pair two asymmetric OR vice versa.

(II) This is similar to type I, the only difference being the polarity of the strands exchanged in the symmetric hDNA formation. The 7:1 formed by this type is indistinguishable in a one factor cross from that of type I. The merging of these equations is most easily seen by knowing that type I symmetric hDNA repair is multiplied by $(1-\delta)$ and in type II the symmetric hDNA repair is multiplied by δ . Because the repair of the symmetric hDNA is identical in both these types, the two equations dealing with which strand is involved [δ or $(1-\delta)$] add up to 1.0 . i.e., $(1-\delta) + \delta = 1.0$. The equation for type II is:

$$\nu_1 \ \nu_2 \ 2\alpha(1-\alpha)\beta \ \delta \ (1-\delta) \ [p^2r^2(1-q) + 2prqs(1-p)].$$

(III) Asymmetric (α) invasion of the mutant chromatid by the 3'-5' strand, (δ), from the wild-type chromatid, (β), and symmetric hDNA formation in the other chromatid pair, ($1-\alpha$), by exchange of 3'-5' strands, (δ). Here repair is such that either both B-X mispairs are repaired, ($\underline{q}^2$), to wild-type, ($\underline{r}$), or one B-X, ($\underline{qs}$), and one A-Y, ($\underline{pr}$), are repaired to wild-type.

(IV) A similar argument for this type and type III merging into one equation exists as existed for types I and II.

Thus the final frequency of 7:1 from asymmetric and symmetric hDNA is :-

$$\nu_1\ \nu_2\ 2\alpha(1-\alpha)\ \beta\ (\ (1-\delta)\ [\underline{p}^2\underline{r}^2(1-\underline{q}) + 2\underline{pr}\underline{qs}(1-\underline{p})] +$$

$$\delta[\underline{q}^2\underline{s}^2(1-\underline{p}) + 2\underline{pq}\underline{rs}(1-\underline{q})]\ ).$$

The complete equation for the frequency of |7:1| asci can be written:

$$f(7{:}1) =$$

$$\nu_1\ \nu_2\ \alpha^2\ \beta^2\ (\ 2(1-\delta)^2\ \underline{pr}(1-\underline{p}) + \tag{10}$$

$$2\delta(1-\delta)\ [\underline{pr}(1-\underline{q}) + \underline{qs}(1-\underline{p})] + 2\delta^2\ \underline{qs}(1-\underline{q})\ ),$$

$$+$$

$$\nu_1\ \nu_2\ (1-\alpha)^2\ (\ 2\underline{p}^2\underline{r}^2\underline{qs}(1-\underline{q}) + 2\underline{pr}\underline{q}^2\underline{s}^2(1-\underline{p})\ ), \tag{11}$$

+

$$\gamma_1\ \gamma_2\ \ 2\alpha(1-\alpha)\ \beta\ (\ (1-\delta)\ [\underline{p}^2\underline{r}^2(1-\underline{q}) + 2\underline{p}\underline{r}\underline{q}\underline{s}(1-\underline{p})] + \tag{12}$$

$$\delta[\underline{q}^2\underline{s}^2(1-\underline{p}) + 2\underline{p}\underline{q}\underline{r}\underline{s}(1-\underline{q})]\ ).$$

Equation (10) comes from asymmetric hDNA in both pairs of chromatids, (11) from symmetric hDNA in both pairs of chromatids, and (12) from asymmetric in one pair and symmetric hDNA in the other pair, at that site.

The equations for the other wide ratio segregation classes which follow can be derived in a similar fashion (see appendix I for diagrams detailing α and β parameters).

$$f(1:7) =$$

$$\gamma_1\ \gamma_2\ \alpha^2(1-\beta)^2\ (\ 2(1-\delta)^2\ \underline{q}(1-\underline{s})(1-\underline{q}) + 2\delta^2\ \underline{p}(1-\underline{r})(1-\underline{p}) + \tag{13}$$

$$2\delta(1-\delta)\ [\underline{p}(1-\underline{r})(1-\underline{q}) + \underline{q}(1-\underline{s})(1-\underline{p})]\ ),$$

+

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ [2\underline{p}^2(1-\underline{r})^2\underline{q}(1-\underline{s})(1-\underline{q}) + \tag{14}$$

$$2\underline{q}^2(1-\underline{s})^2\underline{p}(1-\underline{r})(1-\underline{p})],$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ \delta[\underline{p}^2(1-\underline{r})^2(1-\underline{q}) + \tag{15}$$

$$2\underline{p}(1-\underline{r})\underline{q}(1-\underline{s})(1-\underline{p})] +$$

$$(1-\delta)\ [\underline{q}^2(1-\underline{s})^2(1-\underline{p}) + 2\underline{p}(1-\underline{r})\underline{q}(1-\underline{s})(1-\underline{q})]\ ).$$

$$f(8:0) =$$

$$\gamma_1 \gamma_2 \alpha^2 \beta^2 ((1-\delta)^2 \underline{p}^2\underline{r}^2 + 2\delta(1-\delta) \underline{prqs} + \delta^2 \underline{q}^2\underline{s}^2), \quad (16)$$

$$+$$

$$\gamma_1 \gamma_2 (1-\alpha)^2 \underline{p}^2\underline{r}^2\underline{q}^2\underline{s}^2 ., \quad (17)$$

$$+$$

$$\gamma_1 \gamma_2 2\alpha(1-\alpha)\beta \underline{prqs} ((1-\delta) \underline{pr} + \delta \underline{qs}) . \quad (18)$$

$$f(0:8) =$$

$$\gamma_1 \gamma_2 \alpha^2 (1-\beta)^2 (\delta^2 \underline{p}^2(1-\underline{r})^2 + (1-\delta)^2 \underline{q}^2(1-\underline{s})^2 + 2\delta(1-\delta) \underline{p}(1-\underline{r})\underline{q}(1-\underline{s})), \quad (19)$$

$$+$$

$$\gamma_1 \gamma_2 (1-\alpha)^2 \underline{p}^2\underline{q}^2(1-\underline{r})^2(1-\underline{s})^2, \quad (20)$$

$$+$$

$$\gamma_1 \gamma_2 2\alpha(1-\alpha) (1-\beta) \underline{p}(1-\underline{r})\underline{q}(1-\underline{s}) ((1-\delta) \underline{q}(1-\underline{s}) + \delta \underline{p}(1-\underline{r})). \quad (21)$$

One must also consider the narrower ratio segregation classes which have occurred as a result of four chromatid events. As an example of the methods used in deriving the equations for the 6:2, 5:3, ab4:4, N4:4, 3:5 and 2:6 octad frequencies, there follows the algebraic logic for f(6:2).

If both formations of hDNA are symmetric, $[(1-\alpha)^2]$, there are three types of repair which can lead to a 6:2 segregation:

(1) only two of the four mismatches repair to wild-type leaving the remaining two mismatches unrepaired. These repaired mismatches may both be A-Y, $[\underline{p}^2\underline{r}^2(1-\underline{q})^2]$, both be X-B, $[(1-\underline{p})^2\underline{q}^2\underline{s}^2]$, or be one of each A-Y and X-B (two by two alternatives so multiply by four), $[4\underline{p}\underline{r}\underline{q}\underline{s}(1-\underline{p})(1-\underline{q})]$.

(2) either X-B mismatch repairs to mutant, $[2\underline{q}(1-\underline{s})]$, whilst the other three mismatches (one X-B, two A-Y) repair to wild-type, $[\underline{p}^2\underline{r}^2\underline{q}\underline{s}]$,

(3) either A-Y mismatch repairs to mutant, $[2\underline{p}(1-\underline{r})]$, and the other three mismatches (one A-Y, two X-B) repair to wild-type, $[\underline{q}^2\underline{s}^2\underline{p}\underline{r}]$.

Therefore the f(6:2) arising as a result of symmetric hDNA at the site in question in all four chromatids is represented as :-

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ [2\underline{q}(1-\underline{s})\underline{p}^2\underline{r}^2\underline{q}\underline{s} + 2\underline{p}^2\underline{r}(1-r)\underline{q}^2\underline{s}^2 + \underline{p}^2\underline{r}^2(1-\underline{q})^2$$

$$+$$

$$\underline{q}^2\underline{s}^2(1-\underline{p})^2 + 4\underline{p}\underline{q}\underline{r}\underline{s}(1-\underline{p})(1-\underline{q})].$$

With asymmetric hDNA in both of the chromatid pairs, it is easier to visualise the three alternative results before repair. See Figure 11.

Both invaded chromatids are mutant (β^2)

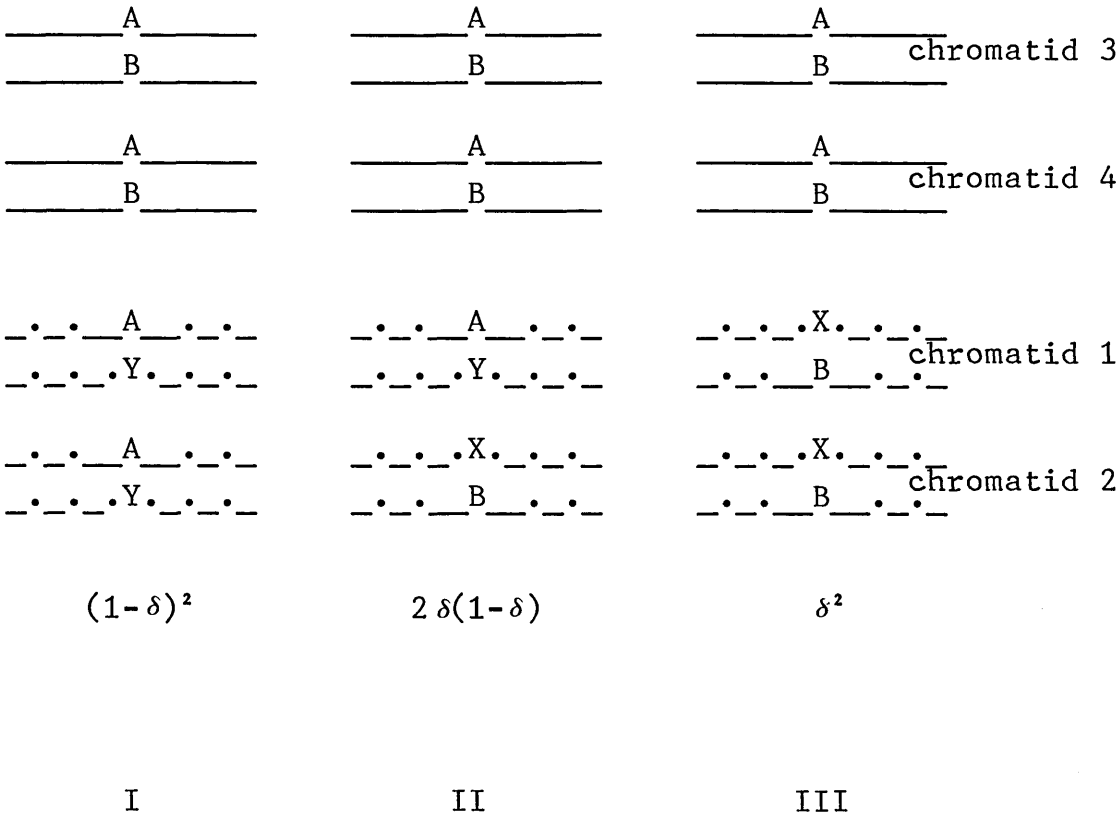


Figure 11.

The three types of double asymmetric hDNA which may lead to a 6:2 segregation class.

Each of the three resulting sets of chromatids in Fig. 11 has a different set of repair requirements to form a 6:2 octad.

(I) $(1-\delta)^2$: either (so multiply by two) of the A-Y mismatches can repair, $(2\underline{p})$, to wild-type, $(\underline{r})$, and the other must repair to mutant, $[\underline{p}(1-\underline{r})]$.

(II) $2\delta(1-\delta)$: either the A-Y mismatch must repair to wild-type, $(\underline{pr})$, and the X-B mismatch must repair to mutant, $[\underline{q}(1-\underline{s})]$, or vice versa, $[\underline{qsp}(1-\underline{r})]$.

(III) δ^2: either (so multiply by two) of the X-B mismatches can repair, $(2\underline{q})$, to wild-type, $(\underline{s})$, and the other must repair to mutant, $[\underline{q}(1-\underline{s})]$.

Also in all three cases above, the option remains of neither of the mispairs repairing, $[(1-\underline{p})^2]$, $[(1-\underline{p})(1-\underline{q})]$ and $[(1-\underline{q})^2]$, respectively.

Therefore the f(6:2) arising as a result of asymmetric hDNA at the site in question in both pairs of chromatids as a result of both invaded chromatids being mutant is represented as :-

$$\gamma_1\ \gamma_2\ \alpha^2\ \beta^2\ (\ (1-\delta)^2\ [2\underline{p}^2\underline{r}(1-\underline{r}) + (1-\underline{p})^2]\ ,$$

$$+$$

$$2\delta(1-\delta)\ [\underline{prq}(1-\underline{s}) + \underline{qsp}(1-\underline{r}) + (1-\underline{p})(1-\underline{q})]\ ,$$

$$+$$

$$\delta^2\ [2\underline{q}^2\underline{s}(1-\underline{s}) + (1-\underline{q})^2]\ )\ .$$

One invaded chromatid is wild-type;
the one invaded in the other pair of
chromatids is mutant.

$2\beta(1-\beta)$

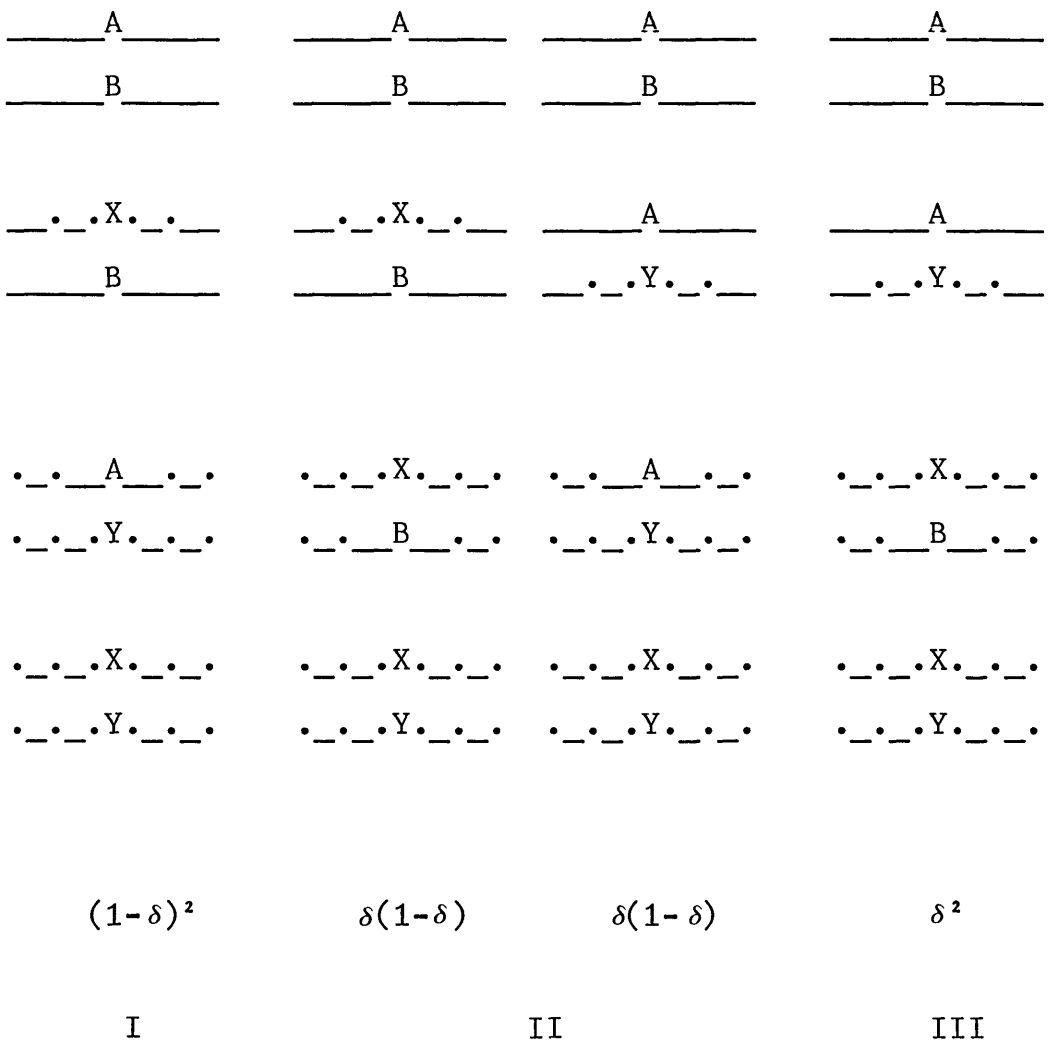


Figure 12.
The three types of double asymmetric hDNA
which may lead to a 6:2 segregation class.

The asymmetric invaded chromatid

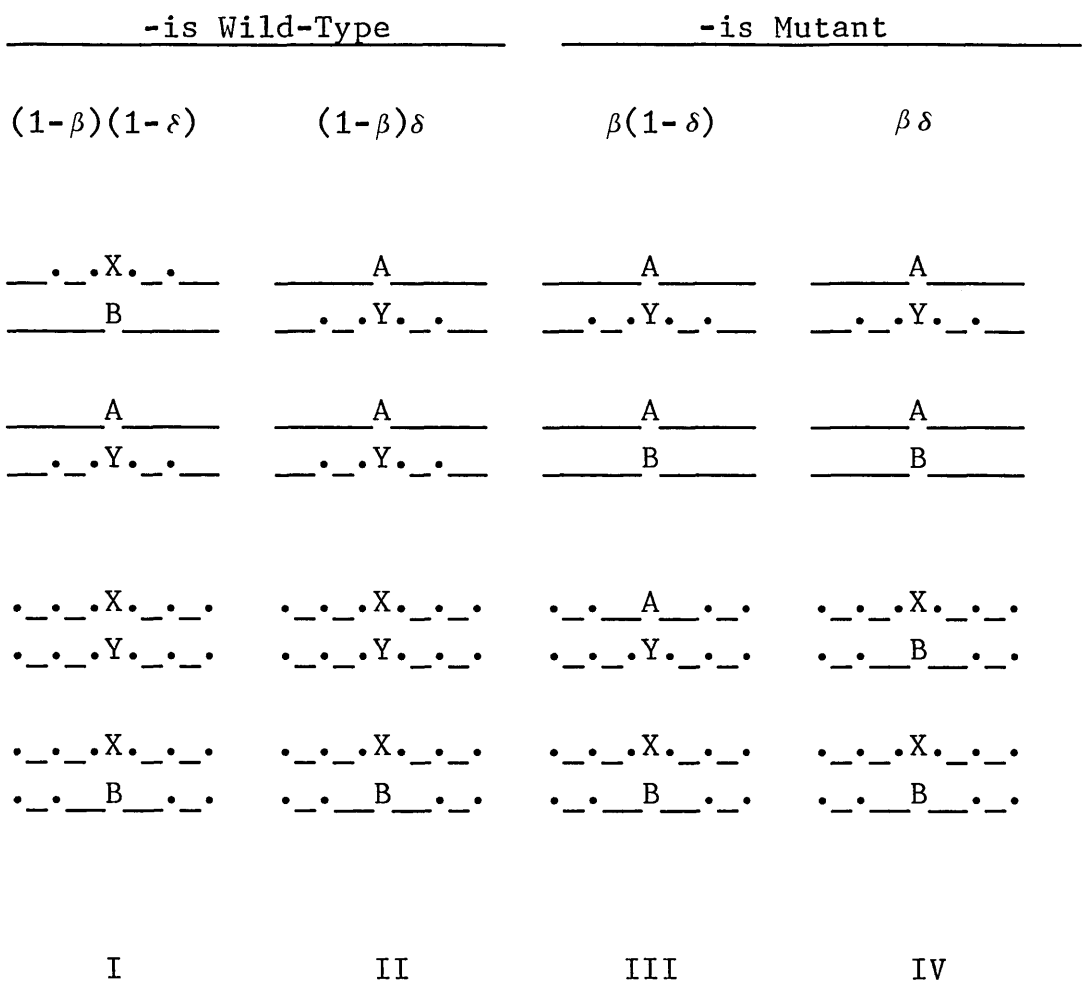


Figure 13.
The four-chromatid stages before repair leading to 6:2, one symmetric, one asymmetric, γ_1 γ_2 $\alpha(1-\alpha)$.

6:2 can also arise from asymmetric hDNA in both pairs of chromatids, with the wild-type chromatid invaded in one pair and the mutant chromatid in the other pair (Figure 12), if both mispairs are repaired to wild-type, which has a frequency of :-

$$\gamma_1\ \gamma_2\ \alpha^2\ 2\beta(1-\beta)\ (\ (1-\delta)^2\ \underline{p}\underline{r}\underline{q}\underline{s}\ +$$

$$\delta(1-\delta)\ [\underline{p}^2\underline{r}^2\ +\ \underline{q}^2\underline{s}^2\ ]\ +\ \delta^2\ \underline{p}\underline{r}\underline{q}\underline{s}\ ).$$

Lastly there is the 6:2 arising from four-chromatid events where one of the hDNA formations is symmetric $(1-\alpha)$ and the other asymmetric (α). In Fig. 13 are shown the basic four possible types at the four-chromatid stage before repair.

(I) $(1-\beta)(1-\delta)$: there is only one way in which this may lead to a 6:2, that is by repair of all the mismatches to wild-type,

$$\underline{p}\underline{r}\underline{q}^2\underline{s}^2\ .$$

(II) $(1-\beta)\delta$: again there is only one way of getting a 6:2, all mismatches repairing to wild-type,

$$\underline{p}^2\underline{r}^2\underline{q}\underline{s}\ .$$

(III) $\beta(1-\delta)$: here there are several ways of getting a 6:2: both A-Y mismatches repair to wild-type, $(\underline{p}^2\underline{r}^2)$, and X-B repairs to mutant, $[\underline{q}(1-\underline{s})]$; one A-Y repairs to mutant, $[2\underline{p}(1-\underline{r})]$, and the other two mismatches repair to wild-type, $(\underline{p}\underline{r}\underline{q}\underline{s})$; or, only one of the three mismatches

repairs to wild-type, the other two remaining unrepaired, $[2\underline{p}\underline{r}(1-\underline{p})(1-\underline{q}) + (1-\underline{p})^2\underline{q}\underline{s}]$, giving a 6:2(2).

(IV) $\beta\,\delta$: there are several ways of yielding a 6:2: both X-B mismatches may repair to wild-type and A-Y repairs to mutant, $[\underline{q}^2\underline{s}^2\underline{p}(1-\underline{r})]$; either of the X-Bs repairs to mutant and the other mismatches both repair to wild-type, $[2\underline{p}\underline{r}\underline{q}^2\underline{s}(1-\underline{s})]$; or, only one of the three mismatches repairs to wild-type and the other two remain unrepaired, $[2\underline{q}\underline{s}(1-\underline{p})(1-\underline{q}) + \underline{p}\underline{r}(1-\underline{q})^2]$, giving a 6:2(2).

The complete set of equations for 6:2 octad generation from four chromatid events where one of the hDNAs is symmetric and one asymmetric is given as :-

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta(\ (1-\delta)\ [2\underline{p}\underline{r}(1-\underline{p})(1-\underline{q}) + \underline{q}\underline{s}(1-\underline{p})^2] + \delta[2\underline{q}\underline{s}(1-\underline{p})(1-\underline{q}) + \underline{p}\underline{r}(1-\underline{q})^2]\ ),$$

$$+$$

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta(\ (1-\delta)\ [\underline{p}^2\underline{r}^2\underline{q}(1-\underline{s}) + 2\underline{p}^2\underline{r}(1-r)\underline{q}\underline{s}] + \delta[\underline{q}^2\underline{s}^2\underline{p}(1-\underline{r}) + 2\underline{p}\underline{r}\underline{q}^2\underline{s}(1-\underline{s})]\ ),$$

$$+$$

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ (1-\delta)\ \underline{p}\underline{r}\underline{q}^2\underline{s}^2 + \delta\,\underline{p}^2\underline{r}^2\underline{q}\underline{s}\ ).$$

Using this same notation, the production of 6:2 asci from two chromatid events may be written as :-

$\gamma_1 (1-\gamma_2) (1-\alpha) \underline{prqs} + \gamma_1 (1-\gamma_2) \alpha\beta (\delta \underline{qs} + (1-\delta) \underline{pr}).$

It is easy to see that it is correct to write $\gamma_1(1-\gamma_2)$, and not $2\gamma_1(1-\gamma_2)$, because all of the alternate forms must add up to 1.0.

Double events are represented by $\gamma_1 \gamma_2$,
Single events are represented by $\gamma_1(1-\gamma_2)$, and
No hDNA events are represented by $1-\gamma_1$

$= 1.0$

There follows the complete set of equations for all the observed narrower ratio segregation classes (6:2 to 2:6) including the normal appearing 4:4 asci resulting from both correction of hDNA and the absence of hDNA at the site in question (termed correction 4:4s and abbreviated to cor4:4).

f(6:2) , including 6:2(2) =

$$\gamma_1 \gamma_2 (1-\alpha)^2 [2\underline{p}^2\underline{r}^2\underline{q}^2\underline{s}(1-\underline{s}) + 2\underline{p}^2\underline{r}(1-\underline{r})\underline{q}^2\underline{s}^2] \quad (22)$$

+

$$\gamma_1 \gamma_2 (1-\alpha)^2 [\underline{p}^2\underline{r}^2(1-\underline{q})^2 + \underline{q}^2\underline{s}^2(1-\underline{p})^2 + 4\underline{prqs}(1-\underline{p})(1-\underline{q})], \quad (23)$$

+

$$\gamma_1 \gamma_2 \alpha^2 \beta^2 ((1-\delta)^2 [2\underline{p}^2\underline{r}(1-\underline{r})] + 2\delta(1-\delta) [\underline{prq}(1-\underline{s}) + \underline{p}(1-\underline{r})\underline{qs}] + \delta^2 [2\underline{q}^2\underline{s}(1-\underline{s})]), \quad (24)$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ \beta^2\ [(1-\delta)^2\ (1-\underline{p})^2 + 2\delta(1-\delta)\ (1-\underline{p})(1-\underline{q}) + \delta^2\ (1-\underline{q})^2], \tag{25}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ 2\beta(1-\beta)\ (\ (1-\delta)^2\ \underline{p}\underline{r}\underline{q}\underline{s} + \delta(1-\delta)\ [\underline{p}^2\underline{r}^2 + \underline{q}^2\underline{s}^2] + \delta^2\ \underline{p}\underline{r}\underline{q}\underline{s}\ ), \tag{26}$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta\ (\ (1-\delta)\ [\underline{p}^2\underline{r}^2\underline{q}(1-\underline{s}) + 2\underline{p}^2\underline{r}(1-\underline{r})\underline{q}\underline{s}] + \delta[\underline{q}^2\underline{s}^2\underline{p}(1-\underline{r}) + 2\underline{p}\underline{r}\underline{q}^2\underline{s}(1-\underline{s})]\ ), \tag{27}$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta\ (\ (1-\delta)\ [2\underline{p}\underline{r}(1-\underline{p})(1-\underline{q}) + \underline{q}\underline{s}(1-\underline{p})^2] + \delta[2\underline{q}\underline{s}(1-\underline{p})(1-\underline{q}) + \underline{p}\underline{r}(1-\underline{q})^2]\ ), \tag{28}$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ (1-\delta)\ \underline{p}\underline{r}\underline{q}^2\underline{s}^2 + \delta\underline{p}^2\underline{r}^2\underline{q}\underline{s}\ ), \tag{29}$$

+

$$\gamma_1\ (1-\gamma_2)\ (1-\alpha)\ \underline{p}\underline{r}\underline{q}\underline{s} + \gamma_1\ (1-\gamma_2)\ \ \alpha\beta(\ \delta\ \underline{q}\underline{s} + (1-\delta)\ \underline{p}\underline{r}\ )\ . \tag{30}$$

---

f(5:3) , including 5:3(3) =

$$\gamma_1 \gamma_2 (1-\alpha)^2 (2\underline{p}(1-\underline{p})\underline{r}(1-\underline{q})^2 + 2\underline{q}(1-\underline{q})(1-\underline{p})^2\underline{s} + 4\underline{p}\underline{r}(1-\underline{p})\underline{q}^2\underline{s}(1-\underline{s}) + 2\underline{p}^2\underline{r}^2\underline{q}(1-\underline{q})(1-\underline{s}) + 4\underline{p}^2\underline{r}(1-\underline{r})\underline{q}\underline{s}(1-\underline{q}) + 2\underline{q}^2\underline{s}^2\underline{p}(1-\underline{r})(1-\underline{p})), \quad (31)$$

+

$$\gamma_1 \gamma_2 \alpha^2 \beta^2 (\delta^2 2\underline{q}(1-\underline{s})(1-\underline{q}) + (1-\delta)^2 2\underline{p}(1-\underline{r})(1-\underline{p}) + 2\delta(1-\delta) [\underline{p}(1-\underline{r})(1-\underline{q}) + \underline{q}(1-\underline{s})(1-\underline{p})]), \quad (32)$$

+

$$\gamma_1 \gamma_2 \alpha^2 2\beta(1-\beta) (\delta(1-\delta) [2\underline{p}(1-\underline{p})\underline{r} + 2\underline{q}\underline{s}(1-\underline{q})] + \delta^2 [pr(1-q) + qs(1-p)] (1-\delta)^2 [\underline{p}\underline{r}(1-\underline{q}) + \underline{q}\underline{s}(1-\underline{p})]), \quad (33)$$

+

$$\gamma_1 \gamma_2 2\alpha(1-\alpha) \beta ((1-\delta) [2\underline{p}^2\underline{r}(1-\underline{r})(1-\underline{q}) + (1-\underline{p})^2(1-\underline{q}) + 2prq(1-s)(1-\underline{p}) + 2\underline{p}(1-\underline{r})\underline{q}\underline{s}(1-\underline{p})] + \delta [2\underline{q}^2\underline{s}(1-\underline{s})(1-\underline{p}) + 2\underline{p}\underline{r}\underline{q}(1-\underline{s})(1-\underline{q}) + (1-\underline{p})(1-\underline{q})^2 + 2\underline{p}(1-\underline{r})\underline{q}\underline{s}(1-\underline{q})]), \quad (34)$$

+

$$\gamma_1 \gamma_2 2\alpha(1-\alpha) (1-\beta) (\delta [\underline{p}^2\underline{r}^2(1-\underline{q}) + 2\underline{p}\underline{r}\underline{q}\underline{s}(1-\underline{p})] + (1-\delta) [q^2s^2(1-p) + 2\underline{p}\underline{r}\underline{q}\underline{s}(1-\underline{q})]), \quad (35)$$

+

$$\gamma_1(1-\gamma_2)\ (\ (1-\alpha)\ [\underline{p}\underline{r}(1-\underline{q}) + \underline{q}\underline{s}(1-\underline{p})] + \alpha\beta[\ \delta(1-\underline{q}) + (1-\delta)(1-\underline{p})]\ )\ . \tag{36}$$

$$\text{f(N 4:4) , including cor4:4} = 1-\gamma_1 \tag{37}$$

+

$$\gamma_1(1-\gamma_2)\ \alpha(\ \beta\ [(1-\delta)\ \underline{p}(1-\underline{r}) + \delta\underline{q}(1-\underline{s})] + (1-\beta)\ [(1-\delta)\ \underline{q}\underline{s} + \delta\underline{p}\underline{r}]\ ), \tag{38}$$

+

$$\gamma_1(1-\gamma_2)\ (1-\alpha)\ [\underline{p}\underline{r}\underline{q}(1-\underline{s}) + \underline{p}(1-\underline{r})\underline{q}\underline{s}], \tag{39}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ \beta^2\ (\ (1-\delta)^2\ \underline{p}^2(1-\underline{r})^2 + \delta^2\ \underline{q}^2(1-\underline{s})^2 + 2\delta(1-\delta)\ \underline{p}(1-\underline{r})\underline{q}(1-\underline{s})\ ), \tag{40}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ 2\beta(1-\beta)\ (\ (1-\delta)^2\ [\underline{p}\underline{r}\underline{q}(1-\underline{s}) + \underline{p}(1-\underline{r})\underline{q}\underline{s}] + \delta^2\ [\underline{p}\underline{r}\underline{q}(1-\underline{s}) + \underline{p}(1-\underline{r})\underline{q}\underline{s}] + \delta(1-\delta)\ [2\underline{p}^2\underline{r}(1-\underline{r}) + 2\underline{q}^2\underline{s}(1-\underline{s})]\ ), \tag{41}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ (1-\beta)^2\ (\ (1-\delta)^2\ q^2s^2 + \delta^2\ p^2r^2 + 2\delta(1-\delta)\ prqs\ ), \tag{42}$$

+

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ (\ 4p^2r(1-r)q^2s(1-s) + p^2r^2q^2(1-s)^2 + p^2(1-r)^2q^2s^2\ ), \tag{43}$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta\ (\ (1-\delta)\ [2p^2r(1-r)q(1-s) + p^2(1-r)^2qs] + \delta\ [prq^2(1-s)^2 + 2p(1-r)q^2s(1-s)]\ ), \tag{44}$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ (1-\delta)\ [2prq^2s(1-s) + p(1-r)q^2s^2] + \delta[p^2r^2q(1-s) + 2p^2r(1-r)qs]\ )\ . \tag{45}$$

---

f(ab 4:4) , including both 4:4(2) and 4:4(4) =

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta\ (\ \delta\ [2q(1-s)(1-p)(1-q) + p(1-r)(1-q)^2] + (1-\delta)\ [2p(1-r)(1-p)(1-q) + q(1-s)(1-p)^2]\ ), \tag{46}$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ \delta\ [2pr(1-p)(1-q) + qs(1-p)^2] + (1-\delta)\ [2qs(1-p)(1-q) + pr(1-q)^2]\ ), \tag{47}$$

+

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ [\ 2\underset{\sim}{p}^2\underset{\sim}{r}(1-\underset{\sim}{r})(1-\underset{\sim}{q})^2 + 2\underset{\sim}{q}^2\underset{\sim}{s}(1-\underset{\sim}{s})(1-\underset{\sim}{p})^2 + 4\underset{\sim}{p}\underset{\sim}{r}\underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{p})(1-\underset{\sim}{q}) + 4\underset{\sim}{p}(1-\underset{\sim}{r})\underset{\sim}{q}\underset{\sim}{s}(1-\underset{\sim}{p})(1-\underset{\sim}{q})\ ], \tag{48}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ 2\beta(1-\beta)\ (\ \delta\ (1-\delta)\ [(1-\underset{\sim}{q})^2 + (1-\underset{\sim}{p})^2] + (1-\delta)^2\ (1-\underset{\sim}{p})(1-\underset{\sim}{q}) + \delta^2\ (1-\underset{\sim}{p})(1-\underset{\sim}{q})\ ), \tag{49}$$

+

$$\gamma_1(1-\gamma_2)\ (1-\alpha)\ (1-\underset{\sim}{p})(1-\underset{\sim}{q}), \tag{50}$$

+

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ (1-\underset{\sim}{p})^2(1-\underset{\sim}{q})^2\ . \tag{51}$$

---

f(3:5) , including 3:5(3) =

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ (\ 2p(1-r)(1-q)^2(1-p) + 2q(1-s)(1-p)^2(1-q) + 2p^2(1-r)^2qs(1-q) + 2prq^2(1-s)^2(1-p) + 4p^2r(1-r)q(1-s)(1-q) + 4p(1-r)q^2s(1-s)(1-p)\ ), \tag{52}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ (1-\beta)^2\ (\ (1-\delta)^2\ 2qs(1-\underset{\sim}{q}) + 2\delta(1-\delta)\ [pr(1-q) + qs(1-p)] + \delta^2\ 2pr(1-p)\ ), \tag{53}$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ 2\beta(1-\beta)\ (\ \delta^2\ [\underset{\sim}{p}(1-\underset{\sim}{r})(1-\underset{\sim}{q}) + \underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{p})] + \tag{54}$$

$$\delta(1-\delta)\ [2\underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{q}) + 2\underset{\sim}{p}(1-\underset{\sim}{r})(1-\underset{\sim}{p})] +$$

$$(1-\delta)^2\ [\underset{\sim}{p}(1-\underset{\sim}{r})(1-\underset{\sim}{q}) + \underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{p})]\ ),$$

$$+$$

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta\ (\ (1-\delta)\ [\underset{\sim}{p}^2(1-\underset{\sim}{r})^2(1-\underset{\sim}{q}) +$$

$$2\underset{\sim}{p}\underset{\sim}{q}(1-\underset{\sim}{r})(1-\underset{\sim}{s})(1-\underset{\sim}{p})] + \tag{55}$$

$$\delta[\underset{\sim}{q}^2(1-\underset{\sim}{s})^2(1-\underset{\sim}{p}) + 2\underset{\sim}{p}\underset{\sim}{q}(1-\underset{\sim}{r})(1-\underset{\sim}{s})(1-\underset{\sim}{q})]\ ),$$

$$+$$

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ (1-\delta)\ [2\underset{\sim}{q}^2\underset{\sim}{s}(1-\underset{\sim}{s})(1-\underset{\sim}{p}) +$$

$$2\underset{\sim}{p}(1-\underset{\sim}{r})\underset{\sim}{q}\underset{\sim}{s}(1-\underset{\sim}{q}) + (1-\underset{\sim}{p})(1-\underset{\sim}{q})^2 + 2\underset{\sim}{p}\underset{\sim}{r}\underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{q})] + \tag{56}$$

$$\delta[2\underset{\sim}{p}^2\underset{\sim}{r}(1-\underset{\sim}{r})(1-\underset{\sim}{q}) + (1-\underset{\sim}{p})^2(1-\underset{\sim}{q}) + 2\underset{\sim}{p}(1-\underset{\sim}{r})\underset{\sim}{q}\underset{\sim}{s}(1-\underset{\sim}{p}) +$$

$$2\underset{\sim}{p}\underset{\sim}{r}\underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{p})]\ ),$$

$$+$$

$$\gamma_1(1-\gamma_2)\ (\ (1-\alpha)\ [\underset{\sim}{p}(1-\underset{\sim}{r})(1-\underset{\sim}{q}) + \underset{\sim}{q}(1-\underset{\sim}{s})(1-\underset{\sim}{p})] + \tag{57}$$

$$\alpha(1-\beta)\ [\ \delta(1-\underset{\sim}{p}) + (1-\delta)\ (1-\underset{\sim}{q})]\ )\ .$$

---

f(2:6) , including 2:6(2) =

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ (\ 2\underline{p}^2\underline{r}(1-\underline{r})\underline{q}^2(1-\underline{s})^2\ +\ 2\underline{p}^2(1-\underline{r})^2\underline{q}^2\underline{s}(1-\underline{s})\ ), \tag{58}$$

+

$$\gamma_1\ \gamma_2\ (1-\alpha)^2\ (\ \underline{p}^2(1-\underline{r})^2(1-\underline{q})^2\ +\ \underline{q}^2(1-\underline{s})^2(1-\underline{p})^2\ + \tag{59}$$

$$4\underline{p}(1-\underline{r})\underline{q}(1-\underline{s})(1-\underline{p})(1-\underline{q})\ ),$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ (1-\beta)^2\ (\ (1-\delta)^2\ 2\underline{q}^2\underline{s}(1-\underline{s})\ + \tag{60}$$

$$2\delta(1-\delta)\ [\underline{p}\underline{r}\underline{q}(1-\underline{s})\ +\ \underline{p}(1-\underline{r})\underline{q}\underline{s}]\ +\ \delta^2\ 2\underline{p}^2\underline{r}(1-\underline{r})\ ),$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ (1-\beta)^2\ (\ (1-\delta)^2\ (1-\underline{q})^2\ + \tag{61}$$

$$2\delta(1-\delta)\ (1-\underline{p})(1-\underline{q})\ +\ \delta^2\ (1-\underline{p})^2\ ,$$

+

$$\gamma_1\ \gamma_2\ \alpha^2\ 2\beta(1-\beta)\ (\ (1-\delta)^2\ \underline{p}(1-\underline{r})\underline{q}(1-\underline{s})\ + \tag{62}$$

$$\delta(1-\delta)\ [\underline{p}^2(1-\underline{r})^2\ +\ \underline{q}^2(1-\underline{s})^2\ ]\ +\ \delta^2\ \underline{p}(1-\underline{r})\underline{q}(1-\underline{s})\ ),$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ (1-\delta)\ [2\underline{p}(1-r)\underline{q}^2\underline{s}(1-\underline{s})\ + \tag{63}$$

$$\underline{p}\underline{r}\underline{q}^2(1-\underline{s})^2\ ]\ +$$

$$\delta\ [2\underline{p}^2\underline{r}(1-\underline{r})\underline{q}(1-\underline{s})\ +\ \underline{p}^2(1-\underline{r})^2\underline{q}\underline{s}]\ ),$$

+

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ (1-\beta)\ (\ (1-\delta)\ [2\underline{q}(1-\underline{s})(1-\underline{p})(1-\underline{q}) + \tag{64}$$

$$p(1-\underline{r})(1-\underline{q})^2 + \delta[2\underline{p}(1-\underline{r})(1-\underline{p})(1-\underline{q}) +$$

$$\underline{q}(1-\underline{s})(1-\underline{p})^2]\ ),$$

$$+$$

$$\gamma_1\ \gamma_2\ 2\alpha(1-\alpha)\ \beta(\ (1-\delta)\ \underline{p}^2(1-\underline{r})^2\underline{q}(1-\underline{s}) + \tag{65}$$

$$\delta\,\underline{p}(1-\underline{r})\underline{q}^2(1-\underline{s})^2\ ),$$

$$+$$

$$\gamma_1(1-\gamma_2)\ (\ (1-\alpha)\ \underline{p}(1-\underline{r})\underline{q}(1-\underline{s}) + \tag{66}$$

$$\alpha(1-\beta)\ [\ \delta\ \underline{p}(1-\underline{r}) + (1-\delta)\ \underline{q}(1-\underline{s})]\ )\ .$$

Within the preceding algebraic equations are several classes which are in fact subclasses of the aberrant classes specified. These subclasses are the postmeiotic segregation classes of 6:2 and 2:6, represented as 6:2(2) and 2:6(2) respectively where the number in brackets is the number of spore-pairs with postmeiotic segregation. The +/o diagram below, representing ordered asci, distinguishes these subclasses.

Parameters

+	+	+	o
+	+	+	+
+	o	o	o
+	+	o	o
o	+	o	+
o	+	o	o
+	o	o	o
+	+	o	o
6:2	6:2(2)	2:6	2:6(2)

Although having the same allele segregation ratios as narrower ratio segregation classes (Lamb and Wickramaratne 1973), 4:4(4), 5:3(3), 3:5(3), 6:2(2) and 2:6(2), come from hDNA formation in two pairs of chromatids at the same site. They can be detected using ordered octads or by using an unlinked marker, as is done to pick up the ab4:4 class in unordered octads.

In practice only a proportion of the aberrant 4:4(2) octads is detected using an unlinked marker in an unordered octad producing organism such as Ascobolus (Ghikas and Lamb 1977). Ghikas and Lamb (1977) have demonstrated that 4:4(2)s are detectable when the unlinked marker and the white spore-colour gene show parental ditypes (PD) and non-parental ditypes (NPD), but only half are detectable from tetratypes (T).

The estimation of two-thirds of the 4:4(2) octads being detected is only true if the alleles are not linked to centromeres. Linkage to a centromere will alter the relative frequencies of parental di type (PD), non-parental di type (NPD) and tetra type (T) formation. Ghikas and Lamb (1977) have estimated the ratio of 4:4(2) events detectable to those occurring. This ratio is represented by the ratio:

$$\frac{(PD + NPD + \tfrac{1}{2}T)}{(PD + NPD + T)}$$

Table 5 shows that a value of 70 % must be used in the case of grn-20 and w78 , and that the two alleles are not linked. In table 5 half the crosses were in repulsion and half in coupling.

Unfortunately 5:3(3) and 3:5(3) events are indistinguishable from events of single postmeiotic segregation origins unless more than two markers are used or if the two used have very small centromere distances (Ghikas and Lamb 1977).

In the same manner as Kalogeropoulos and Thuriaux (1982,1985) used an iterative computing procedure to find solutions giving the best fit, the full sixty-six equations derived earlier in this chapter were put into Fortran notation. Because of the complexity of the

Parameters

Cross	Non-Parental Di-Types	Parental Di-Types	Tetra-Types	Total Asci Scored
1	107	109	285	501
2	95	90	314	499
3	86	94	320	500
4	95	99	306	500
5	106	100	294	500
6	57	78	165	300
7	67	101	233	401
8	101	113	286	500
Totals =	714	784	2 203	3 701

The Ratio $\frac{\text{Detected } 4:4(2)}{\text{Occurring Events}}$,

is proportional to $\frac{PD + NPD + \frac{1}{2}T}{PD + NPD + T}$.

For the w78 allele this equals 70.24 % .

Tetratype frequency = 59.5 %

Recombination Frequency is $\frac{\frac{1}{2}T + NPD}{PD + NPD + T}$,

For the w78 and grn-20 alleles this equals 49.05 %, showing no linkage.

Table 5.
Showing the Tetratype frequency
in the cross between grn-20 and w78

equations single solutions could not be obtained by classic algebraic methods. All the domains with solutions giving a goodness of fit χ^2 lower than 200 were found by forming a grid where each parameter was allowed to vary between 0.0 and 1.0 by steps of 0.1 .

For each domain with a χ^2 less than a hundred, further solutions were explored using steps of 0.01 for the parameters and solutions were found with the smallest χ^2, then steps of 0.001 and so on until the required degree of accuracy was obtained. Unfortunately there is no way to calculate the variance of the best fitting estimates but looking at graphs where one parameter changes and observing the variation in the χ^2 can give an estimate of all the possible solutions.

If N is the number of octads being considered, then N ν_1 is the number of octads where hDNA formation does occur. N ν_1 was allowed to vary between half the number of aberrant asci in the sample and twice that number.

Only the aberrant 4:4 asci due to single postmeiotic segregation are included in the expected frequency total, f(ab4:4), those due to double postmeiotic segregation, 4:4(4), and undetectable aberrant 4:4(2) will be added to the normal 4:4 class, N4:4.

In this way, for each segregation class, there is an estimated frequency of observation. Next, in order

to test the algebraic model, data had to be collected. But first a locus of study had to be considered and an unlinked marker for the detection of aberrant 4:4s .

The most suitable locus seemed to be wI because of the conversion control factors available and the many alleles such as w-78 and w-10. A suitable unlinked marker to detect aberrant 4:4 asci was considered to be one of the spontaneously arising granular mutants which in the wI^+,grn ascospores showed spore pigment to be localised into minute spheroids loosely attached to the spore wall. These spheroids, called granules, vary slightly in size but are estimated to measure about a twentieth of the spore length. In spores carrying white-spore colour mutations the granular phenotype is less consistent, only occasionally being detected as colourless granules floating freely in the gelatinous envelope of the octad, so wI^- is effectively epistatic to the granular locus.

A granular mutant was chosen that had a comparatively low conversion frequency of its own and was easily scored, with no chance of phenocopying the white-spore mutants. A good candidate, grn-20, was chosen with a low conversion frequency. When crossed with wild-type, sk,7,R,7,- , this mutant had a conversion frequency of less than half of one percent. This compares with a ten to fifty fold greater frequency of conversion of the w-78 allele. Whilst a cross granular x wild-type does not pick

Results of conversion of the grn-4 and grn-20 alleles.

	grn-4		grn-20	
Conversion Class	Number of Octads	% of Octads	Number of Octads	% of Octads
6:2	7	0.173	45	0.222
5:3	18	0.445	1	0.005
3:5	16	0.395	2	0.010
2:6	2	0.049	40	0.198
1:7	1	0.025	1	0.005
4:4 N	4 004	98.91	20 141	99.56
aberrants	44	1.09	89	0.44
Total	4 049	100.00	20 230	100.00

Table 6.
From a cross between grn and wild-type sk,7,R,7,-.

up aberrant 4:4 segregation of the granular mutant, post-meiotic segregation is expected to be a high contributer to misidentifying aberrant 4:4 segregation of white-spore mutants, Table 6 shows that the majority of conversion at the grn-20 allele is due to non-postmeiotic events. Comparing these results to the grn-4 one can see that grn-20 is greatly to be preferred. With the latter, the proportion of misidentified ab4:4s at wI will be under half a percent of genuine ^{ab}4:4s at wI.

Of the fifteen germinating white spores isolated from a cross between grn-20 and a wild-type, all were shown to be new spontaneous spore-colour mutations, breeding true for white-spore. This is a good indication that there is less chance of this granular mutant phenocopying the white-spore mutants than of spontaneous white-spore mutations occurring. These grn-20 conversion results come from over twenty thousand octads scored. The total number of white spores seen was twenty four and these always occurred in 4(red or granular):4(white) ratios which also strongly indicates spontaneous mutations rather than mis-scoring of granular spores.

Below are the octad types with the equation reference numbers used in this chapter.

Postmeiotic

Class of Octad	Segregation	Equation References
f(8:0)	no	16 , 17 , 18.
f(7:1)	yes	10 , 11 , 12.
f(6:2)	no	22 , 24 , 26 , 27 , 29 , 30.
f(6:2)(2)	yes	23 , 25 , 28.
f(5:3)	yes	31 , 32 , 33 , 34 , 35 , 36.
fN(4:4)	no	37,38,39,40,41,42,43,44,45.
fab(4:4)	yes	46 , 47 , 49 , 50.
fab(4:4)(4)	double	51 .
f(3:5)	yes	52 , 53 , 54 , 55 , 56 , 57.
f(2:6)	no	58 , 60 , 62 , 63 , 65 , 66.
f(2:6)(2)	yes	59 , 61 , 64.
f(1:7)	yes	13 , 14 , 15.
f(0:8)	no	19 , 20 , 21.

A test for errors in the equations and in the computer programming is possible by seeing if all the expected frequencies of octad classes add up to one when random values are assigned to the parameters. In the final iterative programme this test was passed to four decimal places.

In practice the observed frequencies of 6:2(2) and 2:6(2) octads were too small to permit a full χ^2 goodness of fit test and therefore combined values for all octads segregating 6:2 were made regardless of postmeiotic segregation. This was also done for all octads segregating 2:6 . However, this work shows that the distinction of these classes is possible if the sample is large enough. (Note that not all 6:2(2) octads are recognisable as such with only one marker such as grn-20. This is also true of 2:6(2) octads, see Ghikas and Lamb 1977, for formulae).

It was hoped that by looking at different alleles at the same locus something could be discovered about the parameters. For example, allele specificity is expected for parameters related to mismatch repair (p, q, r and s) but not necessarily for parameters related to the formation of the hDNA (α, β, and δ); γ and α, for example, might change somewhat across the length of a locus, but would probably not change much for very close sites, on most recombination models (Lamb 1986b).

RESULTS:

As controls, wild-type x wild-type crosses were made to test for spontaneous mutations from wild-type to white spore colour, and w-78 x w-78 and w-10 x w-10 crosses were made to test for reversion from white to wild-type spore colour. A check for phenocopies and false clustering was possible by numerous backcrosses and with the aid of secondary unlinked markers such as fpr-1 and yel-7 (fluorophenylalanine resistance and yellow mycelium mutants respectively).

As in previous studies (Lamb and Wickramaratne 1973) there was no reversion to wild-type. Forward mutation from wild-type to white was observed in wild-type x wild-type crosses, varying from 0.0 % (+/-0.01) to 0.02 % (+/-0.01) for 10 000 octad samples (percentages given as proportions of 4:4 octads). These results are comparable to previous studies (Wickramaratne 1974).

In tests for phenocopies and false clusters, all octads tested which displayed aberrant phenotypic ratios (including 4:4(2)) proved to be genuine. Thus it was concluded that phenocopies are very rare and theoretically only occur with a majority of white spores. Because of the large numbers of plates screened, compared to previous studies of wider ratio octads, the influence of unwanted spontaneous mutants was reduced. Care was taken when scoring 1:7 and 0:8 octads that they did not occur over-

frequently on a single plate, which suggested that such clustering was occurring due to new mutations. Only thirty-one such plates were found and on backcrossing it was shown that all the white mutants which germinated and were fertile were unlinked to w-78. The results from these plates were ignored in the analysis.

In germination studies, fpr-1, yel-7 and mating type were used to test false clustering; unfortunately the spore-products were not always fertile.

The results presented in Tables 7 to 16 are based on crosses involving the following strains, which in small test samples were consistent with conversion-control-factor results of previous workers (Helmi 1980): -

sk,7,R,7,- ; sk,9,1,+ ; sk,1,w78,4,+ ; sk,1,w78,5,- ; st,12,fpr1,1,+ ; st,4,fpr1,6,- ; st,4,w78,fpr1,7,- ; and st,4,w78,fpr1,8,+ for the non-super strains carrying the P allele at the ccf-2 locus.

Sup,1,w78,3,+ ; Sup,2,w78,3,- ; Sup,2,R,1,+ ; and Sup,2,R,5,- for the strains carrying the super factors at ccf-3 and ccf-4.

sz,6,w78,(K),2,- ; sz,5,w78,(K),4,+ ; sz,5,R,(K),5,-; and sz,6,R,(K),3,+ for the strains carrying the K alleles at the ccf-2 locus.

sy,3,R,(91),6,+; sy,3,R,(91),8,- ; sy,3,w78,(91),1,+; and sy,3,w78,(91),3,- for the strains carrying the 91 alleles at the ccf-2 locus.

Parameters

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	41	39.544	3.604E-04
2:6	2 585	2 576.014	2.348E-02
3:5	810	817.546	7.450E-03
N4:4	98 823	98 827.220	0.9004
5:3	1 237	1 231.952	1.227E-02
6:2	5 663	5 667.589	5.165E-02
7:1	126	131.442	1.198E-03
8:0	331	324.557	2.958E-03
ab4:4	51	48.085	6.260E-04
0:8	64	67.060	6.111E-04
Total	109 731		

γ_1 = 0.1271 α = 0.7475

γ_2 = 0.1228 β = 0.652 δ = 0.000

$\underline{p}$ = 0.8867 $\underline{q}$ = 0.8624 $\underline{r}$ = 0.9395 $\underline{s}$ = 0.1616

Chi-Square = 0.8487

Table 7.
Results for the cross + x w-78, with P alleles at both ccf-2 site, non-super. See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	8	6.894	1.382E-04
2:6	173	171.162	3.431E-03
3:5	154	163.825	3.284E-03
N4:4	48 573	48 574.960	0.9734
5:3	338	335.577	6.727E-03
6:2	525	521.058	1.044E-02
7:1	39	43.881	8.796E-04
8:0	37	35.372	7.090E-04
ab4:4	36	31.397	8.990E-04
0:8	5	3.882	7.781E-05
Total	49 888		

ν_1 = 0.0329 α = 0.672

ν_2 = 0.1830 β = 0.589 δ = 0.000

p = 0.679 q = 0.793 r = 0.963 s = 0.496

Chi-Square = 2.4488

Table 8.
Results for the cross + x w-78, with K alleles at both ccf-2 site. See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	6	4.311	8.352E-05
2:6	323	329.450	6.382E-03
3:5	197	194.832	3.774E-03
N4:4	49 540	49 539.251	0.9596
5:3	244	251.968	4.881E-03
6:2	1 228	1 217.213	2.358E-02
7:1	16	19.886	3.852E-04
8:0	51	51.200	9.918E-04
ab4:4	12	10.161	2.818E-04
0:8	5	3.734	7.234E-05
Total	51 622		

ν_1 = 0.05315 α = 0.726

ν_2 = 0.08701 β = 0.577 δ = 0.000

$\underline{p}$ = 0.920 $\underline{q}$ = 0.801 $\underline{r}$ = 0.947 $\underline{s}$ = 0.513

Chi-Square = 2.6814

Table 9.
Results for the cross +,K x w-78,P.
See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	3	1.944	4.947E-05
2:6	223	227.841	5.797E-03
3:5	134	139.967	3.561E-03
N4:4	37 625	37 624.134	0.9571
5:3	233	225.092	5.727E-03
6:2	1 031	1 029.286	2.619E-02
7:1	14	13.895	3.535E-04
8:0	29	33.014	8.399E-04
ab4:4	11	9.223	3.352E-04
0:8	3	1.614	4.106E-05
Total	39 306		

ν_1 = 0.0562 α = 0.676

ν_2 = 0.0646 β = 0.583 δ = 0.000

p = 0.912 q = 0.814 r = 0.980 s = 0.546

Chi-Square = 3.2327

Table 10.
Results for the cross +,P x w-78,K.
See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	6	5.614	2.054E-04
2:6	219	222.641	8.144E-03
3:5	185	192.819	7.054E-03
N4:4	25 824	25 823.263	0.9442
5:3	376	375.283	1.373E-02
6:2	631	623.437	2.281E-02
7:1	29	30.985	1.133E-03
8:0	24	26.466	9.682E-04
ab4:4	36	32.088	1.677E-03
0:8	6	3.404	1.245E-04
Total	27 336		

γ_1 = 0.0726 α = 0.6831

γ_2 = 0.1234 β = 0.7671 δ = 0.000

p = 0.749 q = 0.752 r = 0.740 s = 0.290

Chi-Square = 3.3104

Table 11.
Results for the cross w-78, with 91 alleles at both ccf-2 site. See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	15	15.100	2.009E-04
2:6	2 125	2 130.783	2.835E-02
3:5	2 324	2 300.229	3.060E-02
N4:4	55 039	55 038.234	0.7300
5:3	4 628	4 691.401	6.241E-02
6:2	10 313	10 283.129	0.1368
7:1	150	148.374	1.974E-03
8:0	164	164.641	2.190E-03
ab4:4	401	386.045	7.337E-03
0:8	6	7.062	9.395E-05
Total	75 165		

ν_1 = 0.35707 α = 0.5056

ν_2 = 0.04050 β = 0.5390 δ = 0.0000

p = 0.8295 q = 0.7661 r = 0.9492 s = 0.5856

Chi-Square = 1.9649

Table 12.
Results for the cross +,P,super x w-78,P,super.
See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	25	19.789	5.330E-04
2:6	1 463	1 474.438	3.971E-02
3:5	997	1 008.958	2.718E-02
N4:4	28 111	28 116.292	0.7559
5:3	1 848	1 831.538	4.933E-02
6:2	4 320	4 307.612	0.1160
7:1	98	105.343	2.837E-03
8:0	124	127.267	3.428E-03
ab4:4	123	121.835	4.688E-03
0:8	19	14.929	4.021E-04
Total	37 128		

ν_1 = 0.2877 α = 0.7604

ν_2 = 0.0675 β = 0.7334 δ = 0.0000

$\underline{p}$ = 0.7818 $\underline{q}$ = 0.7179 $\underline{r}$ = 0.9468 $\underline{s}$ = 0.0576

Chi-Square = 3.5042

Table 13.
Results for the cross +,P,non-super x w-78,P,super.
See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	25	24.261	4.588E-04
2:6	2 084	2 086.816	3.946E-02
3:5	1 640	1 631.906	3.086E-02
N4:4	40 730	40 732.000	0.7687
5:3	2 519	2 519.909	4.765E-02
6:2	5 472	5 470.972	0.1035
7:1	93	98.021	1.854E-03
8:0	113	108.723	2.056E-03
ab4:4	189	192.589	5.203E-03
0:8	16	15.805	2.989E-04
Total	52 881		

γ_1 = 0.2696 α = 0.7625

γ_2 = 0.0488 β = 0.6976 δ = 0.0000

p = 0.7807 q = 0.6561 r = 0.9379 s = 0.0511

Chi-Square = 0.5619

Table 14.
Results for the cross +,P,super x w-78,P,non-super.
See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	6	7.560	2.815E-04
2:6	443	440.601	0.01640
3:5	388	388.721	0.01447
N4:4	24 630	24 627.800	0.91598
5:3	493	514.225	0.01914
6:2	797	787.761	0.02933
7:1	18	17.874	6.655E-04
8:0	15	13.959	5.197E-04
ab4:4	66	57.129	3.039E-03
0:8	4	4.365	1.625E-04
Total	26 860		

ν_1 = 0.1089 α = 0.641

ν_2 = 0.0628 β = 0.632 δ = 0.000

p = 0.758 q = 0.703 r = 0.771 s = 0.265

Chi-Square = 2.8077

Table 15.
Results for the cross +,91,super x w-78,91,super.
See text for strains used.

Octad Type	Observed Number of Octads	Expected Number of Octads	Expected Proportion of Octad Type
1:7	1*	0.791	1.205E-05
2:6	2 023	2 023.673	3.084E-02
3:5	59	61.156	9.321E-04
N4:4	57 644	57 662.644	0.8789
5:3	79	83.373	1.271E-03
6:2	5 664	5 657.774	8.623E-02
7:1	4	2.986	4.551E-05
8:0	101	104.072	1.586E-03
ab4:4	1*	0.225	4.899E-06
0:8	14	13.314	2.029E-04
Total	65 610		

γ_1 = 0.1601 α = 0.789

γ_2 = 0.0330 β = 0.712 δ = 0.000

p = 0.993 q = 0.981 r = 0.921 s = 0.181

Chi-Square = 3.5071

Table 16.
Results for the cross w-10, with P alleles at both ccf-2 site. * indicates sample too small for χ^2 test.

Where applicable, results for identical crosses (excluding mating types and secondary markers) were pooled in order to increase the size of the sample and to try to cancel out any background effects. This last effect has been evident throughout this work and others (see Helmi 1980 for a comparison of various workers' results for theoretically similar strains thought to be carrying the same conversion affecting genes).

It can be seen that all the results are explicable by the proposed model at the 5% significance level for χ^2 which is 3.84 for one degree of freedom. Many of the goodness of fit tests showed much better agreement between the results and the expected octad ratios than previously found by other workers (Kalogeropoulos and Thuriaux 1985; 1982). In the tables, the expected column represents the values on which the chi-squares were calculated. These expected values have the proportions of detectable octad classes taken into account.

Table 17 summarises the results for the parameter values for crosses involving the non-super genotypes and it is clear that δ has a value of 0.0 indicating that the invading strand always has the same polarity. This is in sharp contrast to the results obtained in Sordaria fimicola at the gray locus where the full set of parameters was unable to fully explain the data when δ = 0.0 (Kalogeropoulos and Thuriaux 1985; they were

Cross	ν_1	ν_2	α	β	δ	p	q	r	s
w78 x +									
P x P	0.1271	0.1228	0.748	0.652	0.0	0.887	0.862	0.940	0.162
K x K	0.0329	0.1830	0.672	0.589	0.0	0.679	0.793	0.963	0.496
P x K	0.0532	0.0870	0.726	0.577	0.0	0.920	0.801	0.947	0.513
K x P	0.0562	0.0646	0.676	0.583	0.0	0.912	0.814	0.980	0.546
91 x 91	0.0726	0.1234	0.683	0.767	0.0	0.749	0.752	0.740	0.290
w10 x +									
P x P	0.1601	0.0330	0.789	0.712	0.0	0.993	0.981	0.921	0.181

Table 17 . Showing parameter values
for crosses involving non-super.

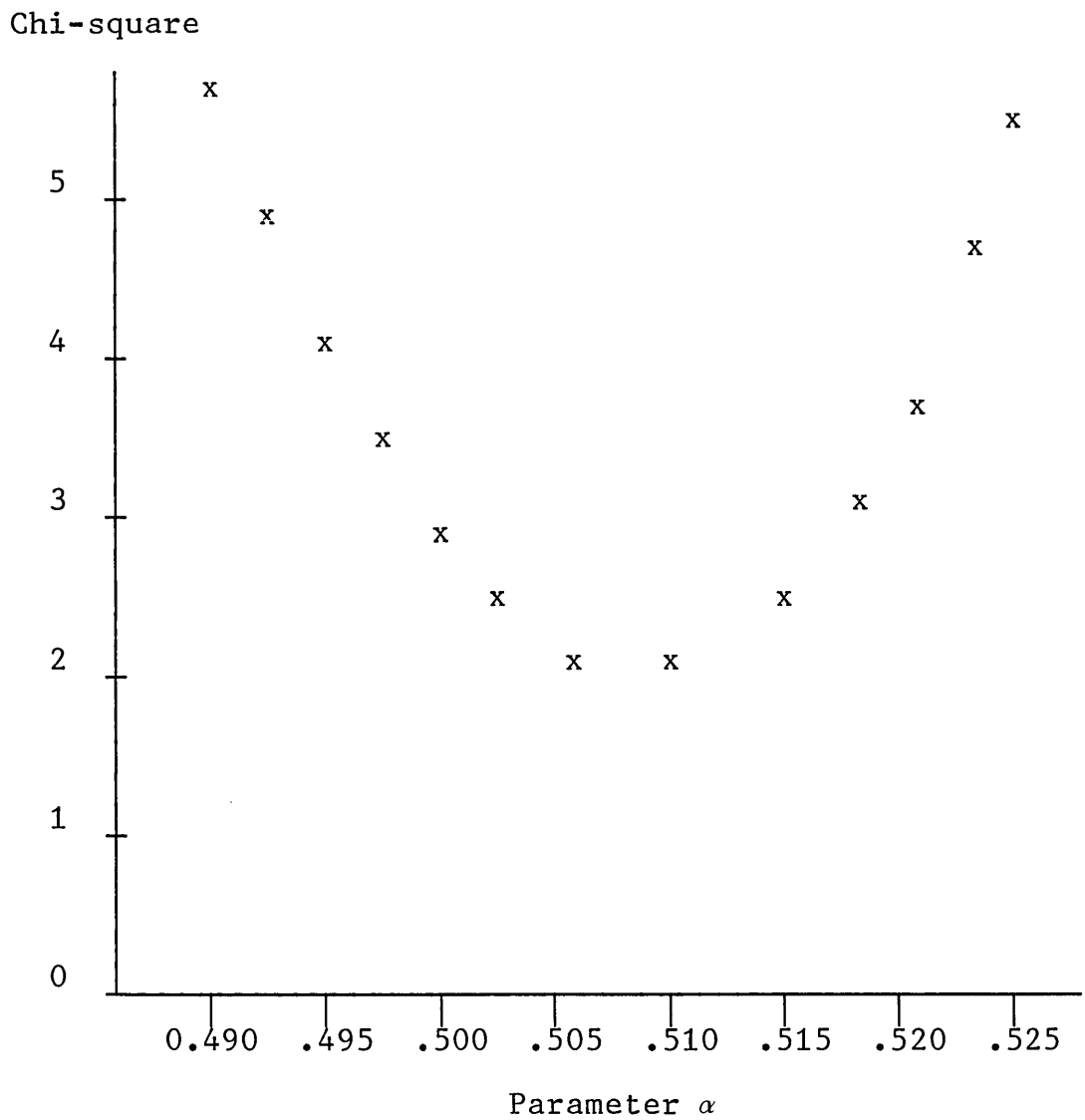


Figure 14.
How goodness of fit varies with α.

unable to explain all the data with any one set of assumptions, but with δ = 0.0 they often got a good fit).

The amount of hDNA which was asymmetric at the w78 site varied with the type of allele at the ccf-2 allele which is closely linked to the wI locus. Figure 14 shows an example of how the goodness of fit varies with the change in α. Whilst the graph is for the super(P) x super(P) cross, graphs for non-super strains analagous to those presented give the same picture, i.e. that α can only vary about +/- 0.0125 before the model fails to explain the data obtained. The results are interesting in that the amount of asymmetric hDNA is extremely similar for the K,w-78 x K,+ cross and the K,w-78 x P,+ and the 91 x 91 cross but different to that in the two crosses P,w-78 x P,+ and P,w-78 x K,+ which are similar to each other in this respect. The parameter ν_1 is similar in the cis/trans crosses involving w-78 and the K/P alleles at the ccf-2 locus but intermediate compared to each homozygous cross, the P x P cross having the highest amount of hDNA formation.

There is no obvious feature for the ν_2 values with no clear indications about how this parameter is affected by the ccf-2 alleles: corresponding-site interference was negative for P,w78 x P,+, was strongly negative for K,w78 x K,+, and was strongly positive for P,w10 x P,+. The probability that the asymmetric hDNA was

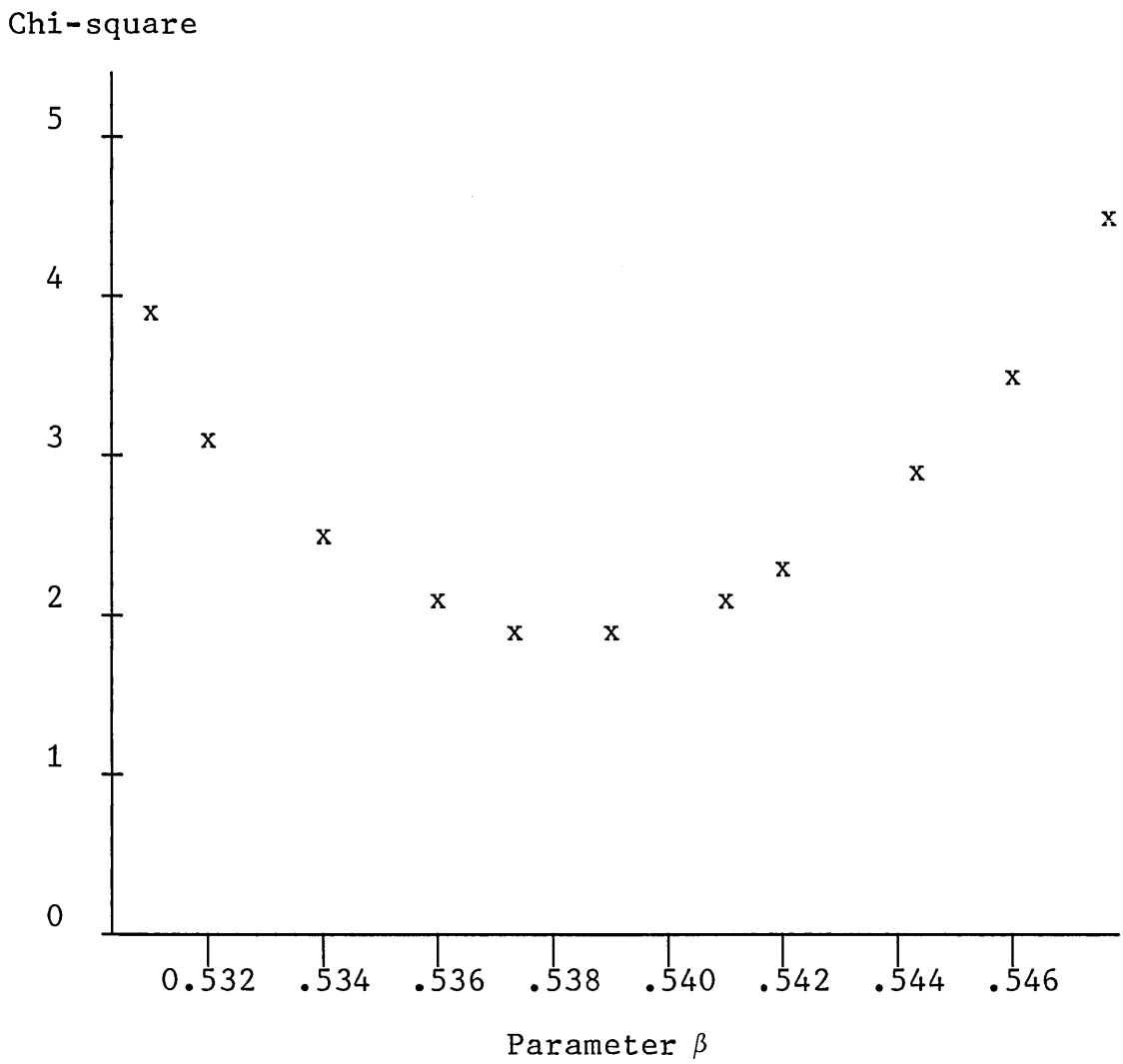


Figure 15.
How goodness of fit varies with β.

formed on one chromatid was different from that on its non-sister chromatid (β not equalling 0.5). Figure 15 shows that the value of β may vary by +/- 0.008 and this would suggest that the bias in chromatid invasion was more extreme for the P x P cross which showed a value for β of 0.652 compared to an average of 0.583 ; this was not a large difference but as far as can be tested this difference has an importance in determining the fit of the model and must be considered as large for this reason. It is highly likely that this model is extremely sensitive to small sampling errors because it can distinguish between such small differences in some of the parameters; alternatively it can be argued that small differences in the values of parameters has a large affect upon octad ratios.

The values for the repair parameters are also very interesting. For the mismatch determined by the parameter p, where a difference in the ccf-2 site occurs, there seems to be a greater chance of repair than if the factor is homozygous, 0.912 or 0.920 compared with 0.679, 0.749 or 0.887 .

This connection between ccf-2 and repair is less apparent for the mismatch determined by q, where the P x P cross differs with q being greater and the 91 x 91 cross being the lowest.

The direction of the mismatch repair (parameter

r) for the hDNA determined by p seems to be independent of the allele at ccf-2 being K or P.

The direction of repair (parameter s) of the mispair determined by q showed similar values, near parity, between the crosses K,w-78 x K,+ and P,w-78 x K,+. In the homozygous cross of P x P there was an obvious disparity in repair towards mutant type (s=0.162). In the cross K,w-78 x P,+ there was a smaller disparity in conversion towards the wild-type (s=0.546).

On the contrary, the 91 x 91 cross differed from the P and K crosses in every single parameter except α where it showed similar values to the crosses which carried w-78 and the K allele on the same chromatids. Of particular interest is the value of β which is 0.767, clearly very different from 0.5 .

The results for w-10 show a remarkable dissimilarity to those for w-78 considering how closely the two markers map to each other. With the ccf-2 alleles being P x P in both crosses for w-10 and w-78 one would expect α and β to be, if not the same, very similar indeed. This is not so. The proportion of asymmetric hDNA formed is larger for w-10 (by 0.041) and the value of β is larger for w-10 also (by 0.06). These differences in themselves do not seem large but they are large enough to render the model such that it would not fit the data (see Figures 17 to 22).

How the "super" factors affect recombination

w78 x + Cross	ν_1	ν_2	α	β	δ	p	q	r	s
P x P									
sup x sup	0.3571	0.0405	0.506	0.539	0.0	0.830	0.766	0.949	0.586
sup x non	0.2877	0.0675	0.760	0.733	0.0	0.782	0.718	0.947	0.058
non x sup	0.2696	0.0488	0.763	0.698	0.0	0.781	0.656	0.938	0.051
non x non	0.1271	0.1228	0.748	0.652	0.0	0.887	0.862	0.940	0.162
91 x 91									
sup x sup	0.1089	0.0628	0.641	0.632	0.0	0.758	0.703	0.771	0.265
non x non	0.0726	0.1234	0.683	0.767	0.0	0.749	0.752	0.740	0.290

Table 18 .Showing parameter values
for crosses involving "super".

Table 18 summarises the parameters determined for the w-78 locus and shows how the super factors at ccf-3 and ccf-4 affect gene conversion. With P at both ccf-2 sites and varying the super factors one can see that there is little cis/trans type difference between super,w-78 x non-super,+ and non-super,w-78 x super,+ as expected because both sites contributing to the super are not linked to w-78. What these two results do display is the variation in parameters determined separately for two 'identical' crosses. One can put down the discrepancies between the two crosses as differences in genetic background of the strains used or to random sampling. The largest difference between these two reciprocal crosses is the value of ν_2 (0.0675 compared to 0.0488). It is heartening to find, even with such different values for four chromatid events, that the other parameters are extremely similar. These results suggest that not too much importance should be placed on the exact values of ν_2.

As expected, the probability of hDNA formation for super x non-super crosses is intermediate between that of super x super and non-super x non-super.

The proportion of hDNA which was asymmetric was much lower for super x super (α=0.506) compared to crosses involving a non-super as one of the parent strains (average α=0.757).

There seems to be a connection between the super

factor and the involvement of a particular chromatid in asymmetric hDNA formation (β). For super x super there seems an almost equal chance between the chromatids as to which one, the mutant carrying or the wild-type carrying chromatid, is involved in asymmetric hDNA formation (β=0.539). There is an over-dominance/heterosis effect of the combined presence of the super factors (so that super x non-super β=0.716, and non-super x non-super β=0.652).

As for when no super factors are involved, δ is equal to zero, indicating that the invading strand generating the hDNA always has the same polarity.

It is in the realm of repair that super has the most remarkable effects, unexpected if it only controls hDNA initiation. The mismatch determined by p shows that one parent being super reduces repair below that of either homozygous cross, repair in super x super having the lower repair frequency of the two homozygous crosses.

The mismatch determined by q again has the super x non-super cross with the lowest probability of being repaired and once more, the super x super cross shows the intermediate value of repair frequency.

The direction of repair of the p determined mismatch is not affected by the presence of super but the repair determined by q shows the largest differences detected in these experiments and is beyond doubt an effect other than sampling. For the cross super x super

the direction of repair is roughly equally to wild-type and to mutant (s=0.586) but for super x non-super the repair is almost entirely to mutant (s=0.055). Where no super factor is present repair is still mostly to mutant (s=0.162) but to a slightly smaller degree.

Table 18 also shows the effects of super on w-78 when the alleles at ccf-2 are both 91. One sees the increase in hDNA formation (ν_1) and the decrease in ν_2 in the presence of super exactly following the pattern produced when the alleles at ccf-2 are P. Other analagous features of super in this cross are a decrease in asymmetric hDNA and a larger change in the probability that the asymmetric hDNA is formed on the mutant chromatid.

The effects of super in the presence of ccf2-91 upon repair are less marked than in the presence of ccf2-P with rather vague and small differences which are not at all clear.

Figures 17 to 22 show how the parameters affect the goodness of fit for the cross super,P,w-78 x super,P,+ and the complete data is represented in Tables 19 and 20.

The present evidence and previous chromatid interference data (Lamb and Wickramaratne 1973) suggest that: all four chromatids can be paired, two by two, at a single site in the synaptinemal complex with no strong negative chromatid interference. Tables 21 to 30 show the

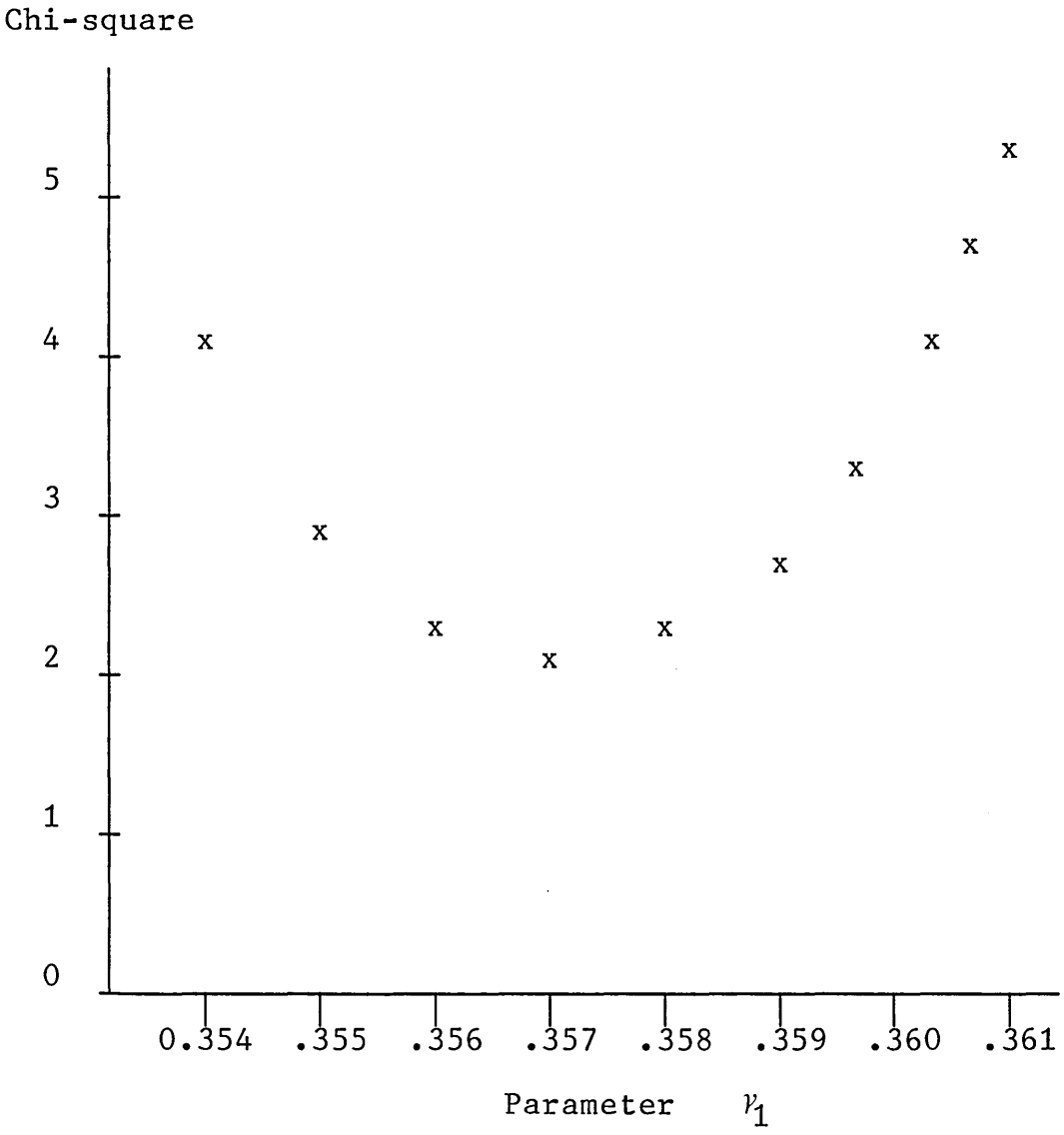


Figure 17.
How goodness of fit varies with ν_1 .
Cross super,P,w-78 x super,P,+ .

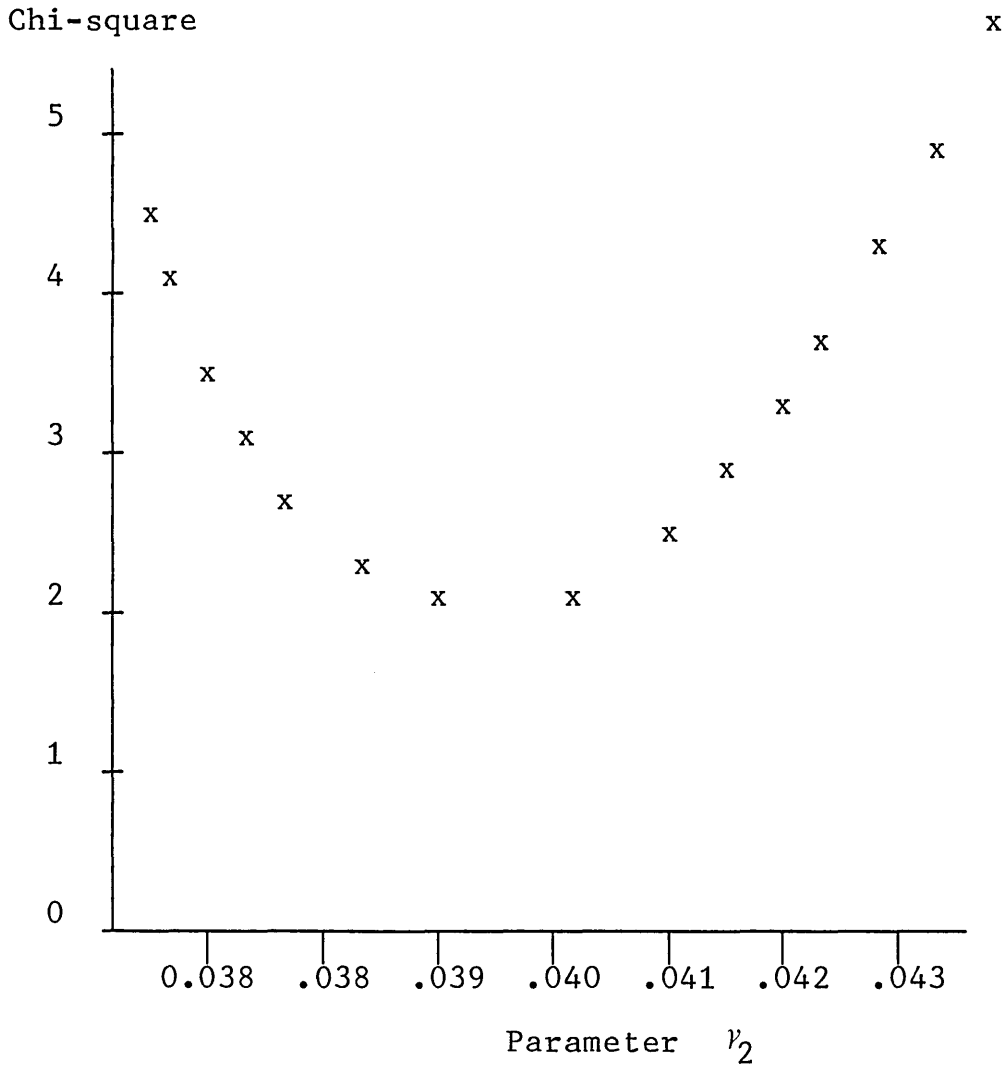


Figure 18.
How goodness of fit varies with ν_2 .

How the goodness of fit varies with parameters.

ν_1 Parameter	Chi-square	ν_2 Parameter	Chi -square
0.353	5.56	0.036	6.72
0.354	4.00	0.037	4.77
0.355	2.89	0.038	3.37
0.356	2.21	0.039	2.46
0.357	1.97	0.040	2.02
0.358	2.15	0.041	2.01
0.359	2.77	0.042	2.40
0.360	3.81	0.043	3.16
0.361	5.27	0.044	4.26
		0.045	5.69

α Parameter	Chi-square	β Parameter	Chi-square
0.491	5.59	0.528	4.78
0.495	4.06	0.530	3.80
0.500	2.72	0.532	3.03
0.505	2.01	0.534	2.46
0.510	1.92	0.536	2.10
0.515	2.45	0.538	1.96
0.520	3.61	0.540	2.02
0.525	5.38	0.542	2.30
		0.544	2.79
		0.546	3.49
		0.548	4.40

Table 19.

For cross w-78,P,super x +,P,super.

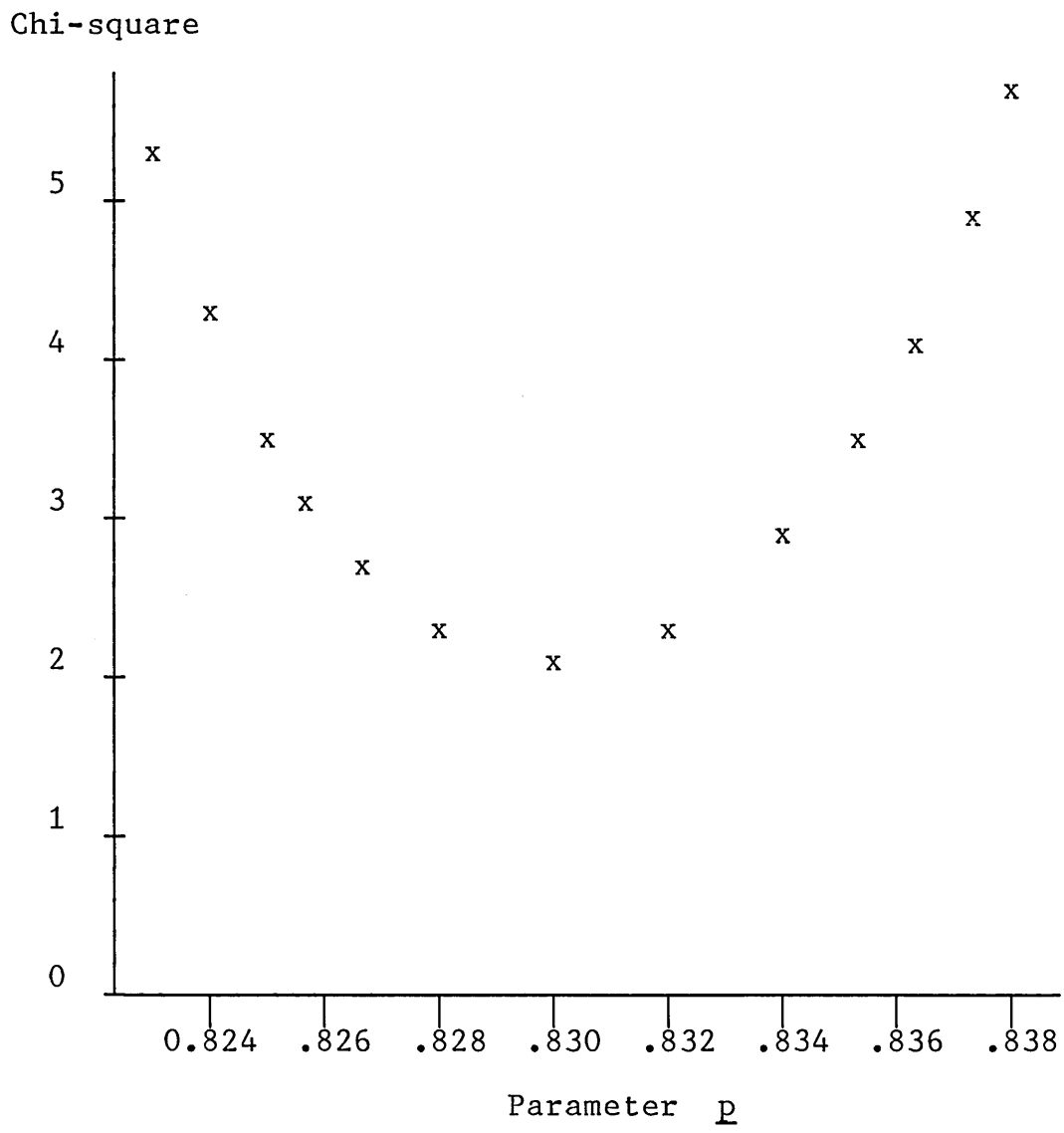


Figure 19.
How goodness of fit varies with p.

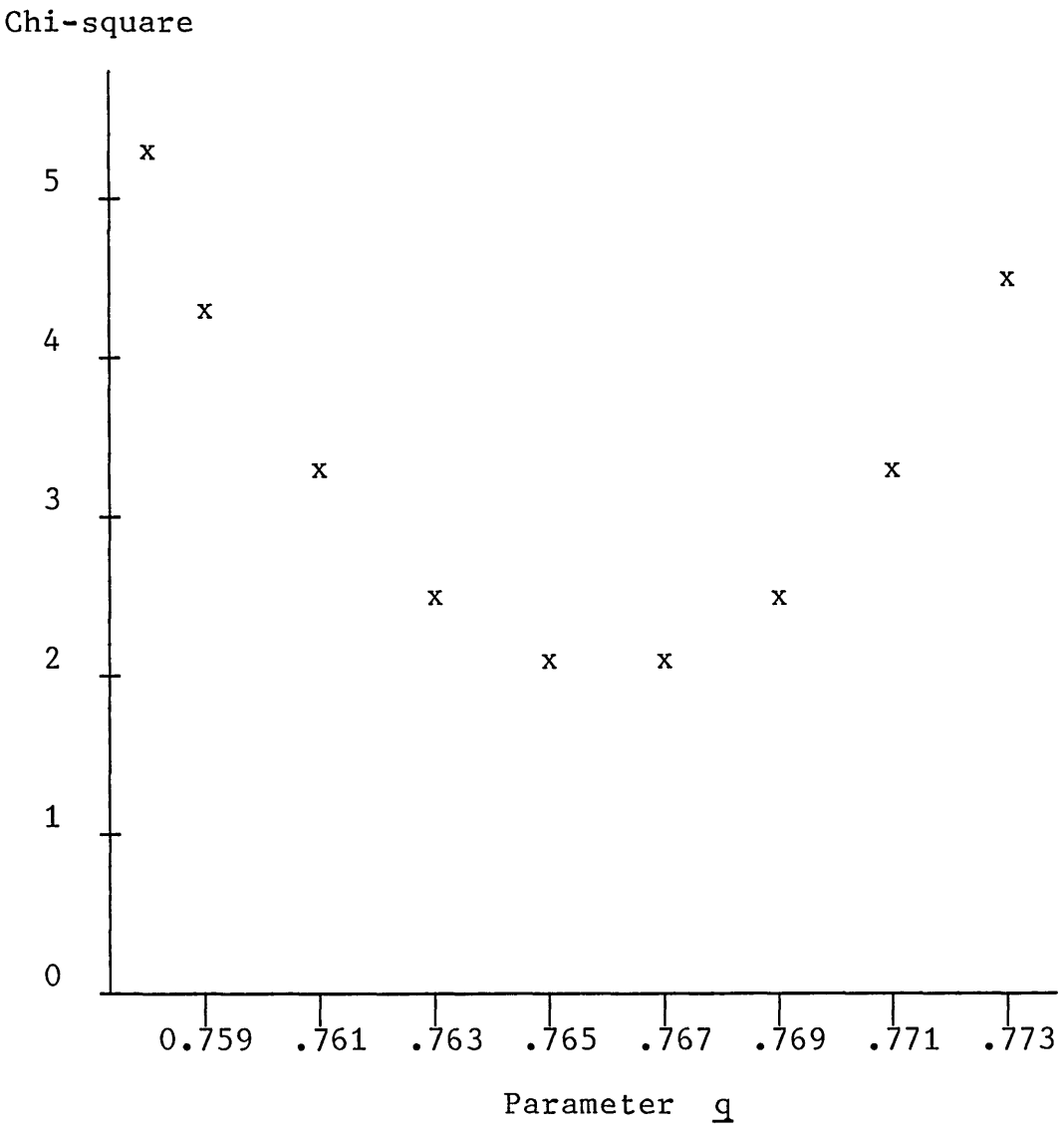


Figure 20.
How goodness of fit varies with q.

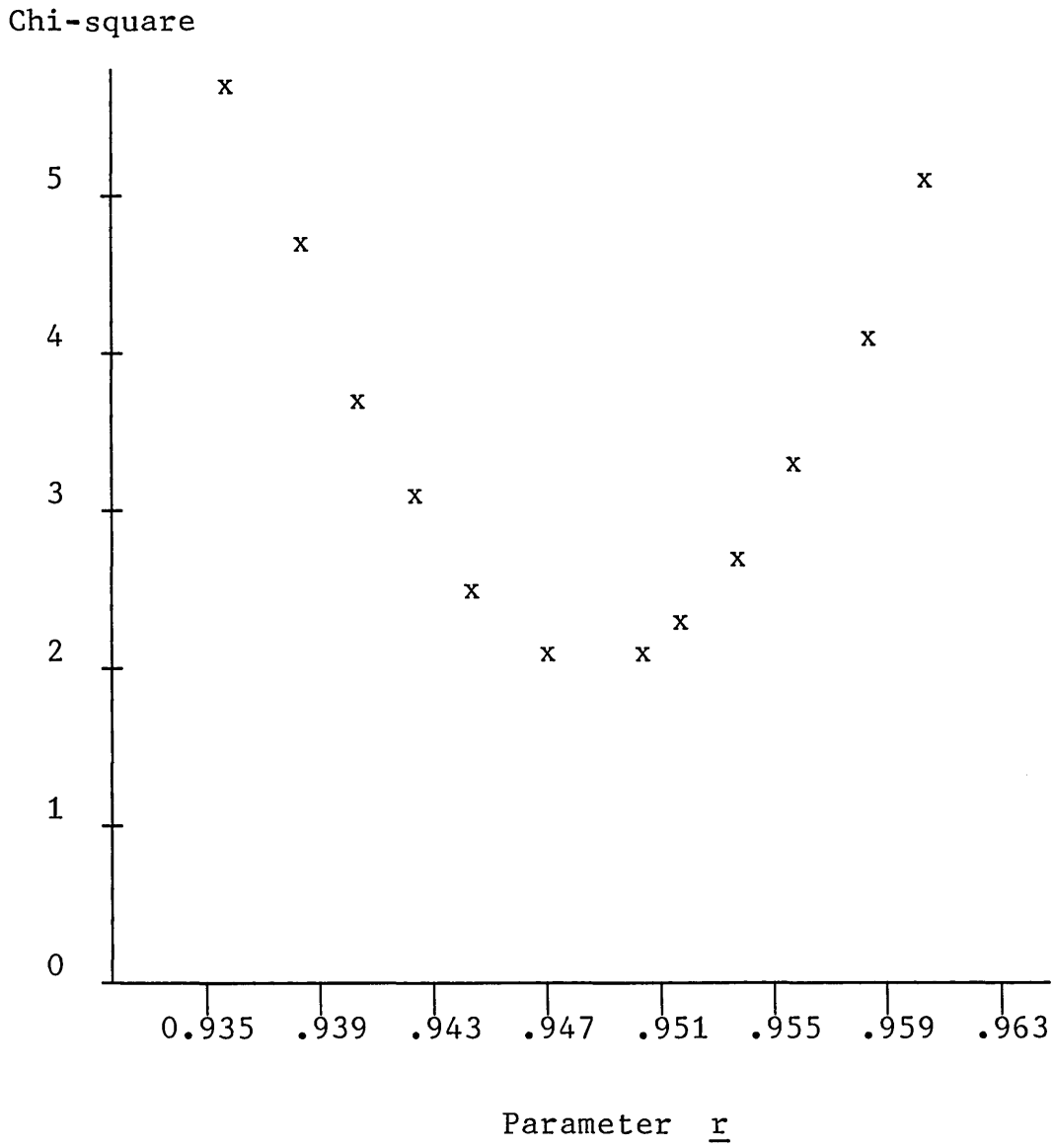


Figure 21.
How goodness of fit varies with r .

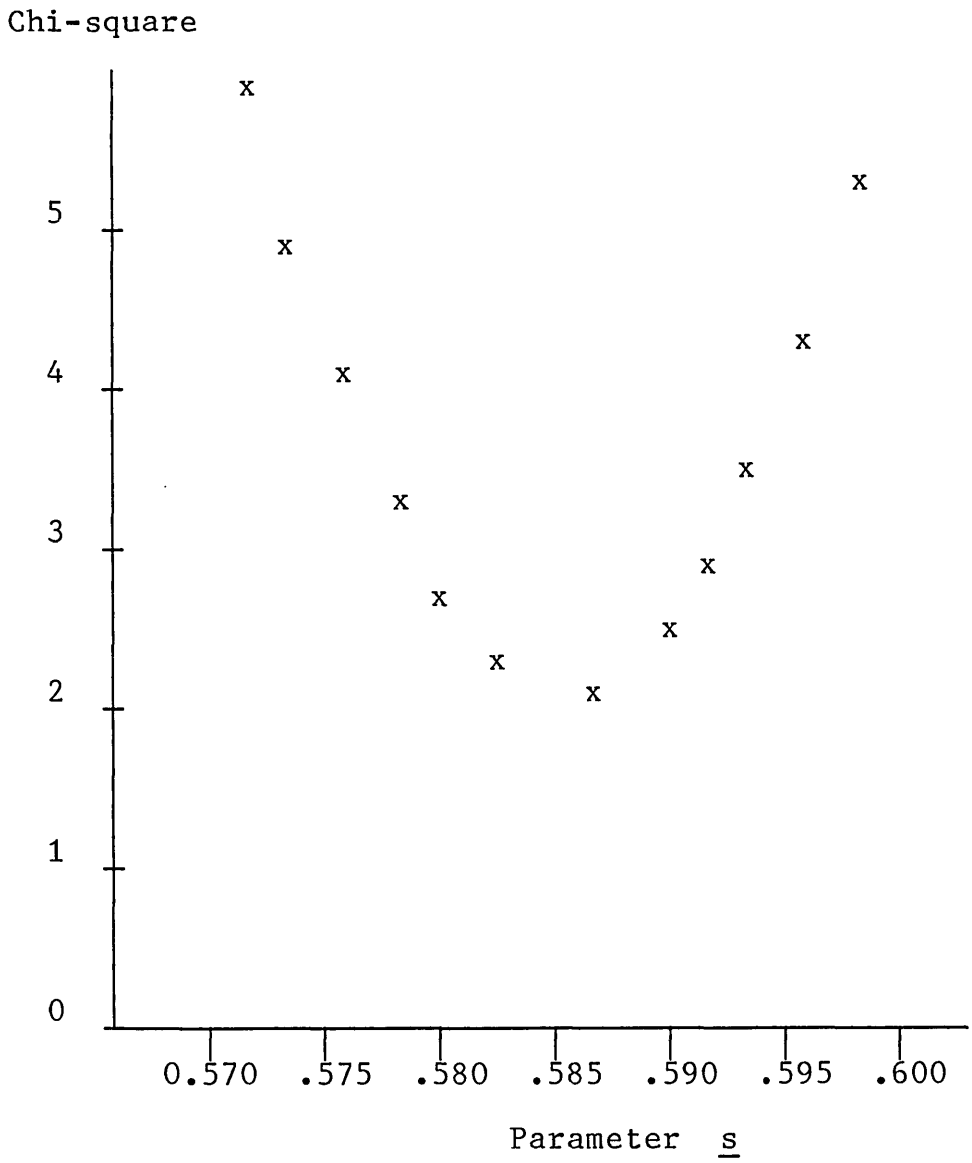


Figure 22.
How goodness of fit varies with s .

How the goodness of fit varies with parameters.

p Parameter	Chi-square	q Parameter	Chi-square
0.822	5.78	0.757	5.79
0.824	4.14	0.759	4.29
0.826	2.95	0.761	3.16
0.828	2.21	0.763	2.40
0.830	1.94	0.765	2.01
0.832	2.14	0.767	2.01
0.834	2.81	0.769	2.39
0.836	3.96	0.771	3.16
0.838	5.60	0.773	4.33
		0.775	5.89

r Parameter	Chi-square	s Parameter	Chi-square
0.936	5.60	0.572	5.88
0.938	4.53	0.574	4.82
0.940	3.64	0.576	3.93
0.942	2.95	0.578	3.20
0.944	2.43	0.580	2.64
0.946	2.10	0.582	2.25
0.948	1.96	0.584	2.02
0.950	2.00	0.586	1.97
0.952	2.23	0.588	2.08
0.954	2.65	0.590	2.37
0.956	3.25	0.592	2.83
0.958	4.03	0.594	3.46
0.960	5.00	0.596	4.28
		0.598	5.26

Table 20.

For cross w-78,P,super x +,P,super.

proportions of narrower ratio octad frequencies arising from four chromatid events and presents the corresponding site interference values for the crosses made. The corresponding site interference coincidence coefficient values given are merely a ratio of ν_2/ν_1 and correspond to the ratio of observed/expected frequencies of conversion in two pairs of chromatids. It is realised that this definition is different from that defined by Lamb and Wickramaratne (1973) who estimated the equivalent of $\nu_1/\frac{1}{2}\nu_2$. One may get comparable values by multiplying the C.S.I.C.C. values in this work by a factor of two.

The used alternative definition for corresponding site interference coincidence coefficient is suggested :-

"Corresponding site interference (C.S.I.) is the interference in hDNA formation suffered at a site in one pair of non-sister chromatids given that hDNA is formed, or is being formed at this site in the other non-sister chromatid pair."

Table 31 summarises the corresponding site interference coincidence coefficient (C.S.I.C.C.) values and the proportion of narrower ratio octads arising from four chromatid events. For w-78, with around one tenth of the narrower ratio octads arising from four chromatid events in crosses excluding the super-factors, any analysis of data excluding four chromatid events is

insufficient. It also seems probable that because the proportion of narrower ratio octads arising from four chromatid events can vary greatly, any model fitted that disregards such events is open to criticism.

What is evident from Tables 21 to 30 is the great differences in the contribution of four chromatid events to the narrower ratio segregation classes. For example in the cross P,+ x P,w-78 presented in Table 21, the proportion of aberrant 4:4s arising from four chromatid events is 0.304 compared to 0.06 for 6:2 . The estimates of C.S.I.C.C. by Lamb and Wickramaratne (1973) and Ghikas and Lamb (1977) are comparable to most of those found in this work. In the Sordaria brevicollis data, C.S.I.C.C. values have all been greater than one (Yu-Sun et al. 1977) indicating only negative corresponding site interference in contrast to that found in Ascobolus where this interference varies from strong and positive to only moderate and negative.

Lamb and Wickramaratne (1973) showed a strong agreement between observed and expected frequencies of 8:0 octads for various temperatures in a w-10 x + cross using their expectation equations which partially ignored 6:2 arising from four chromatid events. No such correlation was found by them for w-78 and in this work this relationship is similarly absent. Clearly the gene conversion factors and the mutant site have an effect on

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads With hDNA
2:6	2 421	155	2 576
3:5	714	103	817
cor4:4	2 647	396	3 043
5:3	1 078	154	1 232
6:2	5 326	342	5 668
ab4:4	48	21	69
Total	12 234	1 171	13 405

Percentage of narrower ratio octads arising from 4 chromatid events is

8.74 %

Corresponding Site Interference = 0.9662

$\hat{C}$oincidence Coefficient

Table 21. Results from the cross + x w-78, with P alleles at both ccf-2 sites.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads With hDNA
2:6	152	19	171
3:5	135	29	164
cor4:4	278	49	327
5:3	285	51	336
6:2	460	61	521
ab4:4	29	2	31
Total	1 339	211	1 550

Percentage of narrower ratio octads arising from 4 chromatid events is

13.61 %

Corresponding Site Interference = 5.5623

$\overset{o}{C}\hat{i}$ncidence Coefficient

Table 22. Results from the cross + x w-78, with K alleles at both ccf-2 sites.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	313	16	329
3:5	181	14	195
cor4:4	615	42	657
5:3	225	27	252
6:2	1 160	57	1 217
ab4:4	11	4	15
Total	2 505	160	2 665

Percentage of narrower ratio octads arising from 4 chromatid events is

6.00 %

Corresponding Site Interference = 1.6371

Coincidence Coefficient

Table 23. Results from the cross +,K x w-78,P.
See text for strains used.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	220	8	228
3:5	132	8	140
cor4:4	500	23	523
5:3	209	16	225
6:2	994	35	1 029
ab4:4	11	2	13
Total	2 066	92	2 158

Percentage of narrower ratio octads arising from 4 chromatid events is

4.26 %

Corresponding Site Interference = 1.1495

$\overset{o}{C}$incidence Coefficient

Table 24. Results from the cross +,P x w-78,K.
See text for strains used.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	205	18	223
3:5	169	24	193
cor4:4	424	34	458
5:3	335	40	375
6:2	572	51	623
ab4:4	34	12	46
Total	1 739	179	1 918

Percentage of narrower ratio octads arising from 4 chromatid events is

9.33 %

Corresponding Site Interference = 1.6997

$\hat{C}$oincidence Coefficient

Table 25. Results from the cross + x w-78, with 91 alleles at both ccf-2 sites.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	2 076	55	2 131
3:5	2 219	81	2 300
cor4:4	6 411	136	6 547
5:3	4 516	175	4 691
6:2	10 022	261	10 283
ab4:4	508	44	552
Total	25 752	752	26 504

Percentage of narrower ratio octads arising from 4 chromatid events is

2.84 %

Corresponding Site Interference = 0.1134

C_{λ}^{o}incidence Coefficient

Table 26.
Results from the cross +,P,super x w-78,P,super.
See text for strains used.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	1 433	41	1 474
3:5	950	59	1 009
cor4:4	1 514	104	1 618
5:3	1 732	100	1 832
6:2	4 185	123	4 308
ab4:4	147	27	174
Total	9 961	454	10 415

Percentage of narrower ratio octads arising from 4 chromatid events is

4.36 %

Corresponding Site Interference = 0.2346

Coincidence Coefficient

Table 27.
Results from the cross +,P,non-super x w-78,P,super.
See text for strains used.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	2 044	43	2 087
3:5	1 569	63	1 632
cor4:4	1 928	97	2 025
5:3	2 417	103	2 520
6:2	5 361	110	5 471
ab4:4	243	32	275
Total	13 562	448	14 010

Percentage of narrower ratio octads arising from 4 chromatid events is

3.20 %

Corresponding Site Interference = 0.1810

Coincidence Coefficient

Table 28.
Results from the cross +,P,super x w-78,P,non-super.
See text for strains used.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	423	18	441
3:5	366	23	389
cor4:4	643	25	668
5:3	484	30	514
6:2	756	32	788
ab4:4	71	11	82
Total	2 743	139	2 882

Percentage of narrower ratio octads arising from 4 chromatid events is

4.82 %

Corresponding Site Interference = 0.5767

Coincidence Coefficient

Table 29.
Results from the cross +,91,super x w-78,91,super.
See text for strains used.

Octad Type	Estimated Number from 2 Chromatid Events	Estimated Number from 4 Chromatid Events	Total Number of Octads
2:6	1 991	33	2 024
3:5	59	2	61
cor4:4	2 462	95	2 557
5:3	80	3	83
6:2	5 566	92	5 658
ab4:4	1	0	1
Total	10 159	225	10 384

Percentage of narrower ratio octads arising from 4 chromatid events is

2.17 %

Corresponding Site Interference = 0.2061

Coincidence Coefficient

Table 30.
Results from the cross + x w-10,
with P alleles at both ccf-2 sites.

Cross			C.S.I.C.C	% nr Octads Arising from 4 Chromatid events
w78	x	+		
P	x	P	0.9662	8.74
K	x	K	5.5623	13.61
P	x	K	1.6371	6.00
K	x	P	1.1495	4.26
91	x	91	1.6997	9.33
w10	x	+		
P	x	P	0.2061	2.17

w78 Cross	x	+	C.S.I.C.C.	% nr Octads Arising from 4 Chromatid events
P	x	P		
sup	x	sup	0.1134	2.84
sup	x	non	0.2346	4.36
non	x	sup	0.1810	3.20
91	x	91		
sup	x	sup	0.5767	4.82

Table 31.
C.S.I.C.C = Corresponding
Site Interference Coincidence Coefficient.
nr = narrower ratio,
(i.e. |6:2|, |5:3| ab4:4 and cor4:4).

ν_2 though from the results it is impossible to perceive how or why.

Discussion:

The value of δ, polarity of the invading strand, has throughout the results been presented as 0.0 but identical results would be obtained if δ took a value of 1.0 except that the values of p and q, and r and s would be interchanged. Because the assignment of δ is purely guesswork it has been given as 0.0 to coincide with the polarity determined by enzymes extracted from eukaryotes (Kmiec and Holloman 1983), where the invading strand has a 3' end. It is significant that in all the results the only fits for the data have been when δ has expressed values of 0.0 (or 1.0). It is important to point out that for different crosses the values of δ may switch from 0.0 to 1.0 and this is undetectable except that one would expect r ans s to vary a lot which has not been the case. Studies with enzymes tend to indicate that where such distinct preferences of strand invasion exist that they will be consistently in one direction for a particular organism (Kmiec and Holloman 1983, Radding 1982).

When a chromatid carries both w-78 and ccf2-K there is a consistently lower proportion of asymmetric hDNA at the w-78 site. This is most easily explained if

one considers the ccf-2 site to lie close to the initiation site of hDNA formation on the opposite side to the w-78 site. In the Avise more model asymmetric hDNA is supposed to progress so far and then switch to symmetric hDNA. It is possible that when the K allele is present at the ccf-2 site, rather than the P allele, that the chance of switching to symmetric hDNA is increased. There is a general trend in organisms for the proportion of asymmetric hDNA to decrease along a gene and this has been shown to be affected by markers along the length of DNA (see introduction for references). The values for α when 91 is at ccf-2 would indicate it acts intermediately with K and P.

The action of the alleles at ccf-2 are more complicated than just proposed because not only do they seem to affect the proportion of asymmetric hDNA but they also influence the probability of hDNA occurring at the w-78 site. This is explainable by their effect upon heteroduplex elongation or initiation, the presence of K at ccf-2 hindering hDNA elongation, perhaps by having a particular sequence with which the enzyme system (initiation or elongation) finds difficult to pair.

With ccf-2 being so close to or even within the wI locus where w-78 and w-10 are located, it is not so surprising that ccf-2 alleles can affect repair at the white spore sites when heterozygous in the cell undergoing

meiosis. Any stretch of hDNA involving heterozygous ccf-2 alleles will have a mispair and this mispair may be several bases long, being recognised by a repair system analogous to recA1 in Escherichia coli. Because the mechanism of repair involves a largish region of excision if the repair system recognises the mispair at either w-78 or ccf-2 there is a likelihood of the repair extending to the other site, leading to co-conversion. Such co-conversion of ccf-2 and wI sites has been found (Helmi and Lamb 1983) and supports this theory but ccf-2 does not frequently co-convert with wI, so it may well initiate hDNA for wI but does not include itself in hDNA. Furthermore the ccf-2 effect should be similar on several wI sites, something which has not been demonstrated.

Whilst one of the two types of mismatches (determined by p) has a frequency of correction to wild-type the same irrespective whether P or K are the alleles at the ccf-2 site this does not mean that they do not affect the direction of repair, especially in the light of the difference in direction determined by 91 and the effect on the other mispair (determined by q). When 91 is on both of the chromatids involved in hDNA, the repair of the mismatch under consideration (determined by p) tends to be less frequently to wild-type than when either P and/or K are involved. A DNA sequence adjacent to a mismatch has been demonstrated to influence recognition of

a mismatch at a different site (Dohet et al. 1986) and now on the evidence above it is demonstrated to be able to influence the direction of repair. This action is presumably achieved by encouraging one strand of the mismatch to be recognised more (or less) by the 'excision machinery' of the repair system.

The effect of the ccf-2 alleles on the repair of the other mismatch (determined by q) is different from that above. Similar methods of influencing repair probability and direction of repair are available to those described above but the effects are different. For some reason when allele P is on both chromatids, repair at the w-78 site is increased, whilst K on one chromatid and P on the other or K on both seems to make no difference in the probability in repair between these cases. The allele 91 affects both types of mismatches by reducing the probability of repair.

The two directions of repair of this second mismatch (determined by q) seem to be roughly equally likely except in the cases of the P x P and the 91 x 91 crosses involving the ccf-2 site. How or why P x P should have an s value of 0.162 and 91 x 91 have an s value of 0.290 compared to approximately 0.5 for the P x K and K x K crosses is difficult to understand.

There is strong evidence that the super factors,

ccf3-E and ccf4-r, affect the probability of asymmetric hDNA at the w-78 site. These factors, one dominant and one recessive, reduce asymmetric recombination to half of all hDNA at the w-78 site from approximately 75 % when the factors are absent or over-ridden by their alternative alleles. Presumably, because ccf-3 and ccf-4 are not linked to w-78, they are involved in the enzymes which not only affect hDNA formation (seen by their effect on ν_1 and ν_2) but also upon repair.

Not only do the super factors affect asymmetric hDNA formation, they also influence which chromatid of the non-sister pair is the one invaded (β). When the super factors are combined the probability of either strand being the invaded one becomes approximately equal but in their absence β reaches values between 0.65 and 0.73 . This effect is less marked with 91 at the ccf-2 site. It is very strange that all β values depart from 0.5 in the same direction (>0.5), suggesting that wI itself is the initiating site for hDNA formation ?

Kalogeropoulos and Thuriaux (1985) found that the efficiency of repair in S. fimicola depends on whether the hDNA is symmetric or asymmetric, being more efficient when the hDNA is asymmetric. If this can be explained then one will perhaps be on the way to understand how the ccf-3 and ccf-4 genes function. At the present one can only guess as to how they work; we can only see their

effects at the moment, perhaps if someone one day is able to clone the genes involved and test their products *in vitro* then their actual mechanics will be understood. Outside markers for crossing-over and extended studies of co-conversion and other events in heteroallelic *wI* x *wI* crosses are obvious further work to suggest.

I N D U C T I O N A N D I S O L A T I O N
O F M U T A N T S

Most fungal genetics is based on the study of mutations at specific sites and without these mutations, loci would usually be unidentifiable. Although wild populations carry a variety of different alleles at many loci (probably ccf-2 included), these are rarely sufficient or at the desired locations. It is therefore essential in the genetic study of any organism that an efficient method is found for the induction, isolation and identification of mutations.

Mutations occur spontaneously but with very low frequencies. Mutagenic treatments include irradiation with ultraviolet light (UV) or with X-rays, or chemical treatment. Most treatments that induce a useful increase in mutation rate also result in death of many cells and reduced fertility. A balance must be made whereby the number of surviving fertile cells is not at the expense of too few mutations and vice versa.

The aim of this study was to find outside markers flanking the locus wI mutations to permit studies of gene conversion and crossing over in relation to each other. Numerous mutants were isolated and tested for

linkage to wI and techniques for their induction and isolation were improved.

Spontaneous Mutation Frequency:

The most easily studied spontaneous mutants in Ascobolus immersus are those which affect ascospore colour and values for frequencies have varied in the past between 0.001% to 0.80% per octad (Helmi 1980). The rate of spontaneous mutation is known to vary in many organisms with environmental conditions and with genetic control (Stanier et al. 1977). The estimate for the frequency of granular and white spore colour mutations ranged in the current studies from 0.001 to 0.050% per octad. These mutants were detected in 9,R,+ x sk,7,R,7,- crosses for octads showing a 4:4 ratio. These values of spontaneous mutations agreed well with those found by previous workers for this organism (Leblon 1972; Helmi 1980).

Mutagenesis:

The original method involving UV described by Helmi (1980) was improved to give a higher frequency of readily observed spore colour mutations and it was assumed that this increase corresponded with a like increase in the frequency of other, not so easily detected mutations such as for drug resistance, auxotrophy and altered morphology of the colonies. Morphological mutations were

detected for using the naked eye and a disecting microscope.

The original method involved the irradiation with UV of both parental inoculants. It was observed that by increasing the dose of UV, the fertility was greatly affected so that few ascospores were produced and many asci containing only four spores were observed: meanwhile the survival of vegetative cells seemed unaffected. By restricting the irradiation to only one of the parental inoculants, one was able to increase the dose of UV without significantly decreasing fertility, as measured by the number of eight spored octads produced. Measuring the number of spore colour mutants produced, it was established that the best results were obtained by increasing the dose of UV four-fold compared with that used when both parental inoculants were irradiated. The frequency of spore colour mutants was increased in this way to about 5% of octads.

The two wild-type strains, 9,R,+ and sk,7,R,7,- , were inoculated on cellophane measuring about four square centimetres placed on culture medium in large (10 cm) petri dishes. This was in order to reduce shielding from UV of subsurface hyphae by culture medium. The inoculants were then grown at 25° C. for two to three days until the cellophane was about two thirds overgrown when the inoculum block was removed and the remainder was

irradiated with UV. The dose of UV was estimated at 800 ergs/sec/cm^2 and irradiation was for four minutes. Immediately following irradiation the petri dishes were incubated at 25° C. for two to four hours in the dark. Next the pieces of cellophane were transferred upside down to petri dishes with crossing medium on which the other strain had been growing at one edge for two days at 17.5° C . The cellophane was then peeled off, leaving the mutagenised hyphae, or occasionally left for up to six hours. Thereafter culture of the cross continued as normal.

Selection of Mutations:

The large ascospores of Ascobolus make the selective isolation of auxotrophs difficult because of the surplus of nutrients carried within the spores. The nutrients within the spore are such that a wild-type spore grown on plain water-agar produces a colony in excess of a centimetre diameter. In addition, the problems in germinating the spores extend to their synchronization, the times of germinating spanning over three days, even for spores from the same ascus. One method used previously was to fragment hyphae from germlings only a couple of days old and to treat the fragments as conidia by filtration enrichment (Helmi 1980; and personal communication, Lamb 1982; and personal communication).

Filtration Enrichment

Filtration enrichment involves the filtering out of growing prototrophic hyphae from liquid minimal medium whilst the non-growing hyphae (including auxotrophs) pass through the filter to be collected and eventually, usually after several cycles of filtration enrichment, transfered to a medium where they can grow (Catcheside 1954).

In the original method ascospores are:

(i) collected and treated for fifteen minutes with 0.15 pronase solution to separate the spores, which are then induced to germinate (see general methods).

(ii) After 22 hours at room temperature with gentle shaking to deter anastomosis, the germlings are filtered off with filter paper (Whatman no. 1). They are washed with sufficient distilled water to remove any nutrients from the surface liquid which cohesively stays with the hyphae.

(iii) The germlings are then re-suspended in about 15 ml of liquid minimal media in a test tube or McCartney bottle and fragmented in a blender for ten seconds with a homogeniser.

(iv) The fragments are passed through a 305 μm pore size nylon mesh filter. The holder was cylindrical, made of aluminium and with a capacity of 100 ml: no support for the filter was used. The filter holder diameter was 4.5 cm and a sterile inverted petri-dish lid was used as a top

for the ho lder to reduce the risk of contamination.

(v) The small fragments which are not caught by this filter are collected on filter paper, washed with 50 ml of distilled water, then resuspended in 50 ml of liquid minimal medium in screw-top conical flasks (150 ml).

(vi) The flasks are shaken at room temperature for 24 hours.

(vii) Then colonies (theoretically most of which are prototrophic) which have grown are filtered off with the same size filter (305 μm).

(viii) The 24 hour growth is repeated a number of times and any remaining small colonies/hyphae are collected on filter paper.

(ix) These are then resuspended in complete liquid medium and further incubated for 24-48 hours. Any colonies growing now, are isolated by Pasteur pipette onto complete medium and then are tested for auxotrophy, slow growth and morphological mutation (e.g. clock, wavy, etc.).

By altering the minimal medium (v), the duration of incubation (vi), the filter sizes (iv and vii) and the number of filtraions (viii) it was possible to improve the method and to identify some of the reasons for poor practical results in the method prescribed by Helmi (1980).

With the original method, using about 3000 spores per experiment, out of approximately 80000 spores

from mutagenised crosses, with just two cycles of filtration, just under a hundred colonies were isolated. Of these only 13 showed consistently slow growth on minimal medium and when crossed with wild-type strains, none showed this character to be heritable. In a non-mutagenised set of spores, results were exactly comparable and the isolation method was considered inadequate.

Possible explanations for the poor practical results were:

(a) The supply of nutrients in the hyphal fragments was sufficient to permit growth, even of mutants, beyond the original filter pore size. By increasing the subsequent filter sizes it was necessary to extend incubation times so that the normally growing colonies would exceed the pore sizes: for the pore size 2000 μm, an incubation time of upto 60 hours was required.

(b) With time, growing hyphae were exuding nutrients into the medium. Incubation times could be reduced between filtering to prevent the build up of leached nutrients from prototrophic colonies.

(c) During the filtration stage many small colonies were being trapped by larger colonies which blocked the filter, thus reducing the effective size of the pores. This was difficult to overcome; separating samples into smaller subsamples so that congestion at the filter was reduced increased the chances of contamination from

external sources.

(d) The faster growing colonies, not being restricted to spherical growth, were producing new colonies by fragmentation during handling, thus diluting the slow/non-growing colonies. The solution here was to reduce the number of cycles of filtration and to increase the overall number of colonies passing through for testing.

(e) Many colonies were lost when fragments were collected on filter paper, and because this loss was expected to be the greatest for small fragments, it was supposed that this could be the cause for the lack of auxotrophs isolated. Microscopic examination of the filter paper after washing off the trapped colonies failed to support this theory and very fine muslin gauze was tried as an alternative. Though no evidence was found that either paper or muslin gave better results, the handling of muslin was easier because of its flexibility. This flexibility of the muslin meant that it was easier to wash off the colonies. A secondary test was performed which showed that hyphae remained trapped to both filter paper and muslin. After washing the filter material it was transferred to plates of complete medium and incubated for a couple of days at 25° C. Both filter materials showed abundant growth of mycelium.

(f) At some stage of the proceedings anastomosis occurred between the prototrophic wild-type hyphae and the

mutant auxotrophic hyphae so that the multinucleate cells grew like wild-type and therefore were indistinguishable and unisolatable.

(g) It is possible that the auxotrophic cells subjected to physical stress are unable to repair the damage sutained during handling and therefore starve to death. This starvation may extend to a general inability of the cells to sustain their metabolism without the required nutrients for which they are auxotrophic. Using the lysine requiring auxotroph lys-25 it was shown, following fragmentaion of hyphae and incubation conditions similar to those used for the filtration enrichment, that this auxotroph was not easily starvable.

Below are presented the incubation times at room temperature required to prevent all the visible colonies, starting from fragments following blending, from passing through the pore sizes used:-

Pore size μm	Hours of incubation
305	16 - 18
501	24 - 26
700	30 - 34
1 000	36 - 40
1 600	42 - 54
2 000	48 - 60

None of these methods gave auxotrophs but with the 501 μmfilter size, the highest proportion of colonies with morphological differences or slow growth habit was obtained. From approximately 70000 spores from mutagenised

crosses, 5 clock, 2 compact and 10 slow growing colonies were isolated. Of all these only five showed Mendelian inheritance: clo-1, clo-2 (clock mutants), slo-1, slo-2 and slo-4 (slow growers). The mutants slo-1 and slo-2 were later renamed mor-1 and mor-2 respectively to indicate their slightly different morphological growth from the wild-type.

Strains carrying mor-1 had a larger proportion of aerial hyphae giving the appearance of a less dense mycelium.

The strains carrying mor-2 had a phenotypic morphology that was most easily distinguished from the wild-type at 14.5° C. The largest difference in the rate of growth was detected at 17.5° C. on the minimal medium of Yu-Sun (1964) supplemented with 2.5 mg of riboflavin. After four days of incubation in small test tubes the wild-type has grown to the top of the tube along the sloped agar (agar at about 30° to the horizontal when the tube is erect) whilst the mutant-type has grown less than half way (a distance of around 1-2 cm). For some reason the difference in growth rate decreased with the age of the strain, being greatest at the time immediately following germination. Supplementation of the minimal medium with numerous amino acids and vitamins showed that no single biochemical was able to complement this mutation; instead there seemed to be a gradual progression

from mutant-type to wild-type with the addition of amino acids.

Use was made of a lysine requiring auxotroph, lys-31, and a mutant producing yellow mycelium, yel-7, to improve the filtration enrichment method. The yel-7 phenotype has bright yellow mycelium when grown in complete medium supplemented with 2.5 mg of riboflavin compared to the off-white wild-type mycelium. Following long incubation (periods over 1 month) the morphologies become indistinguishable, the bright yellow colour of yel-7 diminishing and taking on the wild-type dark brown colour of aged mycelium.

A lys-31 strain of minus mating type was grown in complete liquid medium. Simultaneously a yel-7 strain of positive mating type was also grown in a separate flask. After 2-3 days growth at room temperature each culture was fragmented with the blender and the prototroph was equally separated into a number of flasks and to these was added a diluted sample of the fragmented auxotrophic strain. These mixtures of fragments were then treated as if they were mutagenised germling fragments, filtration enrichment being carried out for the three filter sizes: 305, 501 and 2000 μm.

The filtrations were repeated six times, with 24 hours between each filtering. At the end of each filtration one of the replicate flasks for each filter

size was terminated with regard to the experiment. The colonies in this flask were transfered to complete medium and later subcultured on minimal medium to test for auxotrophy. In all cases white colonies were auxotrophic and yellow ones were prototrophic. This suggested that anastomosis was rare or of no occurrence. As a further test for anastomosis the colonies were plated on crossing medium and incubated separately under conditions favourable for apothecia production. Because the two mixed strains were of opposite mating types, anastomosis between prototrophs and auxotrophs would be expected to produce fertile spore- producing bodies, which not one did. This depended on hyphal fusion being mating-type independent in Ascobolus (Lamb personal communication).

The controls consisted of duplicates of the mixtures started with, cultured throughout the experiment without any filtrations. This control enabled one to test the theory that auxotrophs die of starvation during the experiment. The results are presented in Tables 32, 33 and 34. Whilst the general level of bacterial and fungal contamination during the procedures was very low, it is seen that for pore size 501 μm two flasks were unscorable. In practice it was unnecessary to add antibiotic agents to the procedures as suggested by other workers (Helmi 1980, Lamb 1982).

The results of the controls demonstrate that the

2000 μm pore size

Number of Filtrations	Number of Viable Colonies	Number of Auxotrophs	Percentage of White Colonies	Percentage of Yellow colonies
0	231	99	42.8	57.1
1	156	111	71.2	23.7
2	143	98	68.5	31.4
3	105	45	42.8	57.1
4	97	50	51.5	48.4
5	12	3	25.0	75.0
6	259	43	16.6	83.4
Control	225	104	46.2	53.7

Table 32.
The effect of filtration enrichment on the presence of auxotrophic- (white) and prototrophic- (yellow marker) colonies.

501 μm pore size

Number of Filtrations	Number of Viable Colonies	Number of Auxotrophs	Percentage of White Colonies	Percentage of Yellow colonies
0	294	104	35.3	64.7
1	180	113	63.3	36.7
2	148	59	39.9	60.0
3	168	45	26.7	73.2
4	198	31	15.6	84.3
5 ***	-	-	-	-
6	158	26	16.4	83.5
Control ***	-	-	-	-

*** Indicates that bacterial contamination prevented scoring.

Table 33.
The effect of filtration enrichment on
the presence of auxotrophic- (white) and
prototrophic- (yellow marker) colonies.

305 μm pore size

Number of Filtrations	Number of Viable Colonies	Number of Auxotrophs	Percentage of White Colonies	Percentage of Yellow colonies
0	105	21	20.0	80.0
1	48	15	31.2	68.7
2	67	11	16.4	83.8
3	93	16	17.2	82.8
4	115	10	8.6	91.3
5	142	5	3.5	96.4
6	218	2	0.9	99.0
Control	91	17	18.6	81.3

Table 34.
The effect of filtration enrichment on the presence of auxotrophic- (white) and prototrophic- (yellow marker) colonies.

reduction in auxotrophic colonies is not a result of starvation. The results as a whole show that whilst the proportion of auxotrophic colonies is at a maximum after the first filtration for all filter sizes, not only does the number of auxotrophs decrease thereafter but the number of prototrophs actually increases. This tends to indicate that shearing of the faster growing colonies adds to the number and proportion of prototrophic colonies. In this experiment it must be noted that the proportion of auxotrophs represents between 20 and 50 percent of the total number of colonies, and this is much higher than would be expected in a real isolation experiment but gives one an idea of what is happening. As the pore size increases these effects seem to be less marked but this is likely to be due to the larger number of initial auxotrophs. It is suggested that in practice these effects would be more pronounced and that to allow for a little growth of the auxotrophic hyphae that whilst the original filter size following fragmentaion should remain 305 μm that for subsequent filtrations the filter size should be 501 μm

From these results the following procedure was finally followed:-

(i) Ascospores are collected, treated with 0.1% pronase solution and induced to germinate in liquid medium.

(ii) After 22 hours at room temperature (18-25°C.) with gentle shaking, the germlings are filtered off (Whatman filter paper no. 1), washed with 50 ml distilled water and resuspended in 20 ml of liquid minimal medium.

(iii) The germlings are fragmented in a blender for 5 seconds.

(iv) The fragments are passed through a 305 μmfilter to remove debris and caught on muslin, using an aluminium filter holder, where they are rinsed with 50 ml distilled water.

(v) The fragments are resuspended in 50 ml of liquid medium in flasks (150 ml) and incubated on bench shakers at room temperature for 6 hours.

(vi) Colonies which have grown are filtered off using a 501 μm filter, the small fragments being collected on muslin.

(vii) The procedures v and vi are repeated.

(viii) The final fragments and small colonies are resuspended in 50 ml complete liquid medium in screw-top flasks (150 ml), incubated on a bench shaker for 24 hours and separated, with the aid of a Pasteur pipette and a microscope, into small test tubes of sloped solid complete medium.

(ix) The resulting isolates are tested and identified.

Using this method for about six months no

auxotrophic mutants were discovered, though many auxotrophic isolates were found. Some of these isolates were even characterised with regard to specific nutrient requirements. These auxotrophic isolates were either infertile or did not pass on their biochemical requirements to their offspring.

As a result of the inability to generate auxotrophic mutants using filtration enrichment, even though several morphological mutants were selected, an alternative method was investigated, namely the enrichment of auxotrophs by the selective killing of growing cells, called here, 'vitiation enrichment'.

Vitiation Enrichment

This method is based upon the selective killing of cells which are growing. The antibiotic Nystatin (mycostatin) was used in a similar manner to that originally developed for floculating yeast by Snow (1966).

The concentration of Nystatin which kills growing hyphal fragments but does not kill stationary-phase hyphal fragments of Ascobolus was discovered to be either 10 μg/ml for 15 minutes or 5 μg/ml for 20 minutes. This compares to 10 μg/ml for 30 minutes for yeast (Snow 1966). This concentration was determined by treatment of hyphal fragments in liquid minimal medium of a variety of wild-type strains and of the two available auxotrophic

mutants, lys-25 and lys-31.

Using yel-7 and lys-31 an experiment was designed to test the specificity of Nystatin as well as to test the usefulness of this method in Ascobolus. Auxotrophic germlings carrying lys-31 were fragmented and a series of dilutions was made, each varying from the next by a dilution factor of ten. Prototrophic germlings carrying yel-7 were fragmented and separated into six equal portions of 10 ml, to which were added one ml from one of the diluted suspensions of the auxotroph fragments. These mixtures were treated with 5 μg/ml of Nystatin for 15 minutes in liquid minimal medium, washedon muslin with 50 ml of distilled water, and then incubated in 50 ml complete liquid medium for 48 hours at room temperature on a bench shaker. Colonies were scored by eye. Controls were carried out with water treatment instead of Nystatin treatment. A second control was the the addition of distilled water in place of auxotrophic fragments in suspension. The results are presented in Table 35. In the untreated controls the total numbers of prototrophic colonies numbered between one and two thousand per flask so that a dilution factor of one represents between one in twelve and one in twenty fragments being auxotrophic.

It is evident that vitiation enrichment is extremely selective, being able to multiply the proportion of auxotrophs many times. But when this treatment was

Dilution Factor For Auxotropph	Number of Isolates: Yellow Wild-Type	White Auxotrophic	% Aux.	
(a) With Nystatin (5 μg/ml for 15 minutes)				
No auxotroph	10	0	0	
1 in 2	11	3	21	
1 in 10	13	19	59	
1 in 100	7	5	42	
1 in 1000	3	5	63	
1 in 10000	***	***		Bacterial contamination
(b) Control, Without Nystatin				
No auxotroph	> 1000	0	0	
1 in 2	> 1000	80	< 8	
1 in 10	> 1000	3	<0.3	
1 in 100	> 1000	0	0	
1 in 1000+	> 1000	0	0	

Table 35.

The selective properties of Nystatin with regard to killing growing prototrophic cells. In the controls, of the many colonies growing, auxotrophs were detected as white colonies and prototrophs as yellow colonies (yel-7).

applied to germlings originating from mutagenised crosses no auxotrophs were isolated that showed Mendelian inheritance. However two isolates that did not show any auxotrophy or slow growth showed an ability to survive the Nystatin treatment and were classified as Nystatin resistant: unfortunately the white-coloured ascospores of these isolates produced from crosses with wild-type strains did not germinate under any tried conditions.

A reason for the possible failure of vitiation enrichment to isolate auxotrophs in practice may be that the selective nature of Nystatin is insufficient in that it kills off too many auxotrophic cells, so many in fact that no auxotrophic cells survive. This is somewhat unlikely from the dilution results where a one thousand-fold dilution represents between one and six auxotrophic fragments in a total of nearly two thousand. Contrary to this, it is evident that Nystatin does kill auxotrophic cells because compared to the non-treatment control of 80 auxotrophic colonies at one-fold dilution, with Nystatin treatment only 3 auxotrophic colonies were isolated.

Indirect Selection

This method applies the knowledge that certain drug resistant mutations are pleiotropic for certain auxotrophic mutations. Examples include p-fluorophenyl alanine resistance which can select for amino acid

auxotrophic mutants, and methyl mercury resistance which shows a higher than expected proportion of methionine auxotrophic phenotypes (Singh and Sherman 1975). Methods in obtaining drug resistant mutants can be found under that heading later on.

Total Isolation

This involves looking at a large number of isolates which have not undergone any selective procedure and basically hoping for the best. Nearly 3000 germlings were taken from mutagenised crosses and examined, (i) for morphological differences, and (ii) for auxotrophy by plating onto minimal medium. This yielded the following proportion of persistently mutant phenotype:-

Phenotype	Total Number of Isolates	Number showing Mendelian Inheritance
Compact growth	1	1 com-1
Yellow mycelium	12	4 yel-
Clock morphology	1	1 clo-UA4b
Slow growing	24	2 slo-
Leaky auxotrophs	7	1 4-aux-b2
Granular ascospores	2	2 grn-

Drug Resistance

In fungi the list of drugs and chemicals for which resistant mutants have been found is large and below are just some which may be applicable to Ascobolus.

From Warr and Roper (1965) in Aspergillus nidulans: Nystatin, 2-deoxy glucose, cyclohexamide, acriflavin, sodium azide, Actidione, iodoacetate, fluoroacetate, p-fluorophenylalanine, Malachite green and tequil.

From Fincham et al. (1979) in miscellaneous fungi: seleno methionine, 8-azoguanine, fluoro-acetic acid, fluoro acetamide, chloro-lactic acid, phenyl mercuric acetate, methyl mercuric chloride, 5 fluoro-uracil, β canavanine, caffeine, β-2-thienyl-DL-alanine, L-azetidine-2-carboxilate, quinolinic acid and Agmatine.

From Mortimer and Schild (1980) in Saccharomyces cerevisiae: Antimycin A, Axenomycin, borrelidin, Cryptopleurine, copper, ethionine, Oligomycin, Roccal (benzalkonium chloride), Spermidine, Trichodermin and triazyl alanine.

From Perkins et al. (1982) in Neurospora crassa: amino triazole, Benomyl and Ediene.

Table 36 shows the concentrations of chemicals which inhibited wild-type strains from growing and also the concentrations which permitted the strains to grow normally. The strains tested were 9,r,+ ; sk,7,R,7,- ; sk,4,w-78,7,+ as well as strains carrying the mutants mor-2 and grn-20. The tests were carried out by inoculating the strains on to complete medium (unless otherwise stated), with the drug added, in small test

Drug	Concentration that Inhibits the growth of Wild-Type strains μg/ml	Concentration that Permits Wild-Type Growth μg/ml
Phenyl mercuric acetate	50	10
Caffeine *	-	-
Ethionine	-	-
Cycloheximide	50	10
8 Azaguanine	200	10
Nystatin (5730 units/mg)	10	2
p-Fluoro phenyl alanine	100	10
Seleno-DL-methionine	50	2
L-Azetidine-2-carboxilate	-	1000
Methyl mercuric chloride *	0.01	0.00
Acriflavin *	500	0
Canavanine	200	20
Benlate (50%)	0.50	0.00
Malachite Green	5.0	1.0
Fluoroacetamide	5	2

Table 36.
The concentration of drugs required to completely inhibit the growth of Ascobolus. The medium was complete except where marked *, when minimal medium was used.

tubes. The drugs (added in suspension form in 95% ethanol) were added to molten agar held in a water bath at 45° C. The mixture was shaken and allowed to cool. Inoculation was made within 24 hours of the tubes being prepared and incubation was over four days with the growth being checked every twelve hours. This was important for degradable drugs such as Nystatin.

Certain chemicals proved to be unsuitable for Ascobolus, for example caffeine, 5-fluorouracil, Agmatine and L-azetidine-2-carboxilate. In these cases either there were large differences between different wild-type strains, there was no toxicity expressed or the results were very inconsistent when repeated.

Resistant mutants were selected by suspending approximately 10000 spores in 100 ml of liquid germination medium and inducing them to germinate. The germlings were collected on muslin, washed with 50 ml distilled water and resuspended in a suitable concentration of the drug in liquid medium. The germlings were incubated for 24-36 hours at room temperature and any visible colonies at this time were transferred by Pasteur pipette to complete medium and tested for resistance.

The number of colonies found growing in the presence of a drug varied greatly between experiments and depended on the drug used. Examination with a microscope of germlings which had not grown in the presence of drugs

revealed that they were either dead or had a much altered morphology and this was subsequently confirmed whenever they were plated out and incubated on complete medium when their morphology would return to wild-type.

The following mutants were isolated using this method: Nys-19, Nys-22 (Nystatin resistance at 10 μg/ml), Fac-3, (fluoroacetamide resistance at 10 μg/ml), Fac-5 (at 20 μg/ml), Can-8 (Canavanine at 200 μg/ml), ACR-1 (Acriflavine at 100 μg/ml), pfl-1, pfl-2 (p-fluoro phenyl alanine at 100 μg/ml), and Aza-1 (8-azaguanine at 1 mg/ml).

The approximate frequency of resistant isolates was in the order of 0.2% of all spores. Of these only about ten percent were both fertile and showed their resistance to be inherited Mendelianly. None of the above mutants was isolated from the same flask.

POLYGENIC CONTROL OF GENE CONVERSION

In the preceeding chapters the impression given is that the control of gene conversion at the wI locus is governed by the gene conversion control factors, oligogenes, with characterisable major influences on recombination. It has long been realised that genetic variation in fungi is not the only factor affecting gene conversion. Lamb (1968; 1969) demonstrated in Sordaria fimicola the effects of altering temperature on conversion and similar reports have been made for Ascobolus (Lamb and Wickramaratne 1975, Wickramaratne and Lamb 1978, Lamb 1986).

There can be no doubt that gene conversion is under the control of enzymes, and temperature effects are therefore not surprising. The complex nature of the recombinational process would suggest the involvement of more than one enzyme and it is logical to assume that the function of these enzymes is fundamental for the survival in the wild of the species over a period of time.

The micro-environment to which a fungal organism like Ascobolus is exposed, is expected to vary enormously and it is the continuing event of recombination that

allows an organism to adapt. Because of the many external variations imposed on an organism one must expect many genes to be involved in maintaining a metabolism which allows the many cellular processes to continue in as beneficial way to the organism as possible.

It is known that at least one of the enzymes controlling recombination is ATP dependant (Radding 1982) and one's general understanding of biochemistry would induce one to suppose that this ATP dependence is a general rule of interacting metabolic processes rather than an exception. With this being the case one would assume that gene conversion is affected, not only by oligogenes (affecting the primary enzymes involved directly) but also by scattered genes whose main functions are probably not directly involved with gene conversion itself but which affect the level of ATP in the cell. These scattered genes are likely to affect gene conversion in a continuous way typical of polygenes.

Previous workers have found heterogeneity of results from crosses involving the same gene conversion factors (e.g. Helmi 1980, p197). This variation was a problem in estimating gene conversion parameters because the equations derived in the earlier chapter were so sensitive to small alterations in the spectra of gene conversions. It was intended to demonstrate that the variation between different crosses could be reduced by

selection. It was also wondered if the genetic background could be altered to such a degree as to disguise the different alleles at a conversion control factor such as ccf-2. With this in mind, selection for both higher and lower gene conversion frequencies at w-78 was made in the presence of one allele at ccf-2. The resulting strains were then examined with regard to the effects of this selection upon other loci and with regard to their interaction with other alleles at ccf-2.

Heterogeneity of Results

Table 37 shows the results from five different wI^{+} x $wI\text{-}78^{-}$ crosses replicated through three incubators and using ten replica plates for each cross in each incubator. All the strains carry the P allele at the ccf-2 locus and are non-super. 500 octads were scored from each plate and the body of the Table represents the number of octads displaying non-Mendelian inheritance for the mutation w-78 (i.e. ratios not 4:4).

The crosses were: A) 7,R,7,- x sk,1,w78,4,+

B) 3,r,- x sk,1,w78,4,+

C) 3,w78,3,- x 9,r,+

D) 3,w78,3,- x 25,5,R,+

E) sk,7,R,7,- x sk,4,w78,7,+

Table 38 shows the calculations and reveals that the results for the crosses are significantly

Heterogeneity of Results

	Cross A			Cross B			Cross C			Cross D			Cross E		
	Incubator			Incubator			Incubator			Incubator			Incubator		
Replica	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	47	38	36	45	53	51	49	62	64	51	42	37	46	43	59
2	48	63	39	50	46	44	53	55	44	52	56	43	52	42	46
3	45	39	47	48	53	46	60	60	63	44	48	47	56	45	53
4	57	55	58	45	49	42	50	60	55	48	46	47	45	56	57
5	50	50	52	38	45	41	76	56	59	39	48	42	49	46	56
6	49	48	53	44	43	48	65	54	39	42	41	46	47	43	56
7	43	47	44	45	50	51	60	54	52	43	47	40	58	58	57
8	50	55	50	49	40	47	46	54	59	53	39	52	52	66	51
9	47	54	51	48	48	52	43	58	54	46	49	45	44	49	54
10	49	46	50	57	44	50	54	60	37	54	37	55	51	58	43
Incubator Totals	485	495	480	469	471	472	556	573	526	472	453	454	500	506	532
Cross Totals	1460			1412			1655			1379			1538		
Cross Means	486.7			470.7			551.7			459.7			512.7		

Table 37.
Number of aberrant octads out of 500 scored per plate.

Analysis of Variance of Heterogeneity Data

Source of Variation	df	SS	MS	
Among Incubators	14	1833.6		
Crosses	4	1627.5	406.87	
Among Incubators within Crosses	10	206.1	20.6	= Experimental error
Among Replicas within Incubators	135	5203.4	38.54	= Sampling error
Total	149	7037.0	47.23	

Test for Cross differences given by :-

$$F_c = \frac{\text{Cross MS}}{\text{Experimental Error MS}} = 19.75$$ Very Significant.

Test for Experimental Error is given by :-

$$F_e = \frac{\text{Experimental Error MS}}{\text{Sampling Error MS}} = 0.54$$ Not Significant.

Table 38. Variation in gene conversion frequency between crosses and incubators

heterogeneous even though all the strains involved did not differ for any known ccf alleles which affect locus wI.

Selection

Over one hundred random wild-type spores, originating from different asci, from cross E were germinated and the resulting strains were plated on crossing medium with sk,4,w78,7,+ . Of these, fifty minus mating-type strains were chosen randomly. At the same time as testing mating type, the conversion frequency was scored for a thousand octads.

Similarly, over one hundred white-spore strains were plated with sk,7,R,7,- and fifty of these fertile strains had their conversion frequency scored.

Of the one hundred strains examined, the strain with the highest conversion frequency of each mating type was crossed with the other and similarly the two strains, one from each group, with the lowest conversion frequency were mated. These strains were labelled 'high' and 'low' depending on the direction of selection.

This time twenty-five w-78,+ and twenty-five R,- strains were scored and again pairs of strains with the highest and pairs of strains with the lowest conversion frequencies were crossed using strains of opposite mating types. This was repeated three times until four generations had undergone selection, and five generations

of results were available for analysis.

When the selected strains were being crossed, controls were also being crossed. These controls took the form of unselected generations. The non-selection crosses were made by inoculating twelve random strains, six of each mating type (minus strains being wild-type for ascospore colour and the plus strains carrying w78) on four replica plates and continuing this process for as many generations as for the selection experiment. Randomness was generated using a table of random numbers (White et al. 1977). These non-selection crosses were labelled 'none'. Whenever conversion frequency was determined it was always calculated on the basis of 1000 octads, and the final conversion frequencies for generation 5 were based on two hundred strains.

Table 39 shows the conversion frequencies for each generation with generation zero representing the frequency of conversion in the cross between the two parental strains. Figure 23 displays these results in graphical form and it is obvious that selection has been successful, with a disparity in the selection differences, the response being greater for upward selection than for downward selection.

Figure 24 shows the distribution of the fifth generation conversion frequencies in the form of a histogram with the midpoint of each column represented as

PERCENTAGE GENE CONVERSION FREQUENCIES

	Selection Category		
Generation	'high'	none	'low'
0	10.9	10.9	10.9
1	13.0	11.3	9.0
2	14.1	10.5	8.9
3	14.4	10.6	8.5
4	14.8	11.0	8.3
5	15.3	10.6	8.3

Table 39.
Frequencies of gene conversion at successive generations, represented as percentages, based on 1000 octads .

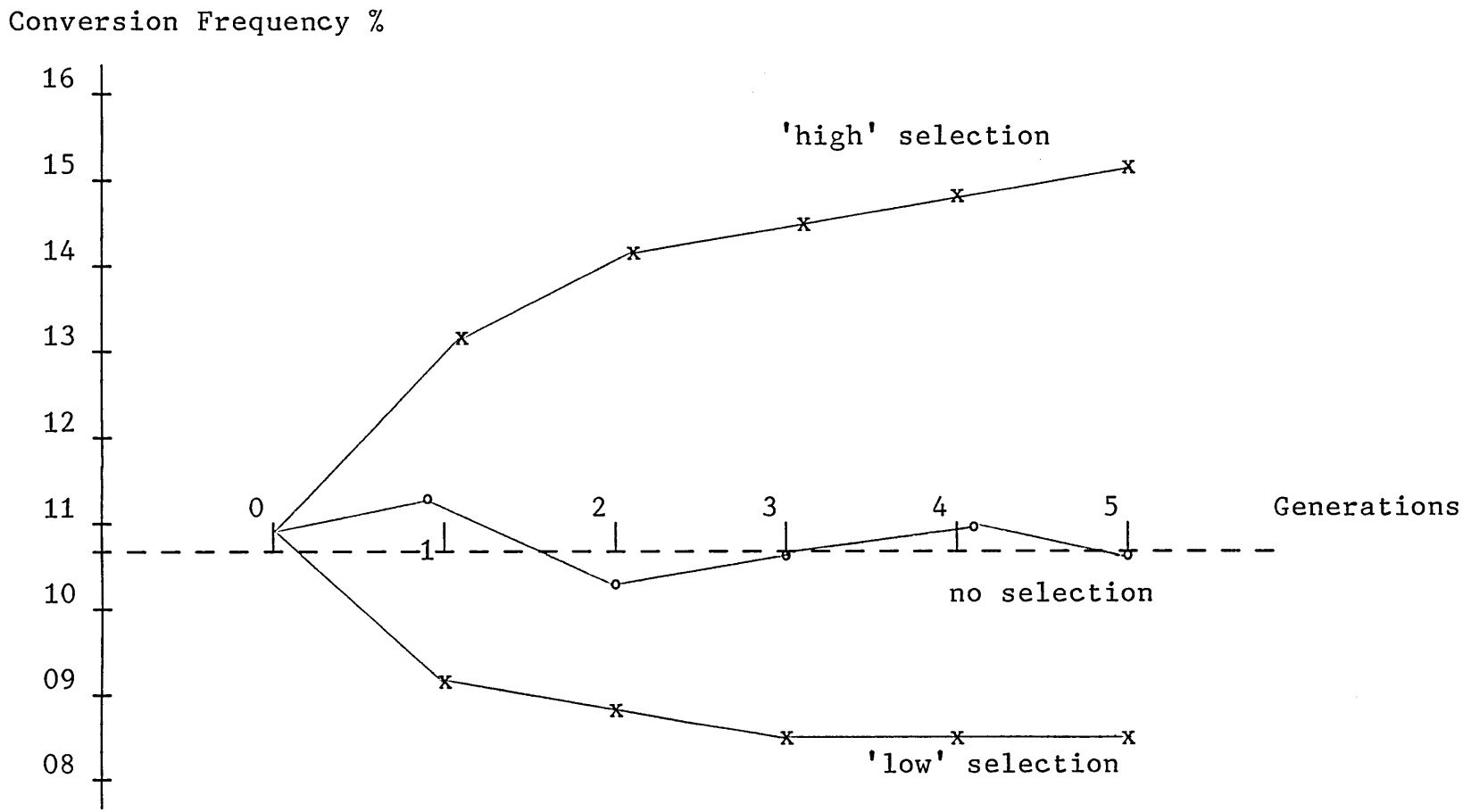


Figure 23. Showing the effects of successive selection on conversion frequency.

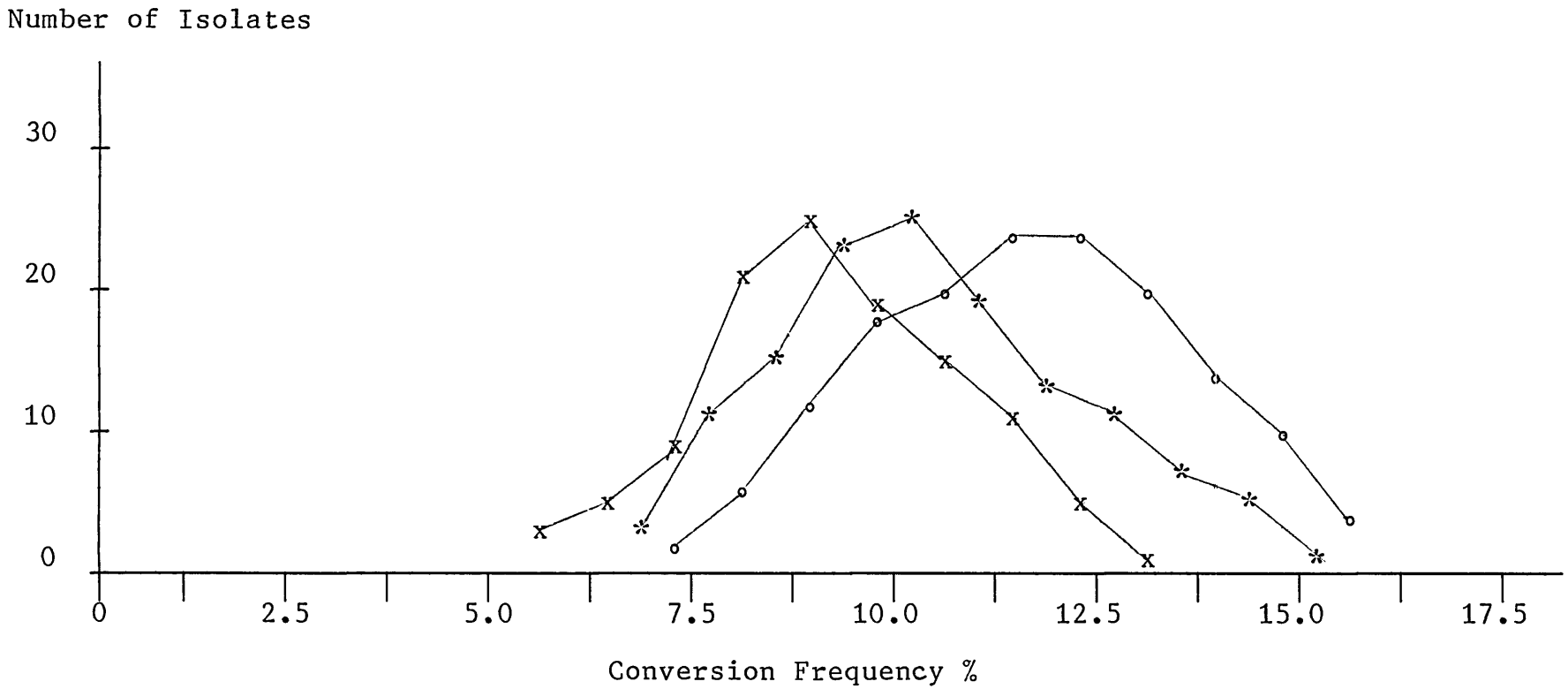


Figure 24.
Showing the results of selection compared to non-selection.
'low' = x ; 'high' = ∘ ; 'non' = * .

a single point. It is clear that the results resemble a normal distribution with a slight skewness towards the direction of selection, the non-selection population having no obvious skewness. The lack of more than one distinct peak within the distribution suggests that there has been no selection for genes with discontinuous variation in conversion, supporting the hypothesis that no oligogenes are segregating here (all carried ccf2-P).

Crossing the selected strains to tester strains permitted the study the interaction of the selected genes with various oligogenes. Table 40 shows the effects of crossing the selected strains with tester strains carrying different alleles at the ccf-2 site. The results are averages of six crosses, using three selected strains of each mating type. The selection strains carried the P allele at the ccf-2 site and it is clear that the selected genes have the expected effects on w-78 if they act independently from the ccf-2 factor. The tester strains were sz,5,w78,(K),3,+ ; sz,5,w78,(K),2,+ ; sz,6,w78,(K),3,+ ; sz,5,R,(K),4,- ; sz,5,R,(K),7,- ; and sz,6,R,(K),1,- .

Table 41 shows the effects of crossing with a 'super' strain (P at ccf-2). The conversion frequencies shown are averages of six crosses, using three selected strains of each mating type, the tester 'super' strains being Sup,1,w78,3,+ and Sup,2,R,5,- .

Selection Category	ccf-2 allele in Tester Strain	Conversion Frequency%		χ^2 2x2
'high' in ccf2-P	P	15.3		48.8
'none' in ccf2-P	P	11.7		
'low' in ccf2-P	P	9.2		30.1
'high' in ccf2-P	K	6.1		80.2
'none' in ccf2-P	K	3.4		
'low' in ccf2-P	K	2.2		22.9
'high' in ccf2-P	91	4.8		31.8
'none' in ccf2-P	91	3.2		
'low' in ccf2-P	91	2.8	*	2.4 NS

Table 40.

The interaction of the selected polygenes with ccf-2 alleles. Results based on 10 000 octads.

TESTER STRAIN IS 'SUPER'

Selection Category	Conversion Frequency %		χ^2 2x2
'high'	14.7		156.9
'none'	8.6		
'low'	8.1	*	2.0 NS

Table 41.

The interaction of the selected polygenes with other ccf-3 and ccf-4 alleles than the ones selected with. The strains selected were ccf3-e, ccf4-r . The tester strain was ccf3-E, ccf4-r so that when the crosses were made the genotype in the meiotically dividing cells was 'super'. Results based on 10 000 octads.

The results show that at least for the selected genes for 'high' conversion frequencies they are functioning in the 'super' genetic background. Whilst the 'low' selected genes have a lower conversion frequency it is not a significant difference from the control, non-selection.

Table 42 shows that the selected genes are not specific with regards to the mutant w-78, as they also affect mutant w3c1 within the same locus. The tester strain was w3c1,+ and the results are from the single cross of the test strain with the wild-type strains of the relevant selection criteria.

Table 43 shows that the polygenes are not specific for wI because they also affect these other two loci, w-BHj and w-9. The test strains were sj,w-BHj,5,+ and BB,9,w-9,+ . The conversion frequencies were calculated from the single crosses of the test strains with the wild-type strains of the relevant selection criterion.

Discussion

It is evident from the total non-specificity of the selected genes, that the selection has been for a general enhancement (in the case of 'high') or a general reduction (in the case of 'low') of gene conversion rather than of a specific nature. It is therefore suggested that

Selection Category	ccf-2 allele in Tester Strain	Conversion Frequency%	χ^2 2x2
'high' in ccf2-P	P	12.1	0.48 NS
'none' in ccf2-P	P	11.8	
'low' in ccf2-P	P	12.0	0.11 NS
'high' in ccf2-P	K	3.4	3.00 NS
'none' in ccf2-P	K	3.0	
'low' in ccf2-P	K	2.0	22.18
'high' in ccf2-P	91	1.3	20.81
'none' in ccf2-P	91	0.7	
'low' in ccf2-P	91	0.7	0.06 NS

Table 42. Results of conversion frequencies at the w3c1 site when selected strains were crossed to w3c1,+ . Results based on 10 000 octads.

Selection Category	Mutant	Conversion Frequency%	χ^2 2x2
'high'	w-BHj	15.1	19.3
'none'	w-BHj	12.8	
'low'	w-Bhj	9.2	60.6
'high'	w-9	12.1	15.8
'none'	w-9	10.2	
'low'	w-9	9.0	7.3

Table 43.

The conversion frequencies of alleles w-Bhj and w-9 which are at different loci from w-78, the allele under which the selection was made. Results based on 10 000 octads.

the genes selected were of a secondary nature in their action. For example, it is possible that in selecting for a change in conversion frequency, one was, at least in part, selecting for a change in the duration of synapsis between the chromatids during meiosis. If this was true, assuming that time was a limiting factor, it would alter the frequency of recombination.

It is suggested that changes in frequency of conversion could be indirectly selected for by selecting for characters such as time of sporulation, growth rate and perhaps even fertility. There was no obvious difference for any of these features.

The effects of selecting over five generations did not make the oligogenic factors indistinguishable and it is unlikely with such a small gene pool available that selection would be able to do this. The fertility of the selected strains was reduced compared to the unselected strains. Whilst no figures are available, all the fifth generation selected strains were evidently less fertile as expressed in the number of octads produced per plate. This may have been due to inbreeding during selection.

Table 44 shows that in selecting for an altered conversion frequency, the spectrum of conversion is unchanged as observed by backcrossing to the original parents. It could be that there are polygenic factors which can be selected for that have effects on disparity

and on the proportion of postmeiotic segregation, and that selection for either of these characters may not affect the frequency of conversion.

Cross	Fractions of Conversion Octads 6:2	2:6	3:5	5:3
'high',w78,+ x sk,7,R,7,-	.667	.226	.048	.071
'high',R,- x sk,4,w78,7,+	.622	.236	.061	.074
sk,4,w78,7,+ x sk,7,R,7,-	.598	.268	.062	.072
'high',w78,+ x 'high',R,-	.629	.245	.075	.094

Cross	Frequencies of Conversion Octads 6:2	2:6	3:5	5:3
'low',w78,+ x sk,7,R,7,-	.579	.263	.063	.074
'low',R,- x sk,4,w78,7,+	.571	.264	.066	.077
sk,4,w78,7,+ x sk,7,R,7,-	.598	.268	.062	.072
'low',w78,+ x 'low',R,-	.575	.276	.057	.057

Table 44.
The conversion spectra for the selected strains when crossed with each other and with the original parents. The body of the table represents the frequencies as a proportion of all aberrant octads from a sample of 1000 octads.

C R O S S O V E R S T U D I E S
I N R E L A T I O N T O C O N V E R S I O N

Of the mutants mentioned in the chapter on mutagenesis and isolation only one, mor-2, is linked to any degree to the wI locus. Octad analysis has indicated a mapping distance of approximately ten recombination units by crossing over in asci segregating 4:4 for the white spore-colour mutant w-78.

Identification of mor-2 and its phenotype are described in the chapter on mutations. It has shown no evidence of co-conversion with w-78 and has no visible effect upon the conversion frequency or spectrum of that mutant (as expected). Whenever the ascospores from an octad segregating for this marker have been germinated and analysed, the first scoring for the character mor-2 has been the correct one and no conversion event of the mutant has been discovered. Strains carrying mor-2 have always bred true so far and only once has a wild-type been isolated from mor-2 x mor-2 crosses, presumably being a reverse mutation.

Preliminary studies have been conducted to measure the crossover frequency in crosses carrying different conversion control factors. These studies have

included measuring crossing over in octads segregating 4:4 and |5:3| for the white spore-colour mutant. This chapter describes a novel method of detecting crossing-over between two sites, one of which is converting, rather than the established method requiring two outside markers, and presents the results found for mor-2 and w-78 in Ascobolus immersus.

It is evident from the chapter on parameters that the proportion of 5:3 events that occur as a result of four-chromatid events is mostly in the excess of ten percent of all 5:3 events for the w-78 mutant. It is therefore not wise to exclude such events from the experiment without due consideration. As a whole it is expected that in the overall analysis of crossing-over and its association with gene conversion the four chromatid events will not alter the conclusions reached.

In associated crossover events the two chromatids involved in the conversion event are also involved in the crossover event. In a dissociated crossover it is the other two chromatids which are involved in the gene conversion; in other words, the crossover is incidental.

Because only one outside marker is available for wI, the frequency of crossovers is not available from these results. Note that associated crossovers occurring on the 'left' of w-78, as drawn, would not be detected

using mor-2.

Figure 25 shows the effect of the two types of crossover event on the types of octads to be expected in the hypothetical case of a base pair X-Y and the alternate allelic base pair A-B. The diagram shows the genotype at the eight strand stage. It represents the 5:3 conversion event arising from asymmetric hDNA.

Figure 26 shows the analogous case where the 5:3 ratio, X-Y:A-B, arises from symmetric (1) hDNA. The diagram represents the eight strand stage following repair of the mismatch on the chromatid originally carrying the outside marker + .

Figure 27 similarly shows the alternate 5:3 eight strand stage arising from symmetric (2) hDNA, following repair of the mismatch on the chromatid originally carrying the outside marker M.

Table 45 summarises the expected octad types as a result of the different crossover events. It can be seen that only dissociated events produce spores with parental genotypes numbering less than four per octad irrespective of the origin of the 5:3 conversion.

From these expected frequencies, given the assumption that crossing over occurs between the two chromatids involved in conversion, one can calculate the expected proportion of incidental crossovers between the two sites involved. It has been possible to study the

Original		Either	Or	The Outside Marker Following a Crossover Type		
				Ass	Non	Dis
A B	M	A B	A B	M	M	+
A B	M	A B	A B	+	M	M
Goes To						
X Y	+	X B	A Y	M	+	+
X Y	+	X Y	X Y	+	+	M

The two centre chromatids, as drawn, undergo conversion by asymmetric hDNA formation and lead to a 5:3 ratio at the A/B X/Y site. The outside marker is either M or +.

Ass = associated, Non = no crossover, and
Dis = dissociated.

Figure 25.

Original	Either	Or	The Outside Marker Following a Crossover Type		
			Ass	Non	Dis
A / B M	A / B	A / B	M	M	+
A / B M	A / Y	X / B	+	M	M
Goes To					
X / Y +	A / B	A / B	M	+	+
X / Y +	X / Y	X / Y	+	+	M

The two centre chromatids, as drawn, undergo conversion by symmetric (1) hDNA formation and lead to a 5:3 ratio at the A/B X/Y site by substitution. The outside marker is either M or +.

Ass = associated, Non = no crossover, and
Dis = dissociated.

Figure 26.

Original	Either	Or	The Outside Marker Following a Crossover Type		
			Ass	Non	Dis
A B M	A B	A B	M	M	+
A B M	A B	A B	+	M	M
Goes To					
X Y +	X B	A Y	M	+	+
X Y +	X Y	X Y	+	+	M

The two centre chromatids, as drawn, undergo conversion by symmetric (2) hDNA formation and lead to a 5:3 ratio by restoration.

Ass = associated, Non = no crossover, and
Dis = dissociated.

Figure 27.

From asymmetric hDNA, octads produced are:-

Crossover	A/B M	A/B +	X/Y M	X/Y +
Associated	3	2	1	2
Not occurring	4	1	0	3
Dissociated	2	3	2	1

From symmetric (1) hDNA, octads produced are:-

Crossover	A/B M	A/B +	X/Y M	X/Y +
Associated	4	1	0	3
Not occurring	3	2	1	2
Dissociated	1	4	3	0

From symmetric (2) hDNA, octads produced are:-

Crossover	A/B M	A/B +	X/Y M	X/Y +
Associated	3	2	1	2
Not occurring	4	1	0	3
Dissociated	2	3	2	1

Table 45.

The octad types arising from asymmetric and symmetric hDNA. M or + is the outside marker and the ratio A/B:X/Y equals 5:3. The parental types are A/B M and X/Y +.

relationship between conversion and crossing-over by monitoring the amount of associated crossing-over when conversion is altered by changing the genetic background of the organisms crossed.

In crossing strains to determine the frequency of crossing over, only narrower ratio octad numbers have been considered. The conversion frequency was determined as the frequency of these octads with non-Mendelian segregation. Linkage was determined from octad analysis of 4:4s. Disassociated crossovers were scored for both 5:3 and 3:5 octads and Table 46 shows the pooled results because independently, the results are too small to give a general picture.

Table 46 shows the recombination results for w-78 and mor-2. Conversion frequencies are calculated from ten thousand octads per cross for the same crosses. The data for dissociated crossovers were limited by the germination of complete, or nearly complete, octads and the occurrence of |5:3| ratios. Recombination in 4:4 octads was calculated using classical octad analysis and the frequency of parental ditypes,

$$\frac{(\frac{1}{2}T + NPD) \times 100}{PD + NPD + T} .$$

Recombination arising from crossing-over in 4:4 octads does not increase with gene conversion frequency in

Cross			4:4 Sample Size	Percent Recomb. in 4:4s	Conversion Frequency of 10000 octads	Total Number of |5:3| Octads Analysed	The Number of Dissociated Crossovers
w-78	x	+					
P	x	P	124	11.29	8.56	28	3
P	x	K	33	9.09	4.12	24	0
K	x	P	29	10.35	4.13	23	1
K	x	K	48	8.33	2.27	38	1
P	x	91	35	8.57	6.24	11	0
91	x	P	32	9.38	6.56	17	1
91	x	91	31	9.68	5.34	26	0
super	x	super	31	12.90	24.15	26	1

Table 46.
The results of conversion studies of w-78
in relation to crossing over with mor-2.
Dissociated crossovers were measured in |5:3| octads.

a way that is clear although the highest recombination frequencies accompanied the highest conversion frequencies. More significant is the incidence of dissociated crossovers for the two mutants studied. The portion of dissociated crossovers for the entire set of results (7 out of 193) shows that crossing-over is not restricted to the chromatid pair involved in the conversion event. An estimate for the overall frequency of dissociated crossovers is 3.63 %. This compares with approximately 10 % in the data from 4:4 octads for recombination occurring in either of two pairs of chromatids; so frequency is 5 % per pair of chromatids minus an allowance for some being associated crossovers in correction 4:4s. In the 5:3 data presented, crossovers are looked for in only one pair of chromatids, the pair not involved in the conversion event. It has been found that an average value of 3.6 % is representative of recombination between mor-2 and w-78 in one pair of chromatids. This is not small in relation to 5 %.

These results would suggest that crossing-over between w-78 and mor-2 is loosely associated with conversion events at w-78, the lack of a strong relationship between crossing-over and conversion frequency is possibly a result of being able to measure recombination frequencies only in 4:4 octads. Results of recombination frequency variation in octads showing

conversion at the w-78 site must await a second outside marker. Alternatively, as ccf-2 rarely converts, one could use heterozygous ccf-2, as one marker, mor-2 as the other, whatever ratio occurs at wI.

Table 47 shows the various comparisons of the recombination values using χ^2, 2x2 tests to judge the significance of recombination frequency changes. All the crosses are compared to the PxP cross and all show a significant change in recombination. The reciprocal crosses (P,w-78 x K,+ and P,+ x K,w-78 etc.) were compared and once again show significant differences. Lastly the crosses involving heterozygosity for the ccf-2 locus were compared to the crosses involving homozygosity and on the whole the heterozygosity of the ccf-2 locus had a significant effect on the amount of recombination. It is extremely difficult to determine whether the changes in recombination frequencies are a direct measure of crossing over or are merely an effect of aberrant 4:4s and correction 4:4s. On the whole whilst the changes in recombination frequency are significant they are relatively small compared to the changes in conversion frequency and it is assumed that the changes are entirely due to changes in the amount of correction and aberrant 4:4s.

Cross w-78x+	Percent Recombination	2x2 χ^2 Tests Comparing with: PxP	KxK	91x91	recip.
P x P	11.29	-	44.7	12.4	-
P x K	9.09	23.7	3.3NS	1.9NS	8.2
K x P	10.35	4.1	21.8	2.2NS	8.2
K x K	8.33	44.7	-	10.1	-
P x91	8.57	37.3	0.3NS	6.8	3.7
91x P	9.38	17.6	6.2	0.47NS	3.7
91x91	9.68	12.4	10.1	-	-
Super	12.90	10.7	98.4	45.9	-

Table 47.
Comparisons of recombination frequencies. between w-78 and mor-2 in 4:4 octads. recip = comparing to the reciprocal cross. See Table 46 for more information.

G E N E R A L D I S C U S S I O N

It has been shown in this work that the processes involved in gene conversion are under the influence of polygenes as well as the well documented major genes. In contrast to the major genes such as ccf-2, the polygenes have a non locus-specific effect. The effects of these polygenes can either heighten or reduce the distinction between the major gene differences and may be a useful tool in finding further major genes which under a random genetic background are possibly unidentifiable. In other respects it is unlikely that the polygenic control will be useful in determining the exact mechanisms of recombination because of their lack of influence on the spectra of conversion, but it is possible that such polygenes do exist and their selection may identify major genes which merely influence repair parameters rather than conversion frequency. This second type of major gene is very likely given the current theories on recombination but because of the large variation of results from crosses it is difficult to find at the moment. Examples of selection to reduce variation are abundant in the evolution of modern crop species (Simmonds 1979) and the advantage of the haploid stage in

fungi suggests that such selection should be relatively easy even though they are diploid at the time of recombination.

Now that methods have been demonstrated which can identify the component parts of recombination (Kalogeropoulos and Thuriaux 1982, 1985; and the present work) it is possible to identify the changes, slight as they may be, in recombination as a result in the altered genetic background of the organisms involved. As usual in genetics, it will be the study of the presence of different genes and their effects that will be the guide to solving the questions posed. In this case the question posed is along the lines of, "How does gene conversion occur and how is it related to crossing over ?".

If we look at the most recent evidence, we find that the double-strand break repair model (DSBR) is unable to explain gene conversion events resulting as direct results of the process. It can not be denied, on the basis of mutants deficient in double-strand gap repair, that double strand gap repair is involved in recombination. The most simple solution to this apparent paradox is that the initiation of gene conversion events is double strand gaps. This would not only explain the involvement of double strand gaps in recombination but would explain why data from most organisms studied, including yeast (Lamb 1987), support the involvement of heteroduplex DNA (hDNA)

in gene conversion.

There are certain confusing results which at the moment are difficult to explain without unsupported hypotheses. These include the unexpected association of a crossover event with the initiation end of hDNA. Hypotheses explaining these results include enzyme migration (Markham and Whitehouse 1982) and double Holliday junctions (Holliday 1978). Of these, it is the latter one which seems most likely on the basis that a mechanism for the production of double Holliday junctions is molecularly sound (see Figures 5a and 5b of the Introduction).

On the more specific topic of the gene conversion control factors (ccfs) in Ascobolus, it is more likely from behaviour, that ccf-2 lies between the initiation site of hDNA and the mutants w-10 and w-78, instead of it being the initiation site itself. This conclusion is based on the results of the present work where it has been shown that ccf-2 influences the amount of hDNA which is symmetric and the amount of hDNA as an entirety. This is in keeping with the ccf-2 site having a variety of base sequences (alternate alleles) which influence the extension of hDNA beyond their position, but does not explain why ccf-2 does not usually co-convert with wI. This lack of co-conversion tends to suggest that ccf-2 is the initiation site itself or lies very close to

it on the opposite side to wI.

The extension of hDNA is normally understood to be a result of either branch-migration of a Holliday junction (in the case of symmetric hDNA) or a result of single strand displacement (in the case of asymmetric hDNA). Energy is expended in the unwinding of duplex DNA and it seems likely that the uni-directional nature of strand displacement/branch-migration is energy related and directed by a balance in the energy differential of duplex unwinding/winding and most likely to be controlled by an enzyme analagous to the recA protein of Escherichia coli. Whilst it has been shown in vitro that mismatches do not seem to influence duplex formation (Dohet et al. 1985) it is unknown whether certain base pair sequencies have an influence and it is not unlikely that this is so. It waits to be discovered if this effect is large enough to make a difference.

Because of the proximity of ccf-2 to the wI mutants it is not too surprising that different alleles at the ccf-2 site have a marker effect on mismatch repair at the wI sites. Dohet et al. (1985, 1986) have shown that marker effects can occur. It is noticeable in this work that repair at a wI mutant site is greatest when there is also heterozygosity at the ccf-2 site, and this is explained by the fact that if either are recognised as a mismatch, the excision-repair tract is large enough to

cover both sites. Evidence to support this comes from postmeiotic segregation co-repair studies (Helmi 1980).

The fact that varying conversion control factors at the w-78 mutant does not significantly alter the frequency of crossing over between this site and that of the linked mutant, mor-2, would suggest that the expected crossover event is occurring on the other side of the white spore-colour mutant with respect to the morphological mutant. With the knowledge, in practice, that crossover points are associated with the initiation end of hDNA formation, and that, in theory, the ccf-2 site lies near (or is) the initiation site, the proposed map sequence along the chromosome is:-

——— ccf-2 ——— w-78 ——— mor-2 ———

This map sequence is also true if ccf-2 is the initiation site itself; if not, the initiation site is on the right of ccf-2 as drawn on the basis of co-conversion. If as predicted by the Meselson-Radding model, the proportion of asymmetric hDNA decreases away from the initiation site then the mutant w-10 is located between ccf-2 and w-78.

The current study has demonstrated that crossover events that occur in octads demonstrating gene conversion do not always involve the same two chromatids that are involved in hDNA formation. It has been assumed that incidental crossovers involve the two chromatids not

involved in conversion. This definition is contrary to that of Kitani et al. (1962) and Whitehouse (1982) where the chromatids are chosen at random irrespective of those involved in the aberrant segregation.

The next step will be to map the wI locus and calculate the various parameters for all the white spore-colour mutants to determine whether, as postulated, there is a polarity in conversion for this locus. It is also probable that postmeiotic segregation will have a gradient along the length of the locus even though PMS will depend on the molecular nature of the mutation, e.g., base substitution or frameshift (Paquette and Rossignol 1978). Combining these studies with outside marker behaviour and conversion control factor influence will then support either one or another model of recombination. Thereafter cloning of the ccf genes which are not linked to the sites that they influence may yield an enzyme system akin to rec-1 in Ustilago and recA in E. coli.

A P P E N D I X

C O N S T R U C T I N G E Q U A T I O N S

This appendix is a supplement to the chapter on gene conversion parameters. In the few pages contained herein it is hoped to assist anyone trying to construct new equations for octads, or even to check those derived in this work. Parameter definitions are not repeated and the reader is referred to the body of this thesis for anything not covered here.

The basic structure of this section is to outline all the possible types of four-chromatid stages arrived at by any formation of heteroduplex DNA. The notation is exactly the same as in the main chapter and each diagram is labelled in such a way that one can see how it was arrived at from the original two pairs of chromatids.

The multiplier in front of some parameters, such as $\phi(1-\phi)$, are needed when there are two alternatives, e.g. either of the two hDNAs being ϕ and the other $(1-\phi)$. This is a common feature of these equations !

There follows a summary of α and β combinations required for the formulation of equations involving both pairs of chromatids.

Before hDNA is Formed

(or no hDNA formed, $1-\nu_1$)

5'.._____A_____..3'
3'.._____B_____..5' (1)

5'.._____A_____..3'
3'.._____B_____..5' (2)

Original Chromatids

5'.._._._.X._._._..3'
3'.._._._.Y._._._..5' (3)

5'.._._._.X._._._..3'
3'.._._._.Y._._._..5' (4)

Events involving only one pair of chromatids

$\nu_1(1-\nu_2)$

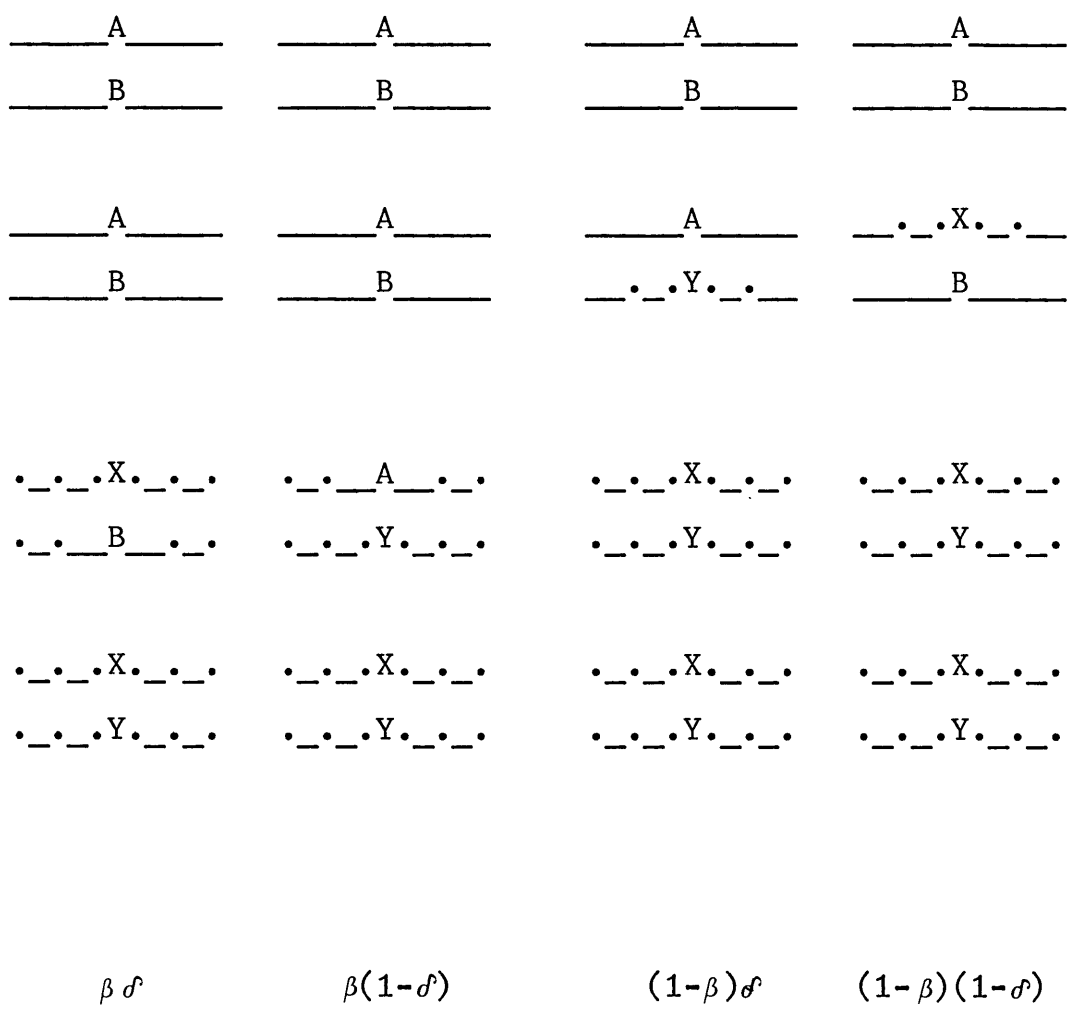
Symmetric $(1-\alpha)$	Symmetric $(1-\alpha)$
A B	A B
A Y	X B
X B	A Y
X Y	X Y
δ'	$(1-\delta')$

Events involving only one pair of chromatids

$\nu_1(1-\nu_2)$

Asymmetric hDNA

α



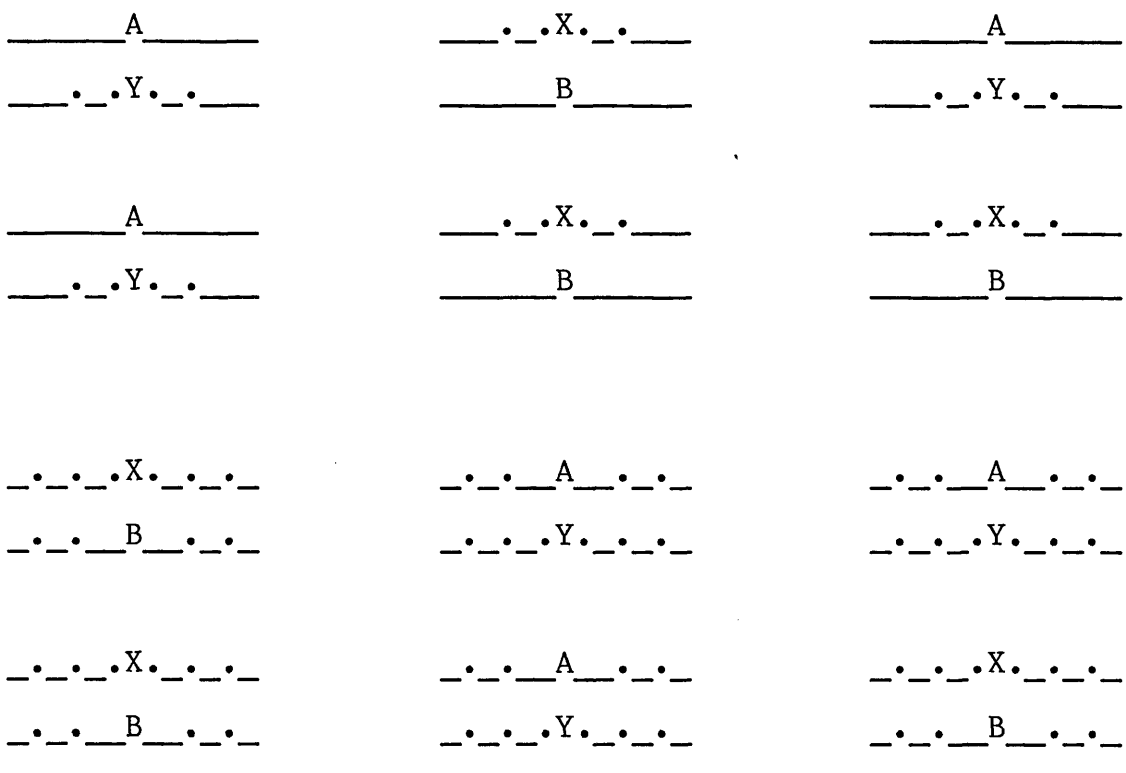
$\beta\delta$ $\beta(1-\delta)$ $(1-\beta)\delta$ $(1-\beta)(1-\delta)$

Events involving both pairs of chromatids

$\nu_1\ \nu_2$

Both hDNA Events are Symmetric

$(1-\alpha)^2$



δ^2 $(1-\delta)^2$ $2\ (1-\delta)$

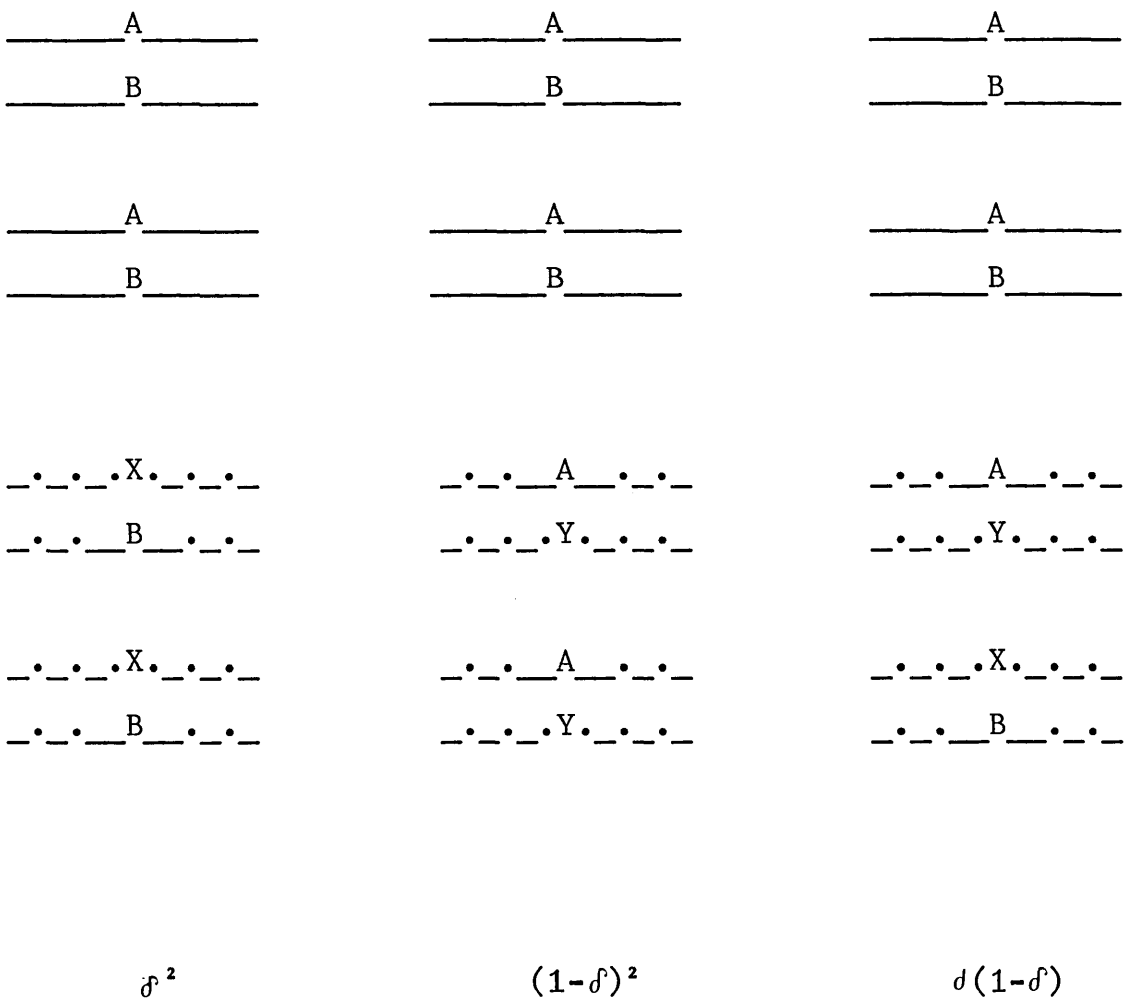
Events involving both pairs of chromatids

$r_1\ r_2$

Both Events are Asymmetric

The Mutant Chromatid is Invaded in Both Events

$\alpha^2 \quad \beta^2$



$\delta^2 \qquad (1-\delta)^2 \qquad \delta(1-\delta)$

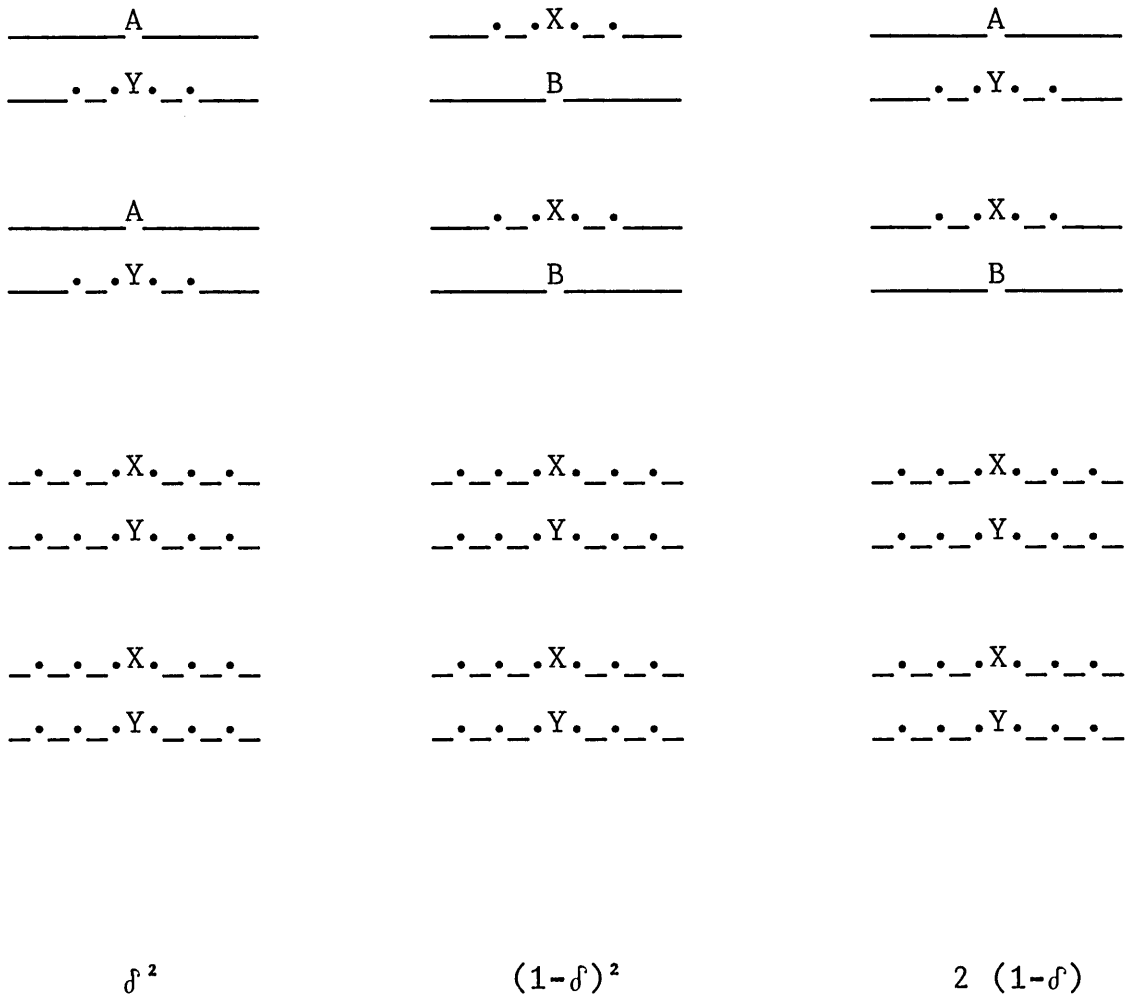
Events involving both pairs of chromatids

$\nu_1 \nu_2$

Both Events are Asymmetric

The Wild-Type Chromatid is invaded in Both Events

$\alpha^2 (1-\beta)^2$



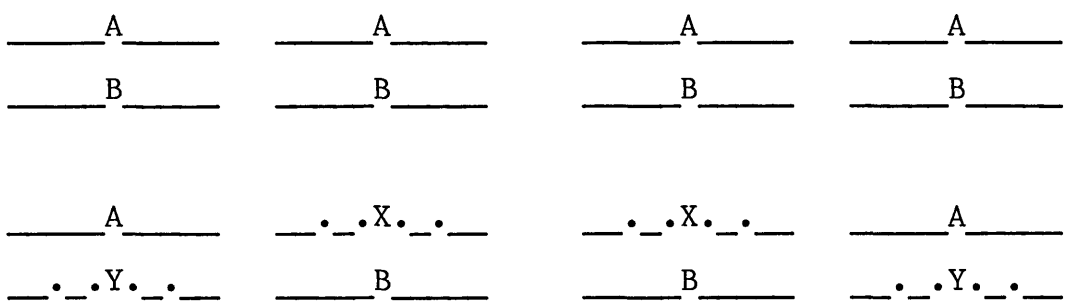
δ^2 $(1-\delta)^2$ $2(1-\delta)$

Events involving both pairs of chromatids

$\nu_1 \nu_2$

Both Events are Asymmetric One Event is the Wild-Type Chromatid being Invaded, the Other Event (in the other pair of chromatids) is the Mutant Chromatid being Invaded

α^2 $2\beta(1-\beta)$



δ^2 $(1-\delta)^2$ $[$ $2\delta(1-\delta)$ $]$

event β with event $(1-\delta)$

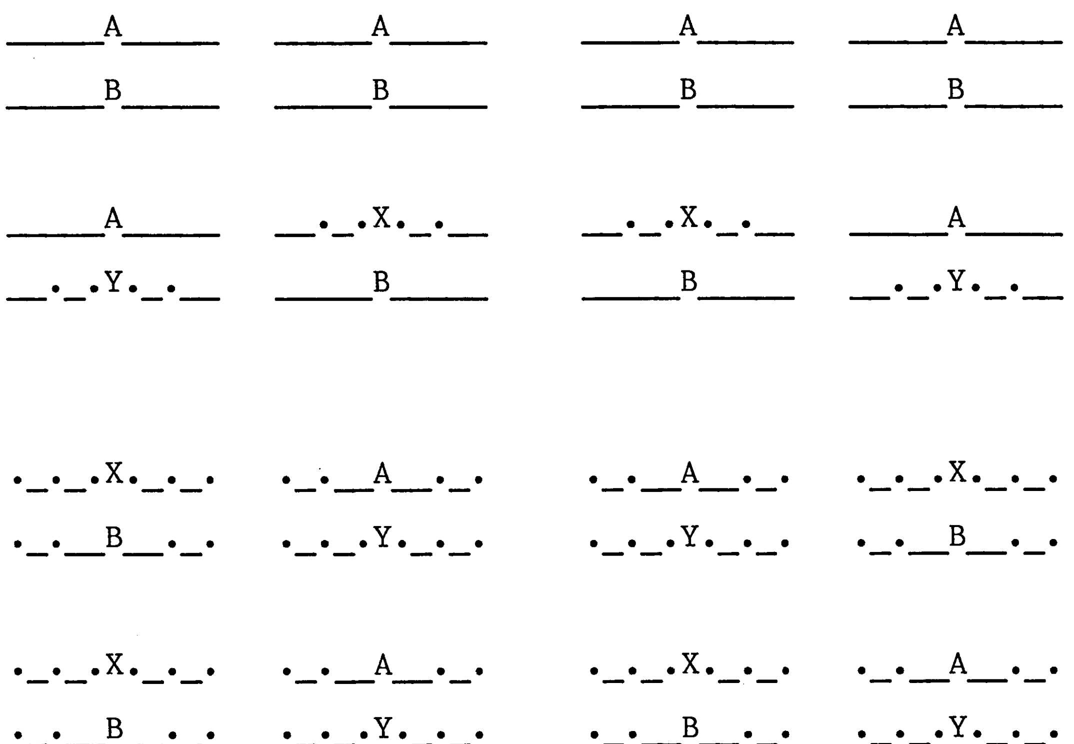
event β with event (δ)

Events involving both pairs of chromatids

$\nu_1 \nu_2$

One Event Asymmetric with the Mutant Chromatid being invaded, and the other Event Symmetric

$2\alpha(1-\alpha)\beta$



δ^2 $(1-\delta)^2$ [$2\delta(1-\delta)$]

event $(1-\alpha)$ with event $(1-\delta)$

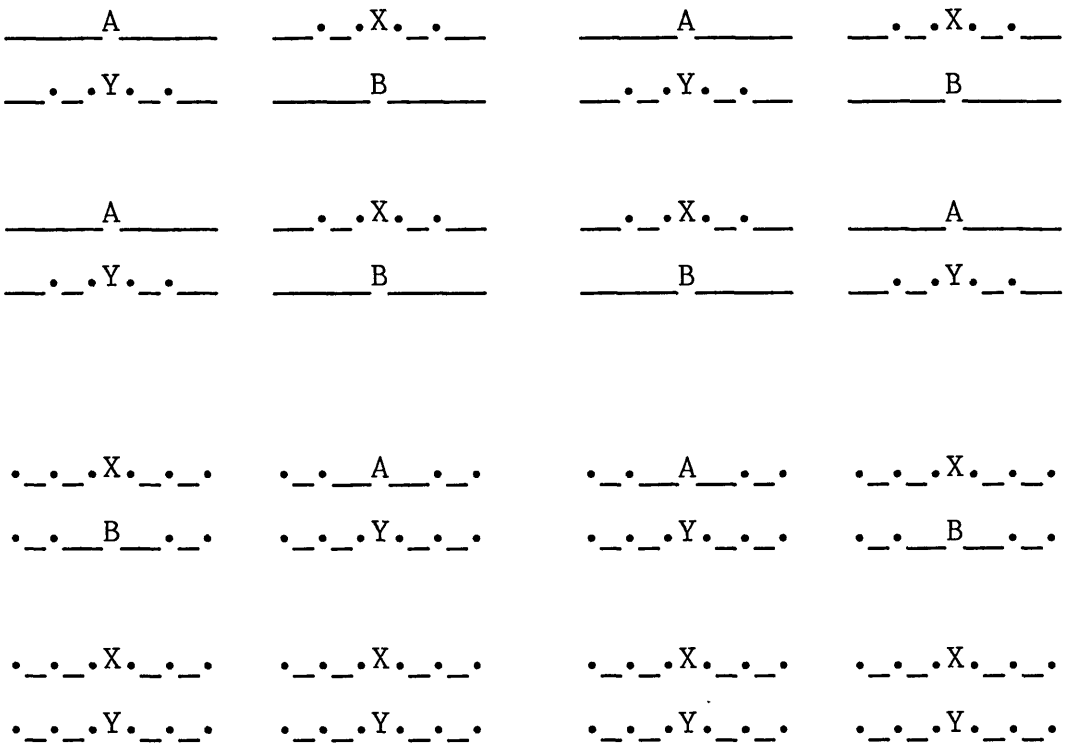
event $(1-\alpha)$ with event δ

Events involving both pairs of chromatids

$\nu_1 \nu_2$

One Event Asymmetric with the Wild-Type Chromatid being Invaded and the other Event Symmetric

$2\alpha(1-\alpha)(1-\beta)$



δ^2 $(1-\delta)^2$ [$2\delta(1-\delta)$]

event $(1-\alpha)$ with event $(1-\delta)$ | event $(1-\alpha)$ with event δ

Summary of α and β Combinations

Class	Events Involving Both Pairs of Chromatids $r_1 r_2$
8:0	$\alpha^2\beta^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$
7:1	$\alpha^2\beta^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$
6:2	$\alpha^2\beta^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$; 2 (1-)(1-β); $^2 2\beta(1-\beta)$
6:2(2)	$\alpha^2\beta^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$
5:3	$\alpha^2\beta^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$; $\alpha^2 2\beta(1-\beta)$; $2\alpha(1-\alpha)(1-\beta)$
N 4:4	$\alpha^2\beta^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$; $\alpha^2(1-\beta)^2$; $2\alpha(1-\alpha)(1-\beta)$; $\alpha^2 2\beta(1-\beta)$.
ab 4:4(2)	$(1-\alpha)^2$; $2\alpha(1-\alpha)(1-\beta)$; $\alpha^2 2\beta(1-\beta)$; $2\alpha(1-\alpha)\beta$
ab 4:4(4)	$(1-\alpha)^2$
3:5	$\alpha^2(1-\beta)^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$; $^2 2\beta(1-\beta)$; $2\alpha(1-\alpha)(1-\beta)$
2:6	$\alpha^2(1-\beta)^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)\beta$; $2\alpha(1-\alpha)(1-\beta)$; $\alpha^2 2\beta(1-\beta)$
2:6(2)	$\alpha^2(1-\beta)^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)(1-\beta)$
1:7	$\alpha^2(1-\beta)^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)(1-\beta)$
0:8	$\alpha^2(1-\beta)^2$; $(1-\alpha)^2$; $2\alpha(1-\alpha)(1-\beta)$

R E F E R E N C E S

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