THE IDENTIFICATION AND CHARACTERIZATION OF FURTHER GENETIC FACTORS CONTROLLING GENE CONVERSION IN *ASCOBOLUS IMMERSUS*

by

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Dedicated

to

My Parents

ABSTRACT

Gene conversion and its genetic controls were studied at three unlinked white ascospore pigmentation mutations in the Pasadena strains of *Ascobolus immersus*.

Conversion of mutation BB9*w*9 was shown to be under the control of a hitherto undetected conversion control factor (*ccf*), *ccf*-5, while the conversion behaviour of unlinked mutation *w*BHj is affected by *ccf*-6. Both *ccfs* are closely linked to the mutations whose conversion they control. Each of these two *ccfs* has two forms, heterozygosity for the *ccf* in a cross, reducing the conversion frequency of the closely linked white mutation in question. Neither *ccf* shows *cis/trans* position effects on the conversion frequency, spectrum or both, of its target mutation. The action of each *ccf* is specific to its closely linked target mutation. Neither control factor appears to interact with the previously detected *ccfs* 2, 3 and 4.

ccfs 5 and 6 are compared with other recombination control factors in the literature and the possible molecular natures and modes of action of the *ccfs* are discussed. It is proposed that *ccfs* 5 and 6 correspond to DNA sequences non-identical between the two forms of each factor, which determine the pairing closeness of DNA duplexes in their vicinity. This close pairing is necessary for the initiation of recombination events from as yet undetected specific sites. On this model local initial pairing would be poorer in crosses heterozygous for the *ccf*, leading to a reduced frequency of intimately paired DNA duplexes (via the binding of a non-specific DNA-pairing protein) and hence H-DNA formation, compared with crosses homozygous for either form of the *ccf*.

One further *ccf* (*ccf*-7) affecting the conversion behaviour of locus II mutation NG*w*18 was identified, but it was not possible to determine the mode of action of this *ccf*.

For all the mutations under study, optimum germination conditions were established. Conversion frequency changes with dehiscence time were investigated for seven mutations. Of the non-locus I mutations only *w*BHj showed a statistically significant pattern of narrower ratio conversion frequency change with time. However, for mutations showing high frequencies of postmeiotic segregation, there was considerable evidence for changes in the PMS/MS ratios with time.

A large number of ascospore colour mutants were isolated. None was linked to the mutations whose conversion behaviour was investigated, but one mutation was very closely linked to locus I. The conversion behaviour of all newly isolated ascospore colour mutants was investigated. No conclusive evidence for further *ccfs* was found, although the new white mutation closely linked to locus I appeared to respond to *ccf*-2. None of the morphological or auxotrophic mutants isolated were linked to any of the mutations under study. By means of reversion studies some evidence of the possible molecular nature of mutation *w*BHj was obtained.

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GENERAL INTRODUCTION

Until the commencement of this work gene conversion studies in the Pasadena strains of *Ascobolus immersus* had primarily been concerned with the conversion behaviour of mutations belonging to ascospore colour locus I. Initial studies on this locus were carried out by Emerson and Yu-Sun (1967), who also investigated the conversion behaviour and genetic controls of a white ascospore mutation (*w*62) unlinked to locus I. Further studies on gene conversion at locus I were by Lamb (1972), Lamb and Wickramaratne (1973), Wickramaratne (1974), Wickramaratne and Lamb (1978), Ghikas and Lamb (1977), Ghikas (1978), Lamb and Ghikas (1979), Helmi (1980) and Helmi and Lamb (1981). Stadler and Towe (1971) and Stadler, Towe and Rossignol (1970) studied the conversion behaviour of mutations mapping at other loci in the same strains of *Ascobolus*.

Lamb and Ghikas (1979) studied the gene conversion properties of 29 induced and 9 spontaneous white ascospore colour mutations, of which 34 did not belong to locus I. Each white mutant strain was crossed to 3 different types of derived wild-type strain. In such crosses 6 mutations showed large differences in total conversion frequency in crosses to different wild-type strains and thus were prime targets for investigating whether these differences in conversion frequency were under genetic control, and if so, whether hitherto unknown genetic control factors were responsible.

Thus the main aim of this work was to investigate the conversion behaviour of the 6 mutations, identify and characterize the conversion control factors which may be responsible and to propose mechanisms for their possible modes of action. For the sake of clarity, the experimental results section is divided into two parts, with the objectives in each part outlined below.

PART ONE

1. Determining whether the detected differences in conversion behaviour of the mutations under study are due to genetic conversion control factors.

2. Investigating and characterizing the nature and behaviour of the factors involved.

3. Determining whether any newly detected conversion control factors are original, or otherwise.

4. Investigating the specificity of these conversion control factors.

5. Proposing possible mechanisms for the action of these conversion control factors.

6. Determining whether the "Super" genetic conversion control system (Helmi and Lamb, 1981) has any effect on the mutations under study.

PART TWO

1. Determining optimum germination conditions for the white mutants used in this study, so improving the initially very poor germination frequencies.

2. Investigating the changes in conversion frequencies with the time of dehiscence for the mutants under study (plus some others) and establishing any possible patterns of change.

3. Attempting to obtain further white mutations linked to those under study and also suitable flanking mutations.

4. Carrying out preliminary investigations of the conversion behaviour of newly isolated ascospore colour mutations.

5. Determining the possible molecular natures of some of the mutations whose conversion control systems were investigated.

LITERATURE SURVEY

Introduction

This survey is concerned with the following topics: genetic recombination, gene conversion, recombination-related phenomena, theories and models for the mechanism of recombination between and within synnemal genes, the biochemistry and molecular basis of hybrid DNA (H-DNA) formation and mismatch repair, the synaptonemal complex and recombination nodules and the genetic controls of recombination and gene conversion.

Two types of organism have, in recent times, made the major contributions to recombination studies - the phages and the fungi (particularly the ascomycetes). The phages have contributed by virtue of the small size of their chromosomes, which encourages physical analysis of intermediates and products of genetic exchange. The ascomycetes offer the opportunity of recovering all four products of individual meioses. Most of the data in this survey will be drawn from studies involving ascomycete fungi and bacteriophage, but where relevant, contributions from bacterial and higher eukaryotic systems will also be considered.

Genetic recombination

Recombination is one of the earliest discovered, yet still least understood, of genetic phenomena. The historic work of Morgan (1911) and Sturtevant (1913) showed that groups of genes could be arranged in a linear series by

using the frequency of recombination between any two as an index of the distance between them. It was not long, however, before the discovery that the frequency of recombination between two linked genes is subject to both environmental (Bridges, 1915) and genetic effects (Gowen, 1919).

Genetic recombination was described by Whitehouse (1973) as the process by which new combinations of parental characters may arise in the progeny. General recombination between non-synnemal genes may come about through the random orientation of non-homologous chromosome pairs on the meiotic spindles; recombination between synnemal genes is mainly by crossing-over between homologous chromosomes, involving the reciprocal transfer of DNA segments, while recombination within genes is mainly by gene conversion. Unlike the latter two cases, there are other types of recombination which do not require extensive homology, such as site-specific recombination, which depends upon the existence of specific sites in one or both molecules and includes the interaction of viral genomes and insertion sequences with the chromosomes of prokaryotes and eukaryotes (Bukhari, 1976; Kamp et al.1978; Landy et al.1978; Chesney et al.1978; Enquist et al.1979). Illegitimate recombination may also occur, requiring neither extensive homology nor special sites (Murray and Murray, 1974; Ikeda et al.1980).

Crossing-over normally takes place at the pachytene stage of meiosis, but the first preparatory stages for this seem to begin in the S-phase of the cell cycle, or

near its end (evidence reviewed by Kushev, 1974). However, in yeast, a limited amount of crossing-over has been reported as early as S-phase DNA synthesis itself (Olson and Zimmermann, 1978a). Both crossing-over and gene conversion can also occur in mitosis, but generally are very infrequent, with mitotic recombination being 100 to 10,000 times less frequent than in meiosis (Holliday, 1977). Mitotic recombination was first reported in *Drosophila melanogaster* by Stern (1936) and has subsequently been demonstrated in many organisms, mainly fungi (Zimmermann and Vig, 1975) and green plants (Vig, 1975), but there is also some evidence for mitotic recombination in mammals (Grünberg, 1966; Bateman, 1967).

Recombination frequencies between any pair of genes are not constant, but are subject to environmental effects, as first reported by Bridges (1915). Variations in recombination frequency following temperature changes prior to or during meiosis were reported in *Neurospora crassa* by McNelly, Lamb and Frost (1966), Landner (1970) and Lamb (1971) and in *Sordaria* by Lamb (1969, a,b). These authors suggested that temperature does not necessarily act directly on the recombination process but can partly work by intermediary substances as recombination is such a complex process. Induced changes in genetic recombination by temperature treatment during pre-meiotic interphase have been reported in maize (Maguire, 1968), *Drosophila* (Grell, 1967, 1978; Grell and Generoso, 1980) and *Coprinus* (Lu, 1974). However, in the latter case, temperature treatment of pachytene nuclei as well as haploid nuclei

undergoing the last round of DNA synthesis prior to karyogamy also affected crossing-over frequencies. Using the moss *Sphaerocarpus donnellii*, Abel (1964, 1965) could influence recombination frequencies by elevated temperature only after DNA synthesis during meiotic prophase. Some authors do conclude that temperature acts directly on the recombination process (e.g. Grell and Generoso, 1980). It has also been reported that meiotic homologous pairing is reduced by high temperature in common wheat (Kato and Yamagata, 1980).

Cold-shock is also able to influence recombination frequencies, as reported in *Chlamydomonas reinhardi* (Lawrence, 1965, 1967) and *Coprinus* (Lu, 1969, 1970, 1974; Lu and Chiu, 1978). In the latter case, cold treatment at $5^{o}C$ at pachytene in "arrest-release" cultures, suspends the progress of meiosis and hence prolongs the pachytene stage, causing an increase in recombination frequency. This gives weight to the argument that recombination frequency is a function of time.

Premeiotic irradiation was also found to have an effect on recombination in several organisms, such as *Drosophila* (Chandley, 1968), and *Chlamydomonas reinhardi* (Lawrence, 1965, 1967), while γ rays have been shown to affect chiasma frequency in the sunflower *Helianthus annus* (Al-Allaf and Goodward, 1979) and *Pennisetum typhoides* (Lal and Srinivasachar, 1979). Non-lethal doses of γ irradiation increase meiotic recombination in *Coprinus lagopus*, when treatments are given at the beginning of karyogamy (Raju and Lu, 1973).

Metabolic inhibitors can alter recombination frequencies in *Chlamydomonas reinhardi* (Lawrence, 1965, 1967), *Saccharomyces cerevisiae* (Silva-Lopez et al. 1975) and *Coprinus lagopus* (Raudaskoski and Lu, 1980) while chiasma frequency has been shown to be similarly affected in *Trigonella corniculata* by Singh and Ahmed (1979).

Morpurgo (1963) showed that certain types of DNA damage increase mitotic crossing-over in *Aspergillus nidulans*. Mitotic crossing-over in yeast (James and Lee-Whiting, 1955) as well as mitotic gene conversion (Roman and Jacob, 1958) can be induced by ultra-violet light. It seems that both types of mitotic recombination (crossing-over and gene conversion) are a regular response to the induction of genetic damage by mutagens (Zimmermann, 1971; Mayer, 1973; Vig, 1975). Simmon (1979) carried out *in vitro* assays for the recombinogenic activity of 101 carcinogens, non-carcinogens, metals and promoters representing a wide variety of chemical classes, to determine whether they increased mitotic recombination in *Saccharomyces cerevisiae*. It is worth mentioning that, at least in yeast, mitotic gene conversion can be induced at frequencies almost reaching the meiotic level (Esposito, 1968).

Breakage and reunion of DNA play a major role in crossing-over, both in eukaryotes and prokaryotes (Meselson and Weigle, 1961; Meselson, 1967; Fox and Allen, 1964; Siddiqi and Fox, 1973; Taylor, 1965). Some features of genetic recombination in prokaryotes are reviewed by Fox (1978).

Genetic recombination is a fundamental property of all living organisms starting from the RNA - containing viruses and ending with higher plants and animals (Kushev, 1974) and as a source of variation is one of the principal factors in evolution. Meiotic-like recombination in vegetative dikaryons of *Schizophyllum commune* has been reported by Frankel (1979). Such processes were common and may be as important as conventional basidial meiosis in the origin of recombinant strains. The role of recombination in the evolution of fungi is discussed by D'Yakov (1980). Recombination mechanisms are a basic factor in variation within a species and have been discussed as playing a significant part in the dynamics of evolution, particularly as it applies to the evolution of co-adaptive gene clusters (Fogel and Mortimer, 1969; Chovnick, 1973). Allard (1963) showed by a high-low selection experiment that chromosomal mechanisms governing crossing-over can be altered by selection. Charlesworth and Charlesworth (1979), using computer models, found that selection for increased recombination is usually weak, while that for decreased recombination is frequently much stronger.

Gene conversion

The term "gene conversion" was introduced by Winkler (1930) to describe a process of interaction between alleles at meiosis. Conversion was observed by Zickler (1934) involving spore colour mutants of *Bombardia lunata*, but this observation was neglected. The term was re-introduced by Lindegren (1953) to account for aberrant ratios in the

products of meiosis in yeast, apparently arising from such interaction between alleles. The occurrence of gene conversion was confirmed by Mitchell (1955) in studies with pyridoxine-requiring mutants of *Neurospora crassa* and has since been detected and extensively studied in other ascomycetes like *Sordaria* and *Ascobolus*, using ascospore and auxotrophic mutants (references cited in Catcheside, 1977; Fincham, Day and Radford, 1979). Several aspects of fungal intragenic recombination were shown to apply to *Drosophila* by Smith, Finnerty and Chovnick (1970) in studies with the *maroon*-like cistron, by Ballantyne and Chovnick (1971) with the *rosy* cistron and by Carlson (1971) with the rudimentary locus. Evidence that recombination between alleles at the *waxy* locus in *Zea mays* is at least partly due to conversion, was provided by Nelson (1962, 1968) while it seems the same is true at the *gl 1* locus (Salamini and Lorenzoni, 1970). Lamb (1981) has defined gene conversion as a process of chemical interaction between alleles, usually at meiosis, where one allele can convert another to being of its own kind in a heterozygote. In octad-producing fungi, gene conversion may produce the following kinds of segregation patterns: the "narrower ratio" classes 6+ : 2*m*, 2+ : 6*m*, 5+ : 3*m*, 3+ : 5*m*, and the "wider ratio" classes 8+ : 0*m*, 0+ : 8*m*, 7+ : 1*m*, 1+ : 7*m* (Lamb, 1972). In tetrad-producing fungi, the postmeiotic segregation classes (including aberrant 4:4s) can only be detected by the production of sectored colonies.

Conversion frequencies at single sites show great variation, not only between species but also within the

genome of a single species. In the European stock *28* of *Ascobolus immersus* the gene to gene conversion frequency shows almost a 100-fold variation (Nicolas, 1979). The data of Lamb and Ghikas (1979) show a similar variation in the Pasadena strains of the same organism. Among fungi the highest frequencies of gene conversion have been reported in *Ascobolus immersus* (both Pasadena and European strains) where up to 30% of octads may be convertant (Nicolas, 1979; Helmi, 1980). High frequencies of gene conversion are also seen in *Saccharomyces cerevisiae* (Fogel et al. 1979) and *Sordaria brevicollis* (Yu-Sun, Wickramaratne and Whitehouse, 1977), where several per cent of asci may show non-Mendelian ratios. In *Neurospora crassa* (Stadler and Towe, 1963; Case and Giles, 1964) and *Sordaria fimicola* (Kitani and Olive, 1967) frequencies tend to be lower, less or much less than one percent, for the markers for which data are available.

Gene conversion is a faithful process, utilizing the pre-existing genetic input and does not generate novel information at the heterozygous site in question. This fidelity has been demonstrated by several authors. Olive (1959) stated that mutant alleles resulting from conversion behave like their parents in + x *m* crosses. Case and Giles (1964) carried out tetrad analysis studies of the *pan-2* locus in *Neurospora crassa* and concluded that in tetrads showing gene conversion, the extra alleles, whether wild-type or mutant, were indistinguishable from the parental alleles on the basis of mutation, recombination and complementation tests. However, the fidelity of gene conversion was most convincingly demonstrated by

Fogel and Mortimer (1970) who in yeast analysed crosses between wild-type and various *ochre* mutants. These mutants show specific repressibility and correspond to a known DNA nucleotide sequence. In crosses between these mutants and wild-type, 1+: 3*m* tetrads always had three mutants of precisely the original *ochre* type. Similarly, in 3+ : 1*m* tetrads, the extra wild-type was always a true wild-type. Thus what is involved in conversion is a precise change in DNA nucleotide sequence, from wild-type to mutant or vice-versa. Stadler and Kariya (1969) concluded from their studies on intragenic recombination at the *mtr* locus of *Neurospora crassa* that gene conversion is not a process generating novel codons, neither is there any mutagenesis involved in conversion that takes place as a non-reciprocal event. McCarron, Gelbart and Chovnick (1974), working on intracistronic mapping of electrophoretic sites, confirmed the fidelity of information transfer by gene conversion in *Drosophila melanogaster*. However, some rare cases have been reported in which alleles which had probably undergone conversion showed altered conversion properties. Such is the case of the h_{2a} isolate of *Sordaria fimicola* reported by Kitani and Olive (1967). The altered behaviour of h_{2a} Lamb (1975) attributed to the loss of nearby cryptic heterozygosity. In a heteroallelic cross, hybrid DNA (H-DNA) may contain two mismatched base pairs at different sites. Independent repair of the mismatches can generate recombinants and since natural populations are heterozygous or polymorphic at many gene loci, Holliday et al.(1978) proposed that

gene conversion could be important in generating new alleles, from the pre-existing genetic input.

Conversion may involve more than one heterozygous site and the term "co-conversion" refers to a conversion event affecting two or more closely linked sites (Fogel and Mortimer, 1969). These authors demonstrated co-conversion within the *arg4* gene of *Saccharomyces cerevisiae*. It has also been demonstrated in *Sordaria fimicola* by Kitani and Whitehouse (1974), in *Neurospora crassa* by Stadler and Kariya (1969) and in *Ascobolus immersus* by Leblon and Rossignol (1973). Co-conversion events involving sites in more than one gene can occur, as reported for the *me-7* and *me-9* genes in *Neurospora crassa* (Murray, 1970), in data involving the *cyc1* (Lawrence et al. 1975) and *SUP6* loci (DiCapricio and Hastings, 1976) in yeast and in *Ascobolus immersus* (Paszewski, 1967). Rossignol and Haedens (1978) and Kalogeropoulos and Rossignol (1980) demonstrated that the length of the repair tract spanning heterozygous sites within a gene in *Ascobolus immersus* does not always cover the whole H-DNA segment. The data of Fogel et al. (1979) from yeast are also consistent with this view. It therefore appears that co-conversion of two heterozygous sites depends upon whether they are included in the same repair tract.

Gene conversion can occur in mitosis, but is generally much less frequent than in meiosis. Such mitotic conversion can occur spontaneously or as a result of the induction of genetic damage by mutagens (Zimmermann, 1971; Mayer, 1973; Vig, 1975). In yeast, mitotic gene conversion has been demonstrated in the G_1 phase of the cell cycle

(Wildenberg, 1971; Fabre, 1978; Kern and Zimmermann, 1978; Esposito, 1978). In the same organism, mitotic gene conversion can be induced at frequencies almost reaching the meiotic level (Esposito, 1968).

The classical cases of gene conversion reviewed above involve genes on homologous chromosomes. More recently yeast workers have demonstrated intrachromosomal gene conversion (Klein and Petes, 1981; Jackson and Fink, 1981), involving repeated copies of genes on the same chromosome. Also working with yeast, Scherer and Davis (1980) have described interchromosomal conversion. Experimental evidence now exists that members of a gene family on sister chromatids, on the same chromatid or on different chromosomes, can pair with one another and one can then convert the sequence of the other. Jackson and Fink (1981) showed that between a closely linked pair of *his4* genes in yeast, gene conversion is much more common than reciprocal recombination. In *Schizosaccharomyces pombe*, evidence has been reported for the specific exchange of information between reiterated tRNA genes, most easily interpreted as intergenic conversion (Munz and Leupold, in press; cited by Egel, 1981). Baltimore (1981) postulates that gene conversion can explain the maintenance of sequence homogeneity among members of a family of genes in a more satisfactory way than unequal crossing-over can. The latter hypothesis (Szostak and Wu, 1980; Petes, 1980) involves a constantly changing number of genes. The occurrence of gene conversion among tandem mammalian genes has been suggested by Slightom et al.(1980) involving β-like globin genes and Liebhaber et al.(1981) for human α-globin genes. Egel

(1981) also considers the possibility of gene conversion playing a role in the generation of antibody diversity.

Gene conversion between repeated DNA sequences may be involved in the phenomenon of mating type interconversion in yeast (Hicks and Strathern, 1977; Klar, 1980; Jackson and Fink, 1981). This process seems to have features in common with gene conversion within gene duplications (Haber et al. 1980; Klar et al. 1980). Mating type in yeast is controlled by two alleles of the *MAT* locus, *MAT a* and *MAT* α. Homothallic strains switch from one mating type to the other at high frequency (Hicks and Herskowitz, 1977; Strathern and Herskowitz, 1979). This switching of mating type requires two other loci, *HML* and *HMR* (Harashima et al. 1974; Harashima and Oshima, 1976). *HML* and *HMR* are unexpressed copies of the *a* and α information, which can donate this information to the *MAT* locus. The replacement of *MAT* information by *HML* or *HMR* does not lead to a change at the donor locus. Klar et al. (1979) reported that gene conversion of the mating type locus in yeast may occur in strictly an orthodox manner, without involving the *HML* or *HMR* loci.

In eukaryotic cells gene conversion can occur involving extra-nuclear DNA. Strausberg et al.(1978) showed that conversion of inserted segments in yeast mitochondrial DNA takes place unidirectionally. Mannella and Lambowitz (1979) described the same phenomenon in *Neurospora crassa* mitochondrial DNA. Two insertions in the *poky* locus were identified as sites of high frequency unidirectional gene conversion which lead to their spread through the

mitochondrial DNA population of a heteroplasmon. Such a phenomenon, the authors believe, might be responsible for the dominance of *Neurospora* extranuclear mutants.

Gene conversion has important implications in population genetics, which have been overlooked until recently (Gutz and Leslie, 1976). Lamb and Helmi (1982) have derived equations for the interaction of conversion, mutation and selection. Using typical fungal conversion data they showed that conversion, with appreciable disparity in its direction, can be a strong force in changing allele frequencies in a population.

Gene conversion is the most important kind of aberrant segregation and its study can be used to elicit molecular details of meiotic recombination. Its demonstration in *Drosophila* and *Zea mays* were important findings, as it showed that conclusions from fungal studies are likely to apply to eukaryotes in general. The recent findings implicating gene conversion between members of families of repeated genes, whether on the same chromosome or not, have further widened the scope and importance of gene conversion studies to include maintaining sequence homology or promoting variability among reiterated mammalian genes. The implications of gene conversion in population genetics are only just beginning to be appreciated.

Recombination related phenomena

A universal theory for genetic recombination should account for those phenomena which usually accompany crossing-over and gene conversion; these phenomena are

conversion-associated crossing-over, polarity in conversion, interference and marker effects such as map expansion.

Conversion-associated crossing-over

It has been shown in several studies that there is a definite correlation between intragenic recombination and the reciprocal recombination of markers flanking the gene in question. The actual value of this correlation is variable between and within species. In *Saccharomyces cerevisiae*, Fogel and Hurst (1967) observed that approximately 50% of conversion events were accompanied by reciprocal exchange of flanking markers. This finding was confirmed in a further study (Hurst, Fogel and Mortimer, 1972). However, Stadler (1973) pointed out that recombination frequencies associated with conversion events must be corrected to allow for the incidental exchanges which occur in the flanking interval. Applying such a correction, Fogel et al.(1979) observed that in yeast the average percentage of conversion directly associated with crossing-over is approximately 35%, with a range from 18% to 66%. Other studies, such as those of Whitehouse and Hastings (1965), Bond (1973), Kitani and Whitehouse (1974), Stadler and Towe (1971), DiCapricio and Hastings (1976), Sang and Whitehouse (1979 a,b) and MacDonald and Whitehouse (1979) showed great variability with respect to the correlation between conversion and associated crossing-over and a frequent significant departure from the previously assumed 50% value. In *Neurospora crassa*, notably for the *me-2* (Murray, 1963) and *am* genes (Fincham, 1967; Smyth, 1973), a clear majority of the intragenic recombinants are

not recombined with respect to the flanking markers. Sang and Whitehouse (1979a) observed that at the *buff* locus in *Sordaria brevicollis* the frequency of flanking marker exchange in conversion asci differed in the odd-ratio asci (5:3 and 3:5) from that in the even ratio asci (6:2 and 2:6).

According to the above pattern of conversion-crossing-over relationships, Radding (1978a) suggested that the region of non-reciprocity is the actual site of chemical interaction between recombining molecules of DNA: the chemical interaction involves some intermediate, the resolution of which determines whether the flanking arms of DNA emerge in their original relationship or in a new recombinant relationship.

In mitosis, gene conversion is not so closely associated with reciprocal exchange (Roman, 1956; Roman and Jacob, 1958). In mitosis in yeast, gene conversion and reciprocal recombination can be separated from each other. Roman (1956) found that UV light irradiation enhanced mitotic reciprocal recombination more than gene conversion, and that nitrosoguanidine enhanced gene conversion preferentially. In addition, Boram and Roman (1976) isolated an allele of *rad18* that enhances spontaneous mitotic gene conversion, but not reciprocal recombination. These considerations and others suggest that there may be mechanistic differences between meiotic and mitotic recombination.

Polarity in gene conversion

Polarity in gene conversion is a term used to describe two situations. Firstly, it is used to describe the case

where conversion frequencies of different alleles in + x *m* crosses decrease in a continuous fashion from one end of a locus to the other; the region in which polarity occurs has been called a polaron. Secondly, the term polarity is used to describe the case where in a linear map of mutational sites within a locus, in m_1 x m_2 crosses, one allele appears to convert much more than the other; the preference depends on the position of the two alleles relative to each other in the map. Lissouba and Rizet (1960) and Lissouba et al. (1962) were the first to describe such a phenomenon in *Ascobolus immersus*, arranging all mutants of series *46* (except one) in a linear order, such that in any cross between two of the mutants, it was the mutant on the "left" of the map which got converted to wild-type to the exclusion of the mutant to the right. Another case of conversion polarity in *Ascobolus* is much less clear-cut and probably has, at least in part, a different explanation. In series *75*, Rossignol (1969) subdivided the mutants into three classes on the basis of their different degrees of conversion asymmetry. Within each class there was a gradient in conversion frequency from one end of the gene to the other, whereas no such order had been apparent before the three classes had been distinguished. The best interpretation of this is that heteroduplex tends to spread into the gene from one side so that there is a gradient with respect to "opportunity to convert", but the extent to which each heterozygous site makes use of this opportunity depends on the nature of the mismatch. Conversion polarity has also been reported in *Neurospora*

crassa (Murray, 1963; Smith, 1965; Fincham, 1967; Smyth, 1971) for several loci and has been observed in mitotic recombination in *Aspergillus nidulans* (Putrament et al. 1971). Murray (1970) showed that in *Neurospora crassa* H-DNA can extend from one opening point through another potential opening point, involving the contiguous genes *me-7* and *me-9*. The pattern of polarity indicated a discontinuity between the genes, with much of the H-DNA that entered *me-7* originating between the genes. It may be that some cases of conversion polarity are not based on a polarized pattern of heteroduplex formation (in the sense of different lengths spreading from a fixed point), but rather depend on features of the conversion process within a long continuous heteroduplex region. Fincham (1976) suggested that polarity in the case of the *am* locus in *Neurospora crassa* (term used in the sense of most wild-type recombinants always arising by conversion of the mutant site to the left in any $m_1 \times m_2$ cross) would occur given the following assumptions: i) H-DNA commonly covers the whole marked gene; ii) nicking, preparatory to the formation of a Holliday structure, occurs predominantly in strands of one chemical polarity; iii) degradation from a mismatch proceeds predominantly in one chemical direction, and iv) co-conversion is sufficiently common for divergent conversion tracts (i.e. those proceeding from a mismatch site away from another site, rather than towards it) to yield substantially more recombinants than convergent tracts. Fogel et al.(1979) observed in yeast that in 5:3 tetrads the majority of the exchanges are in the proximal interval, whereas in 3:5 tetrads, most of the associ-

ated exchanges are distal. This lead them to reject the notion that polarity reflects a fixed initiation site near the high conversion end of the *arg 4* locus with conversion tracts extending from this site into the gene for various lengths. This is the explanation for polarity given by various authors. Alternatively, Fogel et al. (1979) suggested that H-DNA is propagated in two directions starting from one of a number of initiation sites which are more numerous at the high conversion end of the gene.

Interference

Chromosome interference was first described by Muller (1916), who pointed out that the occurrence of one crossover in *Drosophila* interferes with the coincident occurrence of another between the same pair of chromosomes. He termed the phenomenon "interference"; Carter and Robertson (1952) suggested that a more appropriate title would be "chiasma position interference". Interference appears to result from the relative position of crossovers along the chromosome only and not on the chromatids involved (chromatid interference - see below). For this reason, Whitehouse (1973) called it "crossover position interference". This interference can be positive or negative and is usually strong over short intervals, declining to no interference at about 40 recombination units separation. Nearly absolute positive crossover position interference is seen in *Podospora anserina* (Rizet and Engelmann, 1949). However, the patterns of this interference were shown to differ between linkage groups by Marcou

et al.(1979). Pritchard (1955) attributed the excess of apparent double crossovers within relatively short invervals to "negative interference", i.e. a tendency for crossovers to occur in tight clusters. Later, Case and Giles (1958) showed that gene conversion is the main mechanism of recombination between markers within short intervals, rather than crossing-over. High negative interference has also been reported in bacteriophage T5 (Beck, 1980). Again, gene conversion due to mismatch repair has been implicated. However, Gussin et al.(1980) suggest that mismatch correction is not a major pathway for the production of recombinants for close markers in normal bacteriophage λ infection. Various theories have been put forward to explain crossover position interference. Holliday (1977) postulated that a DNA binding protein is required to stabilize a crossover. The protein is contained within the synaptonemal complex and at the site of a crossover the protein is depleted in that region and no crossovers can be formed nearby. Moens (1974, 1978) put forward a mechanistic model on the grounds that purely structural chromosome rearrangements can bring about changes in crossover frequencies and distribution. The stabilization of an exchange event may take place by way of a reorganization of the lateral elements along the crossover strands. Such a reorganization produces coiled synaptonemal structures, or where the synaptonemal structures are coiled prior to lateral element realignment, it can result in a reduction of synaptonemal complex coiling. Carpenter (1979) favoured, in explaining crossover position interference, a hypothesis that the processes which are responsible for the non-random distribution of spherical

nodules (indicating reciprocal events) may well be integral aspects of the function (s) of the spherical nodules themselves. Marcou et al.(1979) reject explanations of crossover position interference which are based on mechanical obstacles to two close crossovers. They favour the hypothesis that genetic exchanges are preferentially localized with chromosomes consisting of regions showing high and low recombination frequencies, due to special regulation of recombination events. This is their explanation for the crossover behaviour in linkage group 6 of *Podospora anserina*, where regions of strong positive crossover position interference occur.

There are several reports that conversion events do not interfere with nearby crossovers, but crossovers associated with conversion events do interfere (Stadler, 1959; Carlson, 1971; Mortimer and Fogel, 1974). Furthermore, conversion events in adjacent regions do not appear to interfere with each other, and these do not interfere with incidental crossovers and *vice versa*. However, from an analysis of large numbers of gene conversion asci for the *g* locus of *Sordaria fimicola*, Kitani (1978) found that neither the conversion event itself nor crossovers associated with such events cause interference (either chiasma or chromatid) with crossing-over in a neighbouring interval. Fincham, Day and Radford (1979) stated that interference cannot be a property of the initial structure formed between recombining chromatids (assumed to be the common precursor of conversion events with and without associated crossing-over). The initial regions of interactions between homologous chromosomes may be established without

interference with one another, but if one such region opts for crossing-over, it is some way discourages the next region from doing so. Fincham et al.(1979) suggested that the 180° rotation of the joined chromatids, which is involved in the isomerization supposedly essential for crossing-over in the Meselson and Radding model (Meselson and Radding, 1975), results in an increased resistance to another such twist occurring nearby in the same chromatid pair. They felt this hypothesis might be more reasonable if isomerization were of the Sobell type in a completed Holliday structure. A complication to be borne in mind is that interference does not only operate between one pair of chromatids, but equally reduces the chance of any pair of chromatids of the same bivalent of taking part in another crossover. MacDonald and Whitehouse (1979) reported a case in *Sordaria brevicollis* where at the *buff* locus the effect of mutant YS17 on outside marker exchange can be explained by the hypothesis of an enzyme aggregate (Sang and Whitehouse, 1979 a,b). They postulated that the modification of the primary event (assumed to be conversion) made by YS17 might affect the likelihood of the second event (the crossover).

A second type of interference was reported by Mather (1933) and termed chromatid interference. This refers to non-random distribution of the non-sister chromatids involved in successive crossovers. Various studies have been undertaken to detect this type of interference in fungi, including *Neurospora crassa* (Lindegren and Lindegren, 1942; Bole-Gowda et al. 1962; Perkins, 1962; Stadler, 1956;

Strickland, 1961), *Aspergillus nidulans* (Strickland, 1958), *Saccharomyces cerevisiae* (Hawthorne and Mortimer, 1960) and *Sordaria fimicola* (Perkins et al. 1963). Considering the data overall, there is little chromatid interference, while there is a good deal of variation between the studies. Perkins (personal communication to Fincham, Day and Radford, 1979) believes that strand relationships are generally random and that such significant deviations from randomness as appear in some data, are mostly due to unwitting bias in the selection of asci.

Lamb and Wickramaratne (1973) introduced the term "corresponding-site interference" to describe a third type of interference which may occur between the two pairs of non-sister chromatids of a bivalent in H-DNA formation at exactly corresponding sites. According to Lamb and Wickramaratne (1973) and Ghikas (1978), positive or negative corresponding-site interference may occur in *Ascobolus immersus*. The results of Yu-Sun et al.(1977) indicate that corresponding-site interference varies from non-interference to strong negative interference between loci in *Sordaria brevicollis*.

Marker effects and map expansion

Marker effects have been reviewed by Stadler (1973), Catcheside (1974) and Hastings (1975). They are the effects of the specific nature of each mutational site on the properties of recombination in its immediate neighbourhood. These effects may be strictly local, acting only on the site itself and not causing dependent effects in the

rest of the locus, as in the case in *Ascobolus immersus*, reported by Rossignol (1969), or they may extend to other parts of the locus as reported by Gutz (1971b) in *Schizosaccharomyces pombe*. In this latter example, the marker effect may be due to the site being involved in a major recombinational event in the affected locus (Goldman and Smallets, 1979), or due to cryptic heterozygosity (Lamb, 1975). The behaviour of *buff* mutation YS17 in *Sordaria brevicollis* (MacDonald and Whitehouse, 1979) is similar to the case reported by Gutz (1971 a,b). Map expansion, which can be considered as a marker effect, is defined as the tendency of recombination observed between two relatively distant sites of difference in a locus to be greater than the sum of the values observed over a number of short intervening distances. Fincham and Holliday (1970) suggested an explanation for map expansion based on the chance of a mismatched base pair in a segment of heteroduplex DNA being corrected independently of another linked mismatched base pair will increase sharply as the distance between the two sites becomes greater than the length of the DNA segment involved in the correction process. This last point is particularly important since it is now known that the length of the correction segment may be much less than the total length of the heteroduplex in which it occurs (Rossignol and Haedens, 1978; Kalogeropoulos and Rossignol, 1980; Fogel et al. 1979). Ahmad and Leupold (1973) proposed another explanation of map expansion: crossovers can be included in long intervals, but not in short intervals. This explanation assumes that a marker effect reduces conversion but does not affect reciprocal events.

Theories and models of reciprocal recombination and gene conversion

Recombination is a process that occurs between two molecules of DNA at any one place. Each chromosome or chromatid is one molecule of DNA consisting of two chains of opposite polarity. A single chain is commonly referred to as a polynucleotide chain. Taylor (1965) obtained the first direct evidence that crossing-over at meiosis takes place by breakage and exchange between non-sister chromatids and it is widely accepted that recombination includes breakage and reunion of DNA. Genetical and biophysical experiments in prokaryotes have shown that reunion is accomplished via the formation of a heteroduplex joint, a molecular splice in which one strand comes from each parent (Enea and Zinder, 1976; Gurney and Fox, 1968). When mutant sites are included in the heteroduplex joint, one or more bases are mismatched. As long as the mismatch persists, the DNA will be heterozygous. In fungi, in the first mitotic division after meiosis, a wild-type and a mutant sometimes segregate, apparently from a single DNA molecule. These observations on postmeiotic segregation suggest that heteroduplex formation is a feature common to both prokaryotic and eukaryotic recombination. The repair of mismatched bases in DNA is thought to contribute to gene conversion. Evidence for the occurrence of mismatch repair has been obtained from genetic studies of bacterial transformation and transfection with artificially constructed heteroduplex DNA (Spatz and Trautner, 1970; Wildenberg and Meselson, 1975; Wagner and Meselson, 1976).

Most models of genetic recombination and gene conver-

sion include these two components of hybrid DNA formation and subsequent mismatch repair. Such models will be reviewed first. There are two seminal models postulating symmetrical hybrid DNA formation on two non-sister chromatids. Holliday (1964) in his model, proposes that two copolar strands are active initially, while Whitehouse and Hastings (1965) proposed that two antipolar strands are active initially. On Holliday's theory one would expect different mispairs to be formed in the two involved chromatids, while the second theory predicts identical mispairs in the two chromatids, but these identical mispairs can be of two different types, depending on which strands initially break. The clearest evidence for H-DNA formation on two chromatids is the occurrence of postmeiotic segregation in two pairs of sister spores in an octad. Such aberrant 4:4 segregations have been detected in *Sordaria fimicola* (Kitani et al. 1962) and *Ascobolus immersus* (Leblon and Rossignol, 1973; Ghikas and Lamb, 1977). Aberrant 4:4 asci are, however, very rare in *Saccharomyces cerevisiae* (Fogel et al. 1979). Such postmeiotic segregations were predicted by Holliday (1964). Correction of the mismatches formed could lead to gene conversion while resolution of the cross-stranded structure, resulting from exchange of strands of like polarity between the two non-sister chromatids (a "Holliday structure" or "half chiasma") could be accomplished with or without flanking marker exchange. Paquette and Rossignol (1978) concluded from their work with *Ascobolus immersus* that the aberrant 4:4/ total PMS ratio can be considered as an index of the frequency of symmetrical H-DNA, provided that the 5:3/6:2

ratio is sufficiently high. Aberrant 4:4 asci can also arise from situations other than symmetrical or dual H-DNA formation Corresponding site events can give rise to aberrant 4:4s from asymmetric H-DNA (Ghikas and Lamb, 1977). In *Sordaria fimicola* (Kitani et al. 1962; Whitehouse and Hastings, 1965) and *Ascobolus immersus* (Leblon and Rossignol, 1973), H-DNA appears to form regularly on two chromatids, whereas in another strain of *Ascobolus* (Stadler and Towe, 1971; Wickramaratne, 1974), in yeast (Fogel and Mortimer, 1970, 1974; Fogel et al. 1971, 1979) and in *Sordaria brevicollis* (MacDonald and Whitehouse, 1979) the results have been explained by assuming that H-DNA forms only, or more frequently, on one chromatid. Models suggesting H-DNA formation at a given site in only one chromatid (known as asymmetrical H-DNA) have been proposed by Paszewski (1970), Stadler and Towe (1971), Sobell (1972), Boon and Zinder (1969) and Hotchkiss (1971, 1974b). Meselson and Radding (1975) have proposed a very versatile model in that H-DNA is initially formed on one chromatid by asymmetric strand transfer, and by subsequent isomerization and branch migration can generate H-DNA on both chromatids. This model is able to account for the rather special yeast data by postulating that the asymmetric phase might predominate in yeast. The *Ascobolus* conversion data of Paquette and Rossignol (1978) relating to the *b2* locus, can be explained well by the Meselson-Radding (1975) model, as they are in agreement with initial asymmetric transfer of one strand, later tending to involve the mutual exchange of both strands. However, in their analysis of H-DNA distribution at the *b2* locus, they treated both mispairs at a given site (with symmetrical H-DNA) as "+/*m*", all with the same parameter values for frequency and direction of correction. In an independent analysis of Paquette and Rossignol's data, Lamb and Helmi (unpublished) found that the data for sites at which H-DNA

had previously been assumed to be asymmetric, fitted well with Holliday's model of symmetric H-DNA formation.

At this point it is fitting to describe the Meselson-Radding model (1975), commonly referred to as the "Aviemore model", because it is the most flexible and versatile model with the widest acceptance today. It is a derivation of Holliday's original model. There are reservations, of course, and they will be discussed later. The initial step is a single break in one strand of one homologue which becomes the site of displacement by a DNA polymerase. The strand on one side of the break grows, displacing the strand on the other side. The displaced strand pairs with the complementary sequence in the homologue and induces a single strand break in the displaced strand. The two fragment strands are then ligated, leaving two other fragments, one of which continues to grow by polymerase activity, while the other is eroded by exonuclease action. These two enzymic properties are conceived of as different catalytic properties of the same complex enzyme. This concerted action results in further strand transfer. It produces a tract of heteroduplex DNA on only one of the two interacting molecules and so is designated as "asymmetrical strand transfer". The necessary rotations of the duplexes are driven quickly by the chemical energy derived from the polymerization reaction. Strand transfer would move in a definite direction, due to the polarized direction of the polymerization. Dissociation of the enzyme allows isomerization of the structure. In one isomeric form the arms that flank the site of exchange are parental, whereas in the other the recombinant strands are flanking. The cross

connection or Holliday structure, is free to migrate, presumably in either direction, as a consequence of rotary diffusion in the two molecules. Hence, while H-DNA was previously formed asymmetrically, strand transfer is now symmetrical and H-DNA may be formed on both interacting molecules. This new structure may also isomerize reversibly. In their assessment of the "Aviemore" model, Fincham, Day and Radford (1979) stated that the model can account for a variety of different recombination patterns, indicating different frequencies of symmetric versus asymmetric H-DNA formation and different degrees of association of H-DNA formation with outside marker recombination. The great strength of the model is that all of its components are more or less feasible from the point of view of molecular mechanism, but its versatility makes it difficult to thoroughly test. Fincham, Day and Radford (1979) gave examples of how the model is able to accommodate some published data. The rare two chromatid events in yeast could be due to a minimal amount of cross-strand migration. The very low frequency of conversion associated with crossing-over in certain *Neurospora* genes (such as *am*) could be due to a minimum amount of isomerization, necessary for crossing-over. The suggestion of Stadler and Towe (1971) based on their *Ascobolus immersus* results, that two-chromatid events always lead to crossing-over, while one-chromatid events never do, could well be true as a very special case. It should be added that Sang and Whitehouse (1979 a,b) and MacDonald and Whitehouse (1979) in their studies of the *buff* locus of *Sordaria brevicollis* observed a higher frequency of conversion-associated crossing-over

in 6:2 and 2:6 aberrant asci than in 5:3s or 3:5s. Paquette and Rossignol's (1978) data and analysis of H-DNA frequency, type and distribution at the *b2* locus in *Ascobolus immersus*, fit every testable prediction of the Aviemore model. However, the reservations of Lamb and Helmi (unpublished) over their analysis should be remembered.

A body of data exists which suggests that a modification of the initial step in the Aviemore model may well be necessary in certain circumstances, i.e. it seems that the strand initially broken can act as a recipient of a homologous DNA strand rather than as a donor. These data come from the studies of Gutz (1971b) on the M26 mutation in *Schizosaccharomyces pombe*, MacDonald and Whitehouse (1979) on the YS17 mutation in *Sordaria brevicollis*, and Angel et al.(1970) with pairwise crosses of *his-3* mutants in *Neurospora crassa*, where the cog^+ chromatid appeared to be the recipient of a nucleotide chain from the *cog* chromatid. Friedman and Sobell (1978) reported additional evidence for sites of initiation of recombination acting as recipients of genetic information in induced mitotic recombination in *Saccharomyces cerevisiae*. Helmi (1980) also suggested that in *Ascobolus immersus*, at ascospore pigmentation locus I, the chromatid carrying a closely linked conversion-promoting factor was itself the chromatid which tended to undergo conversion, implying that it acted as a recipient of a nucleotide chain after having undergone the initial breakage itself. Cunningham et al.(1980) proposed a model for the formation of joint molecules in which the molecule with the first gap acts as a recipient.

The symmetric H-DNA models of Holliday (1964) and Whitehouse and Hastings (1965) account for conversion associated crossovers in the following way. Breakage at the half chiasma of Holliday's model in one isomeric form would lead to recombinant outside markers, while breakage in the other form would maintain a parental configuration. On the Whitehouse model, the occurrence of denaturation on one side or both sides of the break point determines whether heteroduplex formation is accompanied by a crossover.

Theoretically there are five basic types of H-DNA formation possible at any one site: i) strictly asymmetric; ii) strictly symmetric with different mispairs in each hybrid chromatid (Holliday-type); iii) strictly symmetric with the same mispair in each hybrid chromatid, although different meioses may have either of two kinds of mispair (Whitehouse-type); iv) some asymmetric and some symmetric of the Holliday-type; v) some asymmetric and some symmetric of the Whitehouse-type. Quantitative studies of H-DNA models of gene conversion at a heterozygous site have been carried out (Emerson, 1966, 1969; Gutz, 1971a; Fincham et al. 1980) but only considering symmetric H-DNA models. Fincham et al. (1980) admit that only when there is independent evidence that heteroduplex is nearly always symmetrical, can the values obtained from their method of analysis be taken as good estimates of mismatch correction probabilities. Wickramaratne and Lamb (1978) gave detailed estimations of conversion parameters for both symmetric and asymmetric H-DNA models. Quantitative analysis of the five possible kinds of H-DNA formation at a heterozygous site has been undertaken by Lamb and Helmi (unpublished).

The central features of the models discussed so far have been H-DNA formation and mismatch repair. There is considerable justifying evidence for this. The involvement of hybridity has been reviewed by Davern (1971). Broker and Lehman (1971) were able to observe recombination intermediates by electron microscopy, using polynucleotide ligase-deficient and polymerase-deficient mutants of phage T4. Holliday structures have been clearly visualized in prokaryotes (Valenzuela and Inman, 1975; Potter and Dressler, 1976) and more recently in eukaryotic cells also, including yeast (Bell and Byers, 1979) and human HeLa cells infected with adenovirus type 2 (Wolgemuth and Hsu, 1980). In fungi, 5:3, 3:5 and aberrant 4:4 ratios constitute direct evidence for H-DNA formation, while 6:2 and 2:6 segregations do not, and it is only a simplifying assumption that they arise from the same H-DNA as do the odd-numbered ratios. Nevertheless, there are strong reasons for accepting that 6:2 and 2:6 ratios arise, or at least <u>can</u> arise, by correction of mismatches in H-DNA. Leblon and Rossignol (1973) analysed a two-point cross involving two mutants within the *b2* locus of *Ascobolus immersus*. When one of these two mutants was crossed to wild-type, it gave a high frequency of PMS (postmeiotic segregations: 5:3 + 3:5) and very few MS (meiotic segregations: 6:2 + 2:6). The other mutant in a similar cross gave a high frequency of MS and very few PMS. The result from the two-point cross showed that the frequency and pattern of conversion was very similar to the cross involving the mutant which gave high MS and very low PMS, as if this mutant had imposed its conversion pattern on the other.

The clear inference is that a high probability process triggered by the mismatch at one site tends to include the other site as well in the majority of instances, assuming that both sites must have been initially mismatching in the same heteroduplex. Similar results have been reported for the *arg-4* locus in yeast (Fogel et al, 1979) and confirmed for the *b2* locus of *Ascobolus immersus* (Rossignol and Haedens, 1978). This strongly suggests that 6:2 and 2:6 asci result from H-DNA formation and mismatch repair. Further evidence for this comes from the study of Kalogeropoulos and Rossignol (1980) who found that in three point crosses involving mutants of the *b2* locus of *Ascobolus*, two mismatches could undergo independent correction in the same hybrid DNA tract (giving 6:2 segregations) while the medial mismatch could remain uncorrected (giving a 5:3 segregation). Thus there is strong evidence that gene conversion proceeds by H-DNA formation and mismatch repair in fungi. In prokaryotic systems there is very considerable direct evidence for heteroduplex mismatch correction, in bacteriophages T4 (Berger and Pardoll, 1976), f1 (Enea et al. 1975), SPP1 (Spatz and Trautner, 1970) and λ (White and Fox, 1975). Some of this evidence comes from transfection experiments using heteroduplex phage. There is also evidence for heteroduplex mismatch connection in *Pneumococcus* (Roger, 1972, 1977) from a transformation experiment and *Bacillus subtilis* (Bresler et al. 1971).

The findings that one mutation can impose its conversion pattern on another (Leblon and Rossignol, 1973; Rossignol and Haedens, 1978; Fogel et al, 1979) cast doubt

on the conclusions of Leblon (1972 a,b) and Yu-Sun et al. (1977) that the pattern of conversion is dependent on the chemical nature of the mutation used in a cross. Even if only one known mutation is segregating in a cross, nearby cryptic heterozygosity could have the effect of a second scorable mutation (Lamb, 1975). Correction is not a strictly local event, but involves degradation and resynthesis of hundreds or even thousands of nucleotides (evidence reviewed by Fincham, Day and Radford, 1979). Transfection experiments performed by Wagner and Meselson (1976), using λ DNA and *E.coli* revealed that: i) for a given mismatch in a particular heteroduplex, the frequency of repair on one strand can differ considerably from that on the other; ii) repair involving two or more close sites in the same heteroduplex occurs much more often on the same strand than on opposite strands; iii) the pattern of repair suggests that repair events initiate at mismatches and propagate preferentially in the 5' → 3' direction, extending an average distance of 3000 nucleotides. Spatz and Trautner (1970) and Trautner et al. (1973) concluded that in phage SPP1 the specificity of conversion is influenced by the neighbouring base sequence, and that the preference for which strand is cut for correction depends on the position of the mutation in the linkage map.

The term "parity" in fungal genetics means equal, or approximately equal, frequencies of conversion from + to *m* and vice-versa, while "disparity" refers to a bias in one direction. The general rule in yeast was thought to be parity (Fogel and Mortimer, 1969; Fogel et al. 1971),

although some moderately pronounced departures from this have since been reported (Fogel et al. 1979). In other ascomycetes such as *Ascobolus* and *Sordaria*, disparity is the general rule (Leblon, 1972 a,b; Leblon and Rossignol, 1973; Rossignol, Paquette and Nicolas, 1978; Yu-Sun et al. 1977; Lamb, 1981; Lamb and Helmi, 1982). Fincham, Day and Radford (1979) attributed this difference between yeast, and *Ascobolus* and *Sordaria* to the properties of the hypothetical endonuclease which recognizes mismatches. That of yeast would appear to recognize some kinds of mismatch much more efficiently than others because of the very different frequencies of postmeiotic segregation shown by different mutants, but, having recognized a mismatch, it seems almost indiscriminating about which strand it cuts. In *Sordaria* and *Ascobolus*, on the other hand, it would seem that one strand is often corrected preferentially. Catcheside (1977) explained the difference between the yeast data and *Ascobolus* on the basis of possible differences in H-DNA formation rather than repair. Emerson (1966) reported an interesting case in *Ascobolus immersus* in which mutation *w*62 in crosses to wild-type showed a significant preference for conversion to wild-type in MS octads (6:2 > 2:6) and a significant preference in the reverse direction in PMS octads (3:5 > 5:3). Emerson suggested that this difference might be caused by a different kind of mispairing in each chromatid, as Holliday's model predicts. The "ADEL" correction model suggested by Leblon (1974) to explain the conversion pattern of AD-dition and DEL-etion mutations fails to explain the

yeast data of Fink and Styles (1974) who observed approximate parity in conversion direction for a large deletion mutant in the *his 4* gene or the data of Lawrence et al. (1975) who observed the same phenomenon in the iso-1-cytochrome *c* gene of yeast. Perhaps more startling is the work of Hinnen, Hicks and Fink (unpublished; cited by Stahl, 1978) in which the ColE1 plasmid was incorporated into a yeast chromosome and this strain mated with a normal strain and the resulting diploid sent through meiosis. Tetrads were obtained showing conversion of the 8000 nucleotide plasmid, and this conversion showed parity. Radding (1978b) proposed an alternative model for the conversion of additions and deletions. This assumes that after, say, an addition becomes included in a heteroduplex region, DNA synthesis occurs to make the resulting single stranded loop double stranded. The duplex loop and attached heteroduplex region form the equivalent of a Holliday structure and should be susceptible to the same kind of resolution that has been suggested for the Holliday structure. Such resolution would, with equal likelihood, excise the double stranded loop (conversion to + in this case) or integrate it into the chromosome (conversion to *m*). Radding (1978b) goes on to suggest two correction mechanisms that contribute to gene conversion. One mechanism works on small distortions of the helix and resembles excision repair, while the other mechanism works on loops created by large additions or deletions, and resembles reciprocal crossing-over. The hypothesis predicts that in organisms which show disparity in the conversion of mismatched base pairs or small additions/deletions, a tendency towards parity should

correlate positively with the size of the additions or deletions (it is worth mentioning that a very similar mechanism has been proposed by Murray and Holliday (1979) for the splicing-out of introns in the processing of precursor mRNA to mature mRNA, involving "splicer RNA" hybridizing to precursor mRNA, so creating a Holliday-type structure).

Leblon and Rossignol (1979) concluded from their work on the interaction between closely linked allelic frame-shift mutant sites during recombination in *Ascobolus immersus* that: i) the pattern of variation of the disparity values agrees with an equal influence of the associated heterozygous sites in determining the direction of correction; ii) the excision process needed for heteroduplex correction proceeds in both directions from the one stranded nick provoked by the mismatch, i.e. there is no important excision polarity; iii) recognition of the mispair prior to nicking is not part of a polar process. It seems that the *Ascobolus* conversion data obtained by Leblon and his co-workers is a special case in that it gives the mutation under study complete control over its own conversion behaviour and when two mutations are involved, each has an equal share in controlling their co-conversion. Fogel et al.(1979) obtained results from yeast concerning specific *ochre/amber* mismatches at three different sites in the genome. They concluded that the conversion frequencies and correction patterns were characteristics of the location in the genome and not the mismatch itself, adding that the specific region which includes the heterozygosity is of primary importance in determining the parameters of conver-

sion. Gutz (1971b) reported that mutation M26 in the *adenine-6* gene of *Schizosaccharomyces pombe* is able to impress its conversion disparity on other mutations of the gene in two point crosses, whether performed in *cis* or *trans*. This result could be explained if M26 (or the potential mispair) acts as a recognition site for an endonuclease and that mismatch repair takes place primarily on the mutant chain. The other mutations in the gene could not be recognized so readily by the endonuclease. Lamb and Ghikas (1979) studied the conversion properties of 29 induced (by UV, NG and ICR-170) and 9 spontaneous ascospore colour mutations. Each mutant was crossed to three types of derived wild-type strain. Single mutants often gave very different conversion results in the three types of crosses, with changes in any or all of the following: total conversion frequency; percentage of convertants showing conversion to wild-type; percentage of convertants showing postmeiotic segregation. Their data tend to disprove the hypothesis that the nature of a mutation is the only determinant of its conversion pattern and suggest that heterozygosity of nearby cryptic mutations, genetic control factors and the type of H-DNA formed at the site under study, have prominent effects on the conversion of the site in question. As a cautionary note, it should be remembered that apparent postmeiotic segregations are often phenocopies. Gajewski et al. (1968) reported that for certain mutations of locus '*46*' in *Ascobolus immersus*, up to 30% of apparent 5:3 and 3:5 postmeiotic segregations were, in fact, phenocopies and

genotypically 4:4s. Variation in this phenocopy frequency particularly when conversion is infrequent, could lead to apparent changes in conversion pattern and even frequency. Therefore, at present it does not seem possible to predict how a particular mismatch will be corrected. It is evident that many factors are involved, including more than just the physico-chemical nature of the mismatch in question. There are, of course, models of recombination in which gene conversion does not involve repair mechanisms and these should not be overlooked.

Stahl (1969) proposed that conversion and recombination occur in "sex circles" or localized regions of replication. Two exchanges within the replicated regions may result in gene conversion with or without associated outside marker exchange. Limited segments of heteroduplex DNA are postulated to occur at the exchange points and would not necessarily include the heterozygous site. Stahl (1979) suggested that no single model will provide a universal explanation for crossing-over, gene conversion and the correlation between these two events, i.e. cannot accommodate features of both yeast and other fungal data. The conversion parity of large deletions (Fink and Styles, 1974; Lawrence et al. 1975) and integrated plasmids in yeast might seem to tell in Stahl's favour, but the correction process suggested by Radding (1978b) seems to remove the difficulties encountered on heteroduplex repair models. The Meselson-Radding model (1975) can also account for the largely asymmetric H-DNA reported in yeast, given certain special conditions, but difficulties still remain. Orr-Weaver et al. (1981) reported the occurrence of double

stranded break repair during the integration of a gapped-linear plasmid into the yeast genome which already contained an integrated normal plasmid. A conversion-like process seems to have taken place by homoduplex transfer rather than heteroduplex formation plus mismatch repair. Another recombination model which does not involve mismatch repair mechanisms is that of Paszewski (1970). Holliday and Whitehouse (1970) list objections to such models on the basis that they do not explain a large body of genetic data relating to conversion polarity, conversion disparity, postmeiotic segregation frequency and map expansion.

The evolution of recombination models was reviewed by Hotchkiss (1974a) while Catcheside (1977) reviewed and discussed several theories and aspects of such models.

The biochemistry and molecular basis of H-DNA formation and mismatch repair

As outlined in the previous section, there are common features between prokaryotes and eukaryotes with respect to their recombination in which H-DNA formation and the correction of mismatched base pairs are assumed to be the major components of the process by most authors. In this section the proteins, enzymes, biophysical and biochemical reactions, which have been identified or proposed to be involved in the recombination process are briefly described.

Homologous pairing of duplexes

Potter and Dressler (1980) described an enzyme from *Escherichia coli*, which they termed DNA synaptase, which

fuses double-stranded DNA molecules at regions of homology. By bringing two plasmid genomes together in this way, DNA synaptase has the property expected for an enzyme which participates in an early step in genetic recombination. However, the synaptase can be recovered from RecA$^-$ (recombination-deficient) cells and so the enzyme may play a role in another cellular process which involves genome fusion, i.e. the recombinational repair of damaged DNA.

Strand separation

Helix destabilizing proteins, such as gene 32 protein discovered by Alberts and Frey (1970) in phage T4, are capable of destabilizing double-stranded DNA and thus promoting its denaturation, and are implicated in replication, recombination and repair (Broker and Doermann, 1975). A protein in zygotene and pachytene nuclei of *Lilium, Rattus, Bos and Homo*, with properties similar to those of the gene 32 protein was found by Hotta and Stern (1971 a,b). It is assumed that these proteins play a structural part in separating the chains of duplex DNA, by binding to the single chains and holding the otherwise highly folded chains in an extended conformation, and thus facilitate matching of complementary chains.

A second mechanism for separating strands of DNA uses the energy of ATP. Examples of such *DNA-dependent ATPases* have been found in *Haemophilus influenzae* and *E.coli* (references cited in Radding, 1978a). These enzymes act exonucleolytically on double- and single-stranded DNA.

Hoffman-Berling and his colleagues (cited in Radding, 1978a) discovered a DNA-dependent ATPase in *E.coli* which binds to single strands but lacks any nucleolytic activity and is capable of denaturing double-stranded DNA adjacent to a single-stranded region. Cassuto and Radding (1971) showed that in vitro λ exonuclease and its associated protein digest 5' ends of duplex DNA and act at the site where a duplicate length of 3'-terminal chain leaves a stretch of duplex DNA. The enzyme excises nucleotides from the end of the paired chain and allows the unpaired chain to take its place; the excision stops immediately the 3' terminus has fallen into place. Scott et al.(1977) discovered that the *E.coli rep* protein, which is required for the replication of phages ϕ X174 and P2, is a DNA-dependent ATPase, which is capable of strand separation under appropriate conditions.

Endonucleolytic nicking

Endonucleases that specifically nick a DNA molecule prior to H-DNA formation have been postulated in most recombination models. They may recognise specific linear sequences (Holliday, 1968), Gierer loops (Sobell, 1972), a sequence with two-fold symmetry (Sobell, 1975) or a palindromic sequence (Wagner and Radman, 1975). However, Radding (1978a) suggested that the breakage which produces a donor strand is random, but the cutting of a recipient molecule is determined by the structure of the first intermediate formed by homologous pairing. The basic concept is that the DNA strand incision in undamaged

DNA can occur as a response to the invasion of the duplex by single-strands from a homologous DNA molecule. Findings in *E.coli* using homologous and non-homologous DNA as substrates provide support for this concept and attributes the cutting in *trans* of homologous DNA to the RecA protein (Cassuto et al. 1978). Similar endonuclease activity has been reported in *Schizophyllum commune* (Koltin and Shneyour, 1979). Wiegand et al.(1977) showed that the S_1 nuclease specifically recognizes some feature of a D loop in addition to single-strandedness; the D loop is a result of the uptake of the donor single strand by the recipient double helix. Holloman et al.(1975) showed that in vitro the D loop structure is formed by a spontaneous taking up of homologous single-stranded fragments by superhelical DNA of ϕ X174 (RFI). There is little evidence for polarity in recombination in prokaryotes and this probably means that, in general, bacterial and viral recombination can be initiated at any point in the DNA rather than a specific one. Altman and Meselson (1970) purified an endonuclease from phage T4 which induces random single-chain cuts in the DNA.

The role of *E.coli* RecA protein in synapsis and heteroduplex formation

The RecA protein of *E.coli* is essential for genetic recombination in that organism and is a specific protease which regulates an inducible pathway of DNA repair and remarkably is also a DNA-dependent ATPase which promotes

homologous pairing of DNA molecules in vitro. Rec A protein pairs a partially single-stranded molecule and an intact duplex molecule of DNA in two experimentally distinguishable steps: synapsis, which puts molecules in homologous register by a side-by-side interaction of a single-strand with duplex DNA; and strand transfer, which requires a free end and produces long heteroduplex joints by a concerted and polar reaction (Radding, 1981). Single-stranded DNA governs all the activities of the Rec A protein. The effect of single-stranded DNA in stimulating the binding and partial unwinding of duplex DNA by Rec A protein occurs whether the single-stranded DNA is homologous or not, which is consistent with the mechanism designed to search for homology proposed by Cunningham et al. (1979). The amount of single-stranded DNA determines the stoichiometric requirement for purified Rec A protein in the pairing reaction (Shibata et al. 1979) and the binding to single-stranded DNA appears to be co-operative (West et al. 1980; McEntee et al. in press). Less Rec A protein is required in the presence of helix destabilizing proteins, which also bind to single-stranded DNA (McEntee et al. 1980; Shibata et al. 1980). The kinetics of the pairing reaction resemble classical Michaelis-Menton kinetics (Radding et al. 1980) showing that the reaction proceeds via a rate limiting enzyme-substrate complex, unlike the second order kinetics of the pairing of complementary single-strands by helix destabilizing proteins.

Rec A protein will make stable joint molecules from numerous structural variants of DNA, provided that one molecule is at least partially single-stranded and that a

free end exists somewhere in one of the two molecules (Cunningham et al. 1980; DasGupta et al. 1980; West et al. 1981a). Among the products of such reactions are the D loops which are of great importance in the assumed recombination process. It should be stressed that Rec A pairing of two wholly duplex molecules has not been reported.

The pairing of circular single-stranded DNA with linear duplex DNA (DasGupta et al. 1980) has provided an ideal system for studying strand transfer and heteroduplex formation. The circular viral strand of phage DNA displaces the 5' end of the viral strand from linear duplex DNA by a mechanism that is both concerted and polar. The further growth of these heteroduplex joints, which was studied in the presence of the single-strand binding protein of *E.coli*, requires the continued presence of active Rec A protein (Cox and Lehman, 1981). These observations indicate that Rec A protein plays an active role in enlarging heteroduplex joints. West et al.(1981b) and DasGupta et al. (1981) have shown that Rec A protein can cause not only a strand transfer, but also a reciprocal exchange of strands by a reaction that is also concerted. DasGupta et al, (1981) also showed that when Rec A protein pairs linear duplex molecules with molecules that have single-stranded ends, the junctions have the electron microscopic appearance associated with the Holliday structure. These observations support the view that Rec A protein makes stable, joint molecules only by transferring strands and not by the side-by-side pairing of duplex regions suggested by West et al. (1981b).

Although the recognition of sequence homology and the formation of heteroduplex joints are distinguishable experimentally, they can be related in theory to a single mechanism by supposing that Rec A protein is a special type of DNA helicase (Kuhn et al. 1978). According to this hypothesis, Rec A protein begins to unwind duplex DNA in response to any single strand (Cunningham et al. 1979) but completes its action and thereby makes a heteroduplex joint only when a homologous strand is aligned with complementary sequences in register, and when a free end makes the process topologically feasible. It is remarkable that a single small protein of MW 37,800 (Sancar et al. 1980; Horii et al. 1980) can accomplish both of the central steps of recombination, namely, synapsis and heteroduplex formation with such economy.

Strand displacement

Strand displacement by new DNA synthesis is an integral feature of replication forks, in which case the displaced strand itself soon becomes the template for new synthesis (Kornberg, 1974). Fujimura and Roop (1976) showed that phage T5 DNA polymerase promotes strand displacement. Kolodner and Richardson (1977) found that the phage T7 gene 4 protein, which enables T7 DNA polymerase to start at a nick, is a DNA-dependent nucleoside 5'-triphosphatase. They postulated that gene 4 protein promotes strand displacement by unwinding DNA. Nossal (1974) found that T4 gene 32 protein enabled the T4 polymerase to start new DNA synthesis at a nick, presumably because 32 protein promoted strand displacement. Hotta, Ito and Stern (1966)

found from studies of the uptake of ^{32}P that about 0.3% of the total DNA of *Lilium longiflorum* and *Trillium erectum* was synthesized at the zygotene and pachytene stages of meiosis in the anthers and that this DNA synthesis was necessary for chromosome pairing and chiasma formation. Hotta, Parchman and Stern (1968) showed that protein synthesis also took place at this stage of meiosis, and this was necessary for the DNA synthesis to occur.

Strand assimilation

Radding (1978a) suggested that DNA polymerase I of *E.coli* promotes all the requisite functions of strand transfer: synthesis, strand displacement, and 5'→3' exonucleolytic digestion. Gellert et al.(1976) suggested another mechanism that might promote incorporation of a single strand into a helix. Acting on topologically constrained DNA, the enzyme DNA gyrase, which they discovered, should be able to promote extensive uptake of a single strand by increasing the free energy of the superhelix.

Branch migration

Lee, Davis and Davidson (1970) and Meselson (1972) suggested that branch migration is driven by random diffusion, as it entails no net change in the number of paired bases and hence requires little energy. Thompson, Sussman and Warner (personal communication cited by Radding, 1978a) have found that double stranded branch migration in vitro is blocked by gross non-homologies.

Isomerization

There are two types of hypothetical molecular isomerizations. The first kind of isomerization can proceed by rotation of a pair of homologous arms 180° about an axis that is between and parallel to them. This type is possible for structures with a single bridging strand. The second kind of isomerization involves unstacking the base pairs immediately adjacent to the crossed strands, opening out the complex to form a structure in which there are four symmetrically related arms, and twisting the arms so that new pairs are brought into alignment. The two types of isomerization, after the introduction of the idea itself by Sigal and Alberts (1972), have been discussed with respect to their stereochemistry and the possibility that this reaction might occur within the time available for genetic recombination (Sobell, 1974; Meselson and Radding, 1975). The result of isomerization is that the flanking arms would acquire the recombinant configuration

Joining the free ends and sealing the gaps

Weiss and Richardson (1967) purified an enzyme, DNA ligase, from *E.coli* infected with T4. This enzyme catalyses the covalent joining of two segments of a broken chain of a DNA duplex to give a phosphodiester bond, and is coded for by gene 30 in T4 (Fareed and Richardson, 1967). Howell and Stern (1971) demonstrated the presence in *Lilium* pollen-mother cells at zygotene and pachytene, but not at other times, of three enzymes, two of which are: a polynucleotide kinase which phosphorylates 5'-hydroxyl

ends and a polynucleotide ligase which joins adjacent 5'-phosphoryl and 3'-hydroxyl ends if the other chain is intact. They suggested that a non-specific phosphatase dephosphorylates the 3' ends before the ligase can act.

Other theories relating to H-DNA formation

In the above sections of this review, single-strand breaks have been assumed to be required to permit formation of fully coiled heteroduplexes. However, Wilson (1979) has proposed a simple nick-free solution to the topological winding problem. He suggests that homologous duplexes associated by their minor groove surfaces can switch strand pairing to form reciprocal heteroduplexes that coil together into a compact, four-stranded helix throughout the region of pairing. Model building has shown that this fused heteroduplex structure is plausible, being composed entirely of right-hand primary helices with Watson-Crick base pairing throughout. No nicking is involved in this process at all. Kirkegaard and Wang (1978) came to similar conclusions. If both partners in H-DNA formation are covalently closed circular duplex molecules and are negatively superhelically coiled, intermolecular intertwining of a segment of one strand of one molecule to the complementary strand of another molecule, or the formation of two pairs of such intermolecular duplexes, is thermodynamically favourable under certain conditions (Hsieh and Wang, 1975). In this reaction, *E.coli* DNA topoisomerase I (*E.coli* ω protein) may be implicated (Kirkegaard and Wang, 1978).

Mismatch repair

The correction process consists of three steps: i) an endonuclease is supposed to make a single-strand break adjacent to the site of the mismatch; ii) the same nuclease (an endonuclease with exonuclease properties), or a different one (an exonuclease), must digest away a tract of the single-strand including the mismatch and extending some distance beyond it; iii) the gap so created must be filled by repair synthesis and the single-strand ends joined. This mechanism was originally suggested by Holliday (1964) and it has been widely accepted, perhaps because of the close similarity between the suggested repair mechanism and the well studied and proven repair system which recognizes and removes pyrimidine dimers resulting from UV-irradiation of DNA (Braun et al. 1976). The weakness of the mismatch correction hypothesis has been the lack of a direct in vitro demonstration of the enzymes involved. However, enzymes of exonucleolytic and repair synthesis activities (for steps ii) and iii) in the mechanism) have been found, but the search for enzyme(s) responsible for mismatch recognition (step i) is still in progress. Bartok et al.(1974) showed that a nuclease isolated from *Neurospora* recognizes and degrades single-stranded regions in deletion/wild-type heteroduplexes. A similar nuclease (S_1) was isolated from *Aspergillus* by Ando (1966); this enzyme was shown to be specific to mismatch-containing substrates (Shenk et al. 1975). Ahmad et al.(1975) discovered a nuclease that preferred a DNA substrate containing a single purine-pyrimidine mismatch, which presumably causes a very minor distortion in the DNA, rather than an identical substrate

without the mismatch. This nuclease, which was isolated from *Ustilago maydis*, showed greater affinity for deletion/wild-type non-pairs than for one-point mispairs.

The Synaptonemal complex, recombination nodules and recombination

Since the discovery of the Synaptonemal complex (SC) by Moses (1956) further studies have shown that meiotic crossing-over usually, but not exclusively, takes place in the presence of the SC. Models of crossing-over now attempt to involve the SC in the process (Westergaard and von Wettstein, 1972; Holliday, 1977; Moens, 1968). The expectation is that the structure and behaviour of the SC and associated structures such as recombination nodules (Zickler, 1973; Carpenter, 1979) may provide a clue to the timing and distribution of crossovers and the relationship between crossovers and conversions along the bivalent at meiotic prophase. The function of the SC is generally thought to consist of holding the homologues in register throughout the length of the bivalent containing it, so that crossover sites can then somehow be established along it, with their distribution not being controlled by the complex itself. However, Maguire (1977) questioned the general rule that pairing precedes crossing-over, and the data of Olson and Zimmermann (1978a) suggest that synaptonemal complexes are not an absolute requirement for meiotic recombination in *Saccharomyces cerevisiae*. They showed that gene conversion and a limited amount of crossing-over could be completed as early as DNA synthesis and before the formation of synaptonemal complexes.

However, crossing-over of linked and unlinked markers remained at a low level until SCs were formed. Meiosis in the fission yeast *Schizosaccharomyces pombe* appears to be asynaptic (Olson et al. 1978), while SCs have not been detected in four cell types tested from *Ustilago maydis* (Fletcher, 1981). It is well to remember that meiosis does not only bring about interchromosomal recombination but also the orderly separation of homologous chromosomes. Consequently, it is not clear whether SCs are involved in meiotic recombination as well as meiotic segregation (Maguire, 1977; Olson and Zimmermann, 1978b). Mitotic recombination takes place in cells where no separation of homologous chromosomes occurs and, indeed, mitotic recombination has been shown to occur in the absence of SCs in yeast (Olson and Zimmermann, 1978b) and *Ustilago maydis* Holliday, 1971) while SCs and recombination nodules have been reported in a meiotic race of *Meloidogyne hapla* but are absent in a mitotic race (Goldstein and Triantaphyllou, 1978).

Recombination nodules were first reported in ascomycete fungi by Zickler (1973) and have since been observed in a wide variety of organisms including algae, nematodes, rodents, lemurs, lemuroids, man and male *Bombyx* (references cited in Carpenter, 1979). Carpenter (1979) observed two morphologically distinct types of nodule in *Drosophila melanogaster* - spherical and ellipsoidal. She suggested that the spherical nodules correlate with reciprocal events (which may be accompanied by conversion at the site of the event), whereas ellipsoidal nodules correlate with

non-reciprocal events (conversion without exchange of flanking markers). This is supported by the fact that spherical-spherical and spherical-ellipsoidal nodule pairs show interference in their distribution, while ellipsoidal nodules exhibit little, if any interference. Because individual loci show highly significant departures from 50% conversion-associated crossing-over, Carpenter suggested that either the possibility of isomerization on a Meselson-Radding type model differs according to locus, or there are two recombination processes which have different spatial distributions along the chromosomes. A distinction between two types of recombination was indeed suggested from genetical data by Fincham (1970), Fogel and Mortimer (1971), Stadler and Towe (1971) and Stadler (1973). Stadler and Towe (1971) suggested that, on the basis of their results from *Ascobolus*, two-chromatid events always lead to crossing-over, while one-chromatid events never do. The *Sordaria* data of MacDonald and Whitehouse (1979) led them to conclude that modifications of the primary event (assumed to be conversion) might affect the likelihood of the second one (the crossover). This conclusion, if true, could provide a solution to the problem.

The type of arrangement of the chromatids at synapsis which was proposed by Moens (1974), where two non-sister chromatids are adjacent at any point, allows intimate pairing for crossing-over and conversion only along the generally paired sections of these two non-sister chromatids. The other two non-sister chromatids cannot pair at the same point. Lamb (1977) described this arrangement as "linear" and it cannot account for the occurrence of the

three or four chromatid events described in *Ascobolus* by Lamb and Wickramaratne (1973). Wider ratio octads have also been found in *Neurospora* (Case and Giles, 1958), *Sordaria fimicola* (Kitani and Whitehouse, 1974) and *Sordaria brevicollis* (Yu-Sun et al. 1977). These findings of genuine wider ratio octads from conversion, conflict with models of SC structure requiring that only two of the four chromatids pair intimately at one point, such as the model of von Wettstein (1971). Lamb (1977), considering fungal conversion data, has suggested a "compact" arrangement for the chromatids at meiosis, so that all four chromatids can intimately pair. He also estimated the average length of each segment of H-DNA to be at least one gene or more, basing this on co-conversion data. This finding led him to suggest that DNA pairing spreads beyond the small segments aligned at the central component of the SC, rather than being restricted to the central component as suggested by Stern and Hotta (1974) and Stern et al.(1975).

Electron micrograph data quoted by Stern et al.(1975) showed that DNA strands parallel to the lateral component of the SC in *Neurospora*, *Drosophila* and *Zea mays* would represent only 0.3%, 0.2% and 0.015% respectively of their genomic DNA. Gene conversion studies showed that most sites in a number of fungi are included in H-DNA with frequencies of 0.05% to 4%, with these values being underestimates, since not all H-DNA formation gives recognizable conversion. To account for this, Lamb (1977) postulated that there must be either highly selective incorporation of genetic regions into the SC, or detailed DNA matching

between non-sister chromatids must occur largely outside the SC, although it could be initiated near the central component. An experimental analysis of bivalent interlocking in spermatocytes of the newt *Triturus vulgaris* lead Callan and Pearce (1979) to independently suggest that the recombinational process which results in chiasma formation probably takes place outside the SC. These ideas agree well with the body of data which suggests that mitotic recombination (sometimes induced at meiotic levels (see Holliday, 1971))occurs in the absence of SCs and that low levels of meiotic recombination are also possible without SCs. Maguire (1977) may be correct in that the SC is somehow involved in meiotic segregation rather than having the promotion of meiotic recombination as its primary function.

The Genetic controls of recombination

That frequencies of recombination between linked genes are alterable by genotype has been known almost since the discovery of linkage itself. Gowen (1919) was the first to observe that recombination frequencies between pairs of genes varied with the genetic background in *Drosophila melanogaster*. However, an even earlier example of the genetic control of recombination was the classic observation by Morgan (1912) that crossing-over is absent in *Drosophila melanogaster* males. The literature now abounds with reports of the effects of genetic background on chiasma frequency, genetic recombination and gene conversion.

It is evident that in the recombination process there will be enzymes which catalyse the events involved and these enzymes will be specific to particular substrates. Attempts have been made to dissect the recombination process into different components controlled by different genes being correlated with specific enzymic or other molecular functions. There have been two approaches to this: use has been made of mutants defective in some aspect of recombination, while the second approach involves polymorphic genetic variants occurring in natural populations. From the two approaches, genetic factors having quantitative effects on crossing-over and gene conversion have been found in several organisms. These factors can be broadly classified into two groups according to their magnitude of effect and the extent of the genome affected and were termed fine and coarse controls by Simchen and Stamberg (1969). Perhaps a better classification is into genes of general and local effects (Catcheside, 1977). Genes of general effect will be reviewed first.

Genes of general effect

Clark (1973) reviewed several cases of mutants defective in recombination in *E.coli* and other bacteria. The mutants were isolated in the first place as UV-sensitive but also turned out to be defective in recombination. These mutants are usually recessive, virtually eliminate recombination and cause sterility in sexual reproduction. This UV-sensitivity and recombination deficiency relationship suggests that there are common steps between recombination and the repair of UV-induced damage, perhaps excision

repair. Evidence exists for a UV-inducible recombination system in yeast (Fabre and Roman, 1977) and *Ustilago maydis* (Holliday, 1975). Kern and Zimmermann (1978) found in yeast that excision repair of pyrimidine dimers is not required for spontaneous mitotic gene conversion, but the lack of excision reduces UV-induced gene conversion. Their data suggested that the error prone repair pathway represented by *rad-6* competes strongly with the repair activities responsible for mitotic recombination. Holliday (1965), Holliday et al.(1976) and Unrau (1975) succeeded in isolating and characterizing different UV-sensitive mutants in *Ustilago maydis*. The mutant *rec-2* is defective in meiosis and hence sterile when present in both parents of a cross and it shows greatly reduced frequencies of mitotic crossing-over. *rec-1* is a recessive mutation which does not prevent meiosis or mitotic crossing-over, but does confer a deficiency in repair, showing enhanced spontaneous mutation rates but reduced mutagenesis after UV-irradiation. This mutant showed a tendency to lose chromosome segments during meiosis, presumably as a result of unrepaired chromosome breakage. The double mutant *rec-1 rec-2* is, when homozygous, totally defective in mitotic intragenic recombination. The third mutant, *rec-3*, is defective in the excision repair of pyrimidine dimers and it shows an increased frequency of UV-induced gene conversion.

The approach of looking for UV-sensitive mutants has been applied to several other fungi, including *Saccharomyces cerevisiae* (Cox and Parry, 1968) and *Neurospora* (Schroeder,

1970 a,b). A UV-sensitive mutation, *rad1*, was described in *Coprinus cinereus* by Amirkhanian (1979). This mutation, in the homozygous condition, increased spontaneous meiotic recombination by 50% and UV-induced intergenic recombination 5-fold. In *Aspergillus nidulans* UV-sensitive mutations either increasing or decreasing the frequency of spontaneous mitotic crossing-over in homozygous UV-sensitive diploids, have been reported by several authors (Fortuin, 1971 a,b; Jansen, 1970; Shanfield and Käfer, 1969). In *Schizosaccharomyces pombe*, the effect of *rad* alleles of 18 unlinked genes on spontaneous meiotic, mitotic and UV-induced mitotic recombination for a given interval, was measured by Grossenbacher-Grunder and Thuriaux (1981). Only one mutation conferring reduced UV-induced recombination was found. The role of radiation sensitive genes in yeast was reviewed by Game et al.(1980). They concluded that the normal functions specified by *RAD-50*, *-52* and *-57* are not essential for either the initial or terminal steps in meiosis, but are required for successful recombination. The *rad-6* mutant may be recombination defective, or it may fail to progress past DNA replication in the overall sequence leading to the formation and recovery of meiotic recombinants. Prakash et al.(1980) found that *rad-52* diploids undergo premeiotic DNA synthesis and sporulation, but the spores are inviable. No intragenic recombination occurred at two different sites, nor any gene-centromere recombination. They concluded that the *RAD-52* gene function is required for spontaneous mitotic recombination, UV and γ ray induced mitotic recombination and meiotic recombination.

Another approach to the isolation of recombination-defective mutants has been to look for those defective in meiosis, as evidenced by failure to form viable meiotic spores. Bresch et al.(1968) made a large collection of *Schizosaccharomyces pombe* mutants which were unable to sporulate and classified them according to which stage of the sexual cycle they were blocked at, i.e. cell fusion, the first division of meiosis, the second division of meiosis and the delimitation of the ascospores. Esposito and Esposito (1969) performed essentially the same exercise with *Saccharomyces cerevisiae* and isolated numerous mutants unable to complete meiosis. Some mutants blocked in meiosis were defective in recombination. However, three mutants, *spo1* *spo2* and *spo3* were fully competent in recombination, even though incapable of meiosis.

If effects on recombination as such are desired, it is better to look for them directly, rather than through correlated effects on radiation sensitivity or meiosis. Roth and Fogel (1974; 1971; 1976) devised a suitable system to detect this and five mutants were isolated. Three proved, when homozygous, to block gene conversion even though DNA replication prior to meiosis took place apparently normally. The two other mutants were clearly defective in pre-meiotic DNA synthesis. All of the mutants were severely defective with respect to ascospore formation. More recently, Roth (1976) has obtained temperature-conditional mutants blocked in either pre-meiotic DNA synthesis or gene conversion.

An even more direct approach is to select directly for deficiency with respect to an enzyme which may be nec-

essary for recombination. Using this method, Badman (1972) obtained mutations mapping at two loosely linked loci *nuc-1* and *nuc-2* in *Ustilago maydis*. *nuc-1 nuc-2* double mutants, when homozygous, completely block intragenic mitotic recombination without affecting crossing-over between more distant markers. The combined effects of the two genes are necessary for this.

An interesting general effect on recombination has been reported in *Saccharomyces cerevisiae* (Malone et al. 1980) and *Schizosaccharomyces pombe* (Minet et al. 1980). In both species a much smaller mitotic/meiotic recombination ratio is observed for genes unlinked to the centromere than for genes closer to it. Minet et al.(1980) proposed that: i) pairing between homologous chromosomes is the rate-limiting factor in mitotic recombination; ii) mitotic pairing of homologous chromosomes is frequent, possibly occurring in all vegetative cells but is usually restricted to the centromere region; iii) pairing throughout the whole genome is restricted to a small sub-population among the mitotically dividing cells. This is not strictly a genetic effect but does seem to be general in the organisms described.

Genes of local effect

Compared with prokaryotes, eukaryotes show much less recombination per unit of DNA in the haploid set. This reduction could mean that eukaryotes have acquired controls which considerably reduce, by several orders of magnitude, the probability of recombination. The purposes of control

are probably several, but a basic purpose is probably the economy of metabolism effected by reducing the action of nucleases.

The genes in this category affect recombination at specific regions in the genome and may be linked or unlinked to the target region(s). Because of the specificity of such genes with respect to their action on recombination, it is possible that they are involved in regional intimate pairing, comprise specific recognition sites for nucleases or, indeed, code for the nucleases specific to certain recognition sites. This study concerns such local genetic controls of recombination and gene conversion, so a survey of the main types of control systems elucidated is especially relevant, concentrating mainly on fungal data.

1. The *rec, con* and *cog* genes of *Neurospora crassa*. The evidence for local controls of recombination in *Neurospora crassa*, accumulated over many years by Catcheside and his collaborators, was summarized by Catcheside (1977). The system has three main elements. First are the dominant repressors of recombination *rec-1, rec-2* and *rec-3* which are three distinct loci. Two alleles (rec^{+}/rec^{-}) are known at each of the *rec-1* and *rec-2* loci, while three alleles are known at the *rec-3* locus. The alleles at each *rec* locus are specific to a few known targets, which do not usually map close to the *rec* locus and are commonly on different chromosomes. The *rec* loci control allelic recombination at their targets while *rec-1* and *rec-2* also control crossing-over in the neighbourhood of the target.

Catcheside (1979) reported evidence that *rec-2*$^+$ suppresses the production of both wild-type and double mutant recombinants from a heteroallelic cross. Polarity of conversion in the target regions is also affected by the three *rec* loci. The second element in the control system comprises the *con-1*, *con-2* and *con-3* genes which are the postulated recognition sites for the *rec-1*, *rec-2* and *rec-3* gene products respectively. No individual *con* locus has been located by examination of the genetics of a variant gene. The final components of the control system are the *cog* genes, of which only one has so far been identified. It is closely linked to its target region and interacts with *rec-2*. *cog+* is dominant and its enhancing effect on recombination - both intragenic and intergenic - is shown only in the absence of *rec-2*$^+$. The postulated function of *cog* is that it is the locus at which the formation of H-DNA is initiated after synapsis. Catcheside (1979) discussed alternative hypotheses to explain the *rec/con/cog* system, especially the dominance of *rec-2*$^+$.

Genes with properties similar to *cog*$^+$*/cog* were reported in *Neurospora sitophila* by Fincham (1951) and Newcombe and Threlkeld (1972). The Chi element in phage λ also shows remarkable similarity to *cog*. Chi$^+$ is dominant over Chi^0 (Lam et al. 1974) and Chi can act across a heterology (Stahl and Stahl, 1975). Chi has been shown to act directionally (Stahl et al. 1980) and its activity is orientation-dependent (Yagil et al. 1980). Chi-stimulated recombination is specific to the recBC pathway of *E.coli* (Gillen and Clark, 1974; Stahl and Stahl, 1977). Most interestingly, the Chi sequence has been detected in mouse

immunoglobulin genes at a frequency about 10-fold higher than might be randomly expected for an octameric sequence such as Chi (Kenter and Birshtein, 1981). This preliminary observation suggests a role for Chi in the generation of antibody diversity via intrachromosomal recombination.

2. The *rec* genes in *Schizophyllum commune*. In this organism two unlinked systems *A* and *B*, often referred to as two mating type loci, control different aspects of dikaryon formation. Each of *A* and *B* consists of linked genetic loci, with genes of different function, designated α and β and yet other genes can occur between the α and β factors. Simchen (1967) was able to select for high and low recombination between Aα and Aβ, the difference being due to genes of major effect occurring at a *rec* locus linked to the *A* system and to genes of minor effect at other loci. Low recombination frequency was dominant to high. Stamberg (1968) showed that recombination in the *A* and *B* systems was under separate control. Stamberg and Koltin (1973) presented evidence for a genetic difference between strains being due to factors located in the recombining region itself. In this case, high frequency of recombination is dominant to low and they suggest that these sites may be recognition sites for the fine control of recombination, corresponding to the *cog* genes of *Neurospora*. It is also clear that the *rec* factors in *Schizophyllum* are of the same type as those found in *Neurospora*. Tang and Chang (1974) found that in the case of the major genetic factor *rec-1*, the dominant allele reduced the recombination frequency at the *pab-ad-5* region, about 20 map units from *rec-1*.

3. The M26 mutant in *Schizosaccharomyces pombe*. Gutz (1971b) reported a mutant (*ade6*-M26) which greatly increased recombination within the *ade6* locus, by showing a high degree of gene conversion itself and enhanced conversion of other sites in the locus. Gutz (1971b) postulated that M26 is a point of preferential breakage within the *ade6* locus. Goldman and Smallets (1979) demonstrated that M26 uniquely influences both intragenic recombination and gene conversion of other markers within its locus, with two doses of M26 acting in an additive manner. They concluded that conversion results from a sequence of events involving site-specific breakage at M26. Intragenic recombination is signalled with equal probability on each of the chromatids due to the homozygosity of M26 in a cross.

4. The YS17 mutant in *Sordaria brevicollis*. MacDonald and Whitehouse (1979) reported a mutant, YS17, at the *buff* spore colour locus in *Sordaria brevicollis*. This mutant shows high frequencies of gene conversion and greatly increased intragenic recombination in crosses to other *buff* mutants. From these results and others, MacDonald and Whitehouse (1979) concluded that the YS17 mutation is probably acting as a recognition site for an endonuclease that initiates recombination, with the result that the frequency of H-DNA within the *buff* gene is much increased. Furthermore, the recombination initiated at YS17 seems predominantly asymmetric, with the YS17 site acting as a recipient of a nucleotide chain from the other parent. These results most closely resemble the behaviour of the M26 mutant in *Schizosaccharomyces pombe* (Gutz, 1971b).

5. The *rec* genes in *Coprinus cinereus*. Katy and Moore (1980) showed that recombination between alleles of the *ftr* cistron in *Coprinus cinereus* is under the control of two single genes which seem to be independent of one another. The genes are symbolized *RecE* (for recombination enhancement) and *RecR* (recombination reduction). Both genes are fully dominant, non-additive and segregate readily from one another and the *ftr* cistron. Recombination between any pair of *ftr* alleles is increased when *recE* is included in the cross, but *recE* has no effect on recombination between alleles of the *me-5* locus. On the other hand, the reduction of recombination caused by *recR* in the *ftr* cistron is polarized and allele-specific, but *recR* increases the frequency of recombination between *me-5* alleles. Katy and Moore (1980) postulate that both *recE* and *recR* act to increase recombination at the *ftr* and *me-5* loci respectively, but illegitimate binding of the *recR* gene product at an illegitimate binding site in the *ftr* cistron prevents the enhancing activity of the *recE* gene product as its legitimate binding is interfered with. The data are further interpreted on the basis that the *rec* gene products may be involved in chromatid pairing and that polymorphic variants of them cause differences in pairing which, by altering the opportunities for recombination, are observed as differences in allelic recombination frequency.

6. The *cv* modifiers in the European strains of *Ascobolus immersus*. Girard and Rossignol (1974) described four distinct factors, *cv1*, *cv2*, *cv4* and *cv6* (*cv* for conversion modification), each controlling conversion at the *b1*, *b2*,

b4 and *b6* ascospore pigmentation loci respectively. There appear to be two alleles known at each of the *cv* loci, each present in a different wild-type strain. The two alleles are designated *A* and *B*. In crosses of *b2* mutants to wild-type strains, conversion is high in *cv2A* x *cv2A* and *cv2B* x *cv2B* crosses, but low in the heterozygous cross *cv2A* x *cv2B*. This relation holds for all three of the *cv1*, *cv2* and *cv4* systems and it seems likely that *cv6* acts similarly. *cv1* is closely linked to *b1* and *cv2* closely linked to *b2*. It seems that *cv4* and *cv6* are also closely linked to *b4* and *b6* respectively. *cv4* and *cv6* affect crossing-over between the linked loci *b4* and *b6*. These properties are exactly those expected for genes involved in pairing (Girard and Rossignol, 1974; Catcheside, 1977). Intimate molecular pairing would require precise, or almost precise, identity between the segments. Marked molecular differences would inhibit pairing and so not allow any of the succeeding events necessary for recombination to be instituted effectively. Hence heterozygosity of pairing genes would depress conversion and crossing-over locally.

7. The genetic control of gene conversion at the *w62* site in the Pasadena strains of *Ascobolus immersus*. This system was discovered by Emerson and Yu-Sun (1967) and is very similar to the *cv* system described above, but was elucidated first. In crosses heterozygous for a site closely linked to white ascospore pigmentation mutation *w62*, gene conversion of the latter mutation is reduced compared with that found in crosses homozygous for either known form of the factor. This control factor is now

known as *ccf*-1 (*ccf* for conversion control factor), since it was the first genetic control of gene conversion to be reported in the Pasadena strains of *Ascobolus*. As in the *cv* system it would seem that *ccf*-1 influences intimate pairing in its vicinity.

8. The *P/K/91* genetic factors in *Ascobolus immersus*. Studies on this system in the Pasadena strains of *Ascobolus immersus* were commenced by Emerson and Yu-Sun (1967) and continued by Wickramaratne (1974), Wickramaratne and Lamb (1978) and Lamb and Helmi (1978). This system is characterized by the genetic factors *P*, *K* and *91* being closely linked to their target - white ascospore pigmentation locus I (loci numbered by Ghikas, 1978); the three factors show *cis/trans* position effects on the conversion properties of locus I mutations and are incompletely dominant over each other. The system is now referred to as *ccf*-2, since it was the second to be elucidated in the Pasadena strains of *Ascobolus*. Helmi (1980) postulated that the *P*, *K* and *91* factors affect hybrid DNA formation and possibly the correction process involved in mismatch repair. He suggested that *ccf*-2(*P*) is a nucleotide sequence recognized with high efficiency by an endonuclease which initiates recombination, while *ccf*-2(*K*) and *ccf*-2(*91*) sequences are recognized less readily. After endonucleolytic cutting of the *ccf*-2(*P*) sequence, the chromatid bearing this sequence would become the recipient of genetic information from the homologous chromatid.

9. The "Super" genetic factors in *Ascobolus immersus*. This system was described by Helmi and Lamb (1979), Helmi (1980) and Helmi and Lamb (1981), again in the Pasadena strains of *Ascobolus*. The "Super" phenotype, i.e., the enhancement of conversion frequency at locus I was found to be due to the effect of two distinct control factors, *ccf*-3 (unlinked to locus I) and *ccf*-4 (loosely linked to locus I). *ccf*-3 has two alleles, *E* and *e*, while *ccf*-4 has alleles *R* and *r*, where *E* is a dominant enhancer of gene conversion and *R* is a dominant reducer. *ccf*-4(*R*) is completely epistatic over *ccf*-3(*E*), such that *ccf*-3(*E*) will have no enhancing effect on locus I conversion if *ccf*-4(*R*) is in the cross. Similarly, the recessive allele *ccf*-3(*e*) is completely epistatic to the dominant allele *ccf*-4(*R*), such that *ccf*-4(*R*) will have no effect on locus I conversion if the cross is homozygous for *ccf*-3(*e*), i.e., there is mutual epistasis of 4*R* and 3*e*. The "Super" combination and *P*, *K* and *91* act as mutual modifiers. Helmi (1980) proposed that *ccf*-3(*E*) acts by coding for a product which enhances H-DNA formation at locus I, either by increasing the frequency of intimate pairing before H-DNA formation actually starts or by enhancing a step during H-DNA formation. The dominant allele *ccf*-4(*R*) could act either by coding for a product which combines with the product of the *ccf*-3(*E*) allele, or *ccf*-4(*R*) is itself a preferable binding site for the enhancer product. The "Super" factors seem to bear some resemblance to the *rec* genes in *Coprinus cinereus* (Katy and Moore, 1980).

10. The *ss* genetic factors in *Neurospora crassa*. Recently, Catcheside (1981) described a gene that suppresses recombination when heterozygous in *Neurospora crassa*. Three alleles of this controlling gene (ss^E, ss^S and ss^C) were found distributed as a polymorphism in wild and laboratory populations of *Neurospora*. The *ss* (for synaptic sequence) gene is closely linked to the *nit-2* locus and may even lie within it as an intervening sequence. When heterozygous, *ss* reduces recombination 2- to 20-fold within the *nit-2* locus, but crossing-over in intervals flanking *nit-2* is not affected. The magnitude of intragenic recombination reduction depends upon the *ss* alleles involved. *ss* acts multiplicatively with *rec-1* — this gene is unlinked to *nit-2* and the dominant allele reduces allelic recombination at both the *his-1* and *nit-2* loci, but not at 24 other loci or chromosomal regions tested (Catcheside, 1977). Together these genes modulate recombination within the *nit-2* locus over a range exceeding 100-fold. Catcheside (1981) proposed that the *ss* factors act similarly to the *cv* modifiers described by Girard and Rossignol (1974) and the *w62* system described by Emerson and Yu-Sun (1967), both in *Ascobolus*: i.e. the *ss* alleles determine the pairing closeness of homologous DNA duplexes in the vicinity of the *nit-2* gene. Thus the *ss* genotype would determine the frequency of formation of intimately paired duplexes, a necessary prelude to recombination and the formation of a substrate upon which enzymes promoting recombination can act. He proposed that the *ss* site might also, or instead, influence an event later in recombination. Asymmetry in

the paired duplexes (due to *ss* heterozygosity) could interfere with D loop formation or with the migration of single- or double-strand cross connections (according to the Meselson-Radding model). A need for close sequence symmetry in any of the other steps in recombination, is not apparent. Catcheside's data led him to reject this alternative explanation in favour of the first hypothesis of the *ss* alleles being involved in intimate pairing.

Other examples of local recombination controls have been reported: in yeast (Simchen, Ball and Nachshon, 1971), *Schizosaccharomyces pombe* (Thuriaux et al. 1980), *Drosophila melanogaster* (Chovnick et al. 1971; Carpenter, 1973; Carpenter and Baker, 1974; Hawley, 1980) and *Zea mays* (Freeling, 1978), while the genetic controls of recombination in the silkworm (*Bombyx mori*) have been discussed by Turner (1979) and Ebinuma and Yoshitake (1981). Similar recombination hotspots have been described in prokaryotes, for example, *Escherichia coli* (Bresler et al. 1978). In the latter case, regions with frequent recombination exchange (*fre*) are postulated to contain many sites of recombination initiation, where a *recF* dependent endonuclease starts the process. In the above survey of genes with local effects on recombination, genes implicated in regional intimate pairing or comprising specific recognition sites for nucleases or coding for such nucleases have been discussed. It would seem that genes involved in intimate pairing are widely distributed in euka-

ryotes, having been found in two strains of *Ascobolus* (Emerson and Yu-Sun, 1967; Girard and Rossignol, 1974), *Neurospora* (Catcheside, 1981), *Zea mays* (Freeling, 1978) and perhaps *Drosophila* (Chovnick et al. 1971). In diploid organisms in which pairing of genes may be important for gene regulation (Jack and Judd, 1979), such genes could have a rôle in somatic cells. It is also evident that there are several cases where variability in recombination is clearly controlled (at least to a great extent) by closely linked parts of the chromosome. This supports the theory of the recombination-operator proposed by Whitehouse (1966) but with the proviso that the recombination-operator does not necessarily occupy one specific end of each gene. It can be within the gene. The rôle of DNA sequence in yeast recombination was studied by Moore and Sherman (1977). They suggested that the nature of the mismatched bases influences the recombination process. In a further yeast study, Fogel et al.(1979) concluded that the specific region which includes the heterozygosity is of primary importance in determining the parameters of conversion.

Since this Literature Survey was written, marker effects in the *his1* locus of yeast have been reviewed by Savage and Hastings (1981). Hilliker and Chovnick (1981) have made further observations on intragenic recombination in the *rosy* locus of *Drosophila melanogaster*. They confirm that a conversion event has a markedly less than 50% probability of resulting in flanking marker exchange. In the same work it is shown that the frequency of co-conversion within the *rosy* locus is inversely proportional to the distance between co-converting sites. As in fungi, real conversion frequency

differences were observed among *rosy* mutant alleles and the data suggest that there may be a relationship between allele conversion frequency and map position. Hilliker and Chovnick (1981) also report the occurrence of post-meiotic segregation in *Drosophila*. These analogies between *Drosophila* and fungi provide further evidence in support of the theory that eukaryotes share common molecular mechanisms of meiotic recombination.

Markham and Whitehouse (1982) put forward a new theory for the initiation of genetic recombination in eukaryotes, which involves the recognition of initiation sites by an endonuclease capable of migrating along a DNA duplex and cutting in *trans*. This theory accommodates conversion data from the *his3* gene of *Neurospora crassa* (Catcheside and Angel, 1974), the conversion patterns of the *ade6*-M26 mutation in *Schizosaccharomyces pombe* (Gutz, 1971b) and the *buff*-YS17 mutation in *Sordaria brevicollis* (MacDonald and Whitehouse, 1979).

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS

The Organism

The Pasadena strains of the fungus *Ascobolus immersus* were used throughout this work. All strains were derived from wild-type ascospores collected in 1963 from a sample of dung in Pasadena, California, by Emerson and Yu-Sun (Yu-Sun, 1964a). Most genetic investigations in this genus have involved ascospore colour mutants (Bistis and Olive, 1954; Bistis, 1956). In *Ascobolus immersus* more than 2000 ascospore mutants are known, differing from wild-type by only a single gene (Lissouba, Mousseau, Rizet and Rossignol, 1962). Auxotrophic, drug resistance and morphological mutants of this species have also been isolated (Yu-Sun, 1964b; Ghikas, 1978; Helmi, 1980). The first study of gene conversion in *Ascobolus*, using the European strains, was by Rizet, Lissouba and their collaborators, summarized in Lissouba et al.(1962). The Pasadena strains were first used in gene conversion studies by Emerson and Yu-Sun (1967).

Ascobolus immersus is a coprophilous discomycete, the Pasadena strains of which may be cultured on completely defined nutrient medium (Yu-Sun, 1964a). It seems to be cosmopolitan and the commonest member of the genus, its distribution being limited by the occurrence of herbivorous animals. *Ascobolus immersus* has been isolated from dung of

the cow, horse, sheep, goat, nilgai, antelope, elephant, dog, hare and rabbit. The mycelium of pale, almost white colour is coenocytic and the hyphae are septate (the septa bearing pores) and branched. The apothecia may be scattered or gregarious, first often immersed, then erumpent or superficial, about 1.0 mm in diameter and 0.7 mm high with a globular or pear-shaped receptacle, which is first closed and then irregularly opens near the top (Plate 1). The asci are cylindrical and show a great increase in volume during ripening, when their walls are stretched enormously and their tips curve towards the maximal light intensity (they are positively phototropic). Development of the asci is asynchronous and the dehiscence period may cover two weeks.

The ascospores typically possess only one haploid nucleus (Nicolas et al. 1981; Lamb, personal communication), usually number 8 per ascus, are oval shaped, being about 60μ long and 32μ wide (Esser and Kuenen, 1967), unordered and covered with a gelatinous envelope. The contents of the ascus can be shot away over a distance 500 times the length of the stretched ascus (about 35 cm). In this way the ascospores may be collected in octads on an agar screen collecting lid (Plate 2) and studied by means of unordered tetrad analysis. Wild-type ascospores are dark red, but white, granular and pink ascospore colour mutants have been isolated, normally giving a Mendelian 4:4 allele segregation in crosses to wild-type strains. The wild-type dark red pigmentation of the spore was found to be affected by light intensity and contamination with certain species of bacteria or fungi (Helmi, 1980). Also, this red

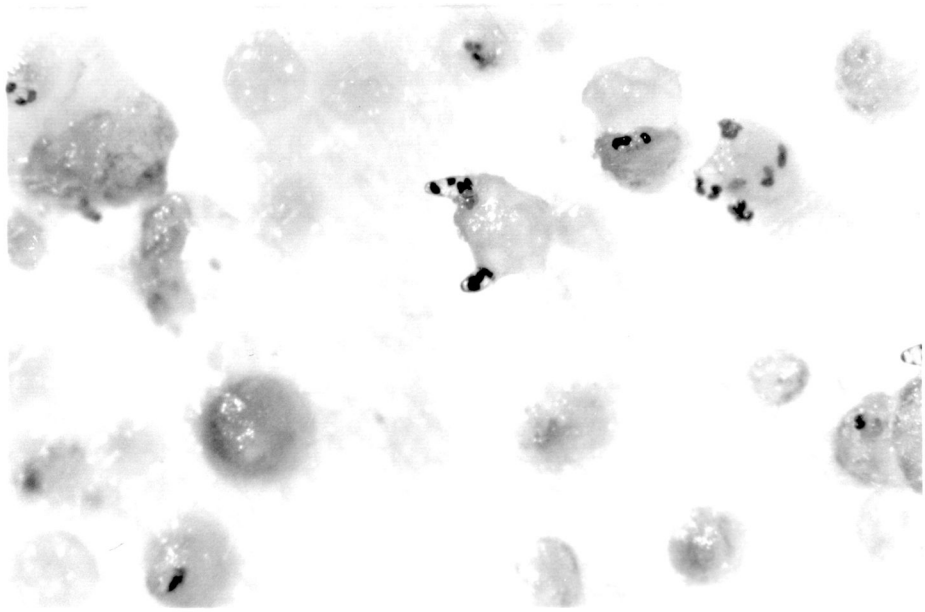


Plate 1: Apothecia with intact asci from cross BB9*w*9+ × 92-.

Plate 2: Spore octads arising from a cross between two linked white ascospore colour mutants. Note 1+:7*w* and 2+:6*w* octads.

Courtesy of Dr. B.C. Lamb

pigment, or one of its precursors, could pass from a wild-type red spore into a mutant white spore through a protoplasmic bridge if the two spores were partly connected (Stadler, Towe and Rossignol, 1970; Ghikas, 1978). In abnormal cases binucleate spores are produced, which are larger than usual - generally the lesser the number of ascospores per ascus, the larger the ascospores are. Such large ascospores are difficult to germinate (Wickramaratne, 1974; Ghikas, 1978; Helmi, 1980) and when they do germinate have always given rise to cultures which were infertile when crossed to several wild-type and mutant strains. During this work, clusters of ascospores containing such large spores were rare, and thus could be neglected during scoring. Occasionally abnormally shaped ascospores were produced. These germinate normally and by crossing to wild-type normal-shaped ascospore strains, were shown to be genotypically wild-type and presumably arose due to developmental errors (Helmi, 1980). In the European strains of *Ascobolus immersus*, several mutants with altered ascospore shape are known (Paquette, 1978; Nicolas et al. 1981).

Ascobolus immersus is haploid and the chromosome number varies according to different authors from 12 (Zickler, 1973) to 14 (Rossignol, personal communication to Lamb) to 16 (Esser and Kuenen, 1967). Linkage relationships in the Pasadena strains are not well known (Yu-Sun, 1964b; Emerson and Yu-Sun, 1967; Wickramaratne, 1974; Ghikas, 1978), but the situation is much better in stock *28* of the European strain where 370 ascospore mutations have been mapped to at

least 75 loci, of which 56 have been mapped to 13 linkage groups corresponding to at least 9 chromosomes (Nicolas et al. 1981).

Ascobolus immersus has many advantages as an experimental organism for genetic research:

1) it has a rapid life cycle, sometimes requiring only 12-16 days;

2) it has large and easy to handle spores;

3) whole octads are dehisced which can be collected without the need to dissect apothecia;

4) it possesses a simple incompatibility system of plus and minus mating types, making controlled crosses possible;

5) many ascospore pigmentation mutants are easily obtainable and since these correspond to autonomously expressed genes, make very useful markers, especially in gene conversion studies;

6) auxotrophic mutants may be selected since the original strains were culturable on completely defined medium. Drug resistance mutants can also be selected for;

7) certain European strains and also the Pasadena strains have high frequencies of gene conversion at the sites of some ascospore colour mutants, making them particularly suited to gene conversion studies; and

8) the detection of existing genetic factors affecting gene conversion and intragenic crossing-over (Emerson and Yu-Sun, 1967; Stadler, Towe and Rossignol, 1970; Girard and Rossignol, 1974; Lamb and Helmi, 1978;

Helmi and Lamb, 1981) facilitates studying the possible mechanisms of genetic recombination.

However, there are disadvantages too:

1) a lack of asexual spores has hindered the use of selective methods, such as filtration enrichment, for isolating auxotrophic mutants (Fincham, Day and Radford, 1979);

2) the production of unordered rather than ordered octads. Nevertheless, by using indirect methods, it is possible to obtain information on second division segregation frequencies, centromere distances and the frequency of aberrant 4:4s and other unique octad ratios such as 6:2(2), 2:6(2), 5:3(3) and 3:5(3) with postmeiotic segregation resulting in the number of non-identical spore pairs shown in parentheses (Ghikas and Lamb, 1977); linkage relations can be determined through two-factor crosses by comparing the ratio of parental ditype (PD), non-parental ditype (NPD) and tetra-type (T) octad classes, and from recombination frequencies amongst random spores;

3) loss of fertility; fertility can, however, often be maintained by periodic re-isolation of ascospores from crosses, although this involves possible changes in genotype;

4) poor and irregular ascospore germination which was overcome for wild-type spores by the introduction of

horse-dung germination medium by Helmi (1980) and by the establishment of optimum germination conditions for various white ascospore mutants in the present work;

5) conversion frequencies can change over the period of ascospore dehiscence. Thus spore collection over the whole dehiscence period is necessary for a true picture of the conversion behaviour of a cross to be determined.

The Strains

All stocks were derived from the original Pasadena strains of *Ascobolus immersus* (Yu-Sun, 1964a), supplied to Dr. B.C. Lamb by Professor Sterling Emerson. The following strains (with their ancestry) were used in this work:

TABLE 1: Strains and their ancestors.

Wild-type strains

Strain	Parental cross	Octad type isolated from	Isolated or induced and isolated by	Reference
P5-	Original isolate from dung sample Pasadena, California	-	Emerson and Yu-Sun	Yu-Sun, 1964a
K5+	"	-	"	"
91-2R+	-	-	"	Emerson, personal communication to Lamb
BBR1-	EC11*w*78, 7- × 1WT3+	5:3 false cluster	Lamb	Lamb, personal communication
BHR2-	EC11*w*78, 7- × *w*10+	2:6	"	"
9r+	92-*w*10- × 1WT3+	-	Ghikas	Ghikas, 1978
25-5R+	*w*78- × 9r+	6:2	"	"
1D2+ and 2D2+	PA8dm- × 23+	8:0	Helmi	Helmi, 1980
7R7-	1D2+ × BHR2-	8:0	"	"
KIII-	*w*78- × *w*10+	1:7	"	"
KIV+	" "	1:7	"	"
42-	91-2R+ × BHR2-	8:0	"	"
61-	" "	8:0	"	"
92-	" "	8:0	"	"
93+	" "	8:0	"	"
352+	" "	8:0	"	"
391-	7R6+ × 421-	8:0	"	"
474-	" "	8:0	"	"
659-	" "	8:0	"	"
1114+	" "	8:0	"	"
1116-	" "	8:0	"	"
7114-	" "	8:0	"	"
421-	NG*w*5+ 42-	4:4	Mafi	Mafi, personal communication

Strain	Parental cross	Octad type isolated from	Isolated or induced and isolated by	Reference
RS8-1-	NGω5+ × 42-	4:4	Mafi	Mafi, personal communication
RS17-1-	" "	4:4	"	"
RS17-3-	" "	4:4	"	"
RS27-1+	" "	4:4	"	"
4P-	25-5R+ × 7R7-	8:0	Howell	Present work
5P-	" "	8:0	"	"
6P-	" "	8:0	"	"
7P-	" "	8:0	"	"
9P-	" "	8:0	"	"
11P-	" "	8:0	"	"
16P-	" "	8:0	"	"
17P-	" "	8:0	"	"
18P-	" "	8:0	"	"
24P-	" "	8:0	"	"
257R-8-	" "	8:0	Mafi	Mafi, personal communication
257R-47+	" "	8:0	"	"
257R-115-	" "	8:0	"	"
257R-154+	" "	8:0	"	"
257R-158+	" "	8:0	"	"
275R-194+	" "	8:0	"	"
257R-210+	" "	8:0	"	"
BH19-1-	ωBHj- × 25-5R+	4:4	Howell	Present work
RM6-1-	" "	4:4	"	"
RM9-2+	" "	4:4	"	"
RM11-2+	" "	4:4	"	"
RM11-4-	" "	4:4	"	"
RM19-4-	" "	4:4	"	"
BH3-	BH19-1- × 25-5R+	8:0	"	"
BH5-	" "	8:0	"	"
BH7-	" "	8:0	"	"
BH9-	" "	8:0	"	"
BH13-	" "	8:0	"	"
BH22-	" "	8:0	"	"
BH43-	" "	8:0	"	"
BH45-	" "	8:0	"	"

Strain	Parental cross	Octad type isolated from	Isolated or induced and isolated by	Reference
92-19+	BB9*w*9+ × 92-	4:4	Howell	Present work
BB92-92-	" "	4:4	"	"
BB92-94-	" "	4:4	"	"
9225-5-	92- × 25-5R+	8:0	"	"
9225-14-	" "	8:0	"	"
9225-16-	" "	8:0	"	"
9225-27-	" "	8:0	"	"
9225-51-	" "	8:0	"	"
White ascospore mutant strains				
*w*62+	P5- × K5+	-	Spontaneous, by Emerson and Yu-Sun	Emerson and Yu-Sun, 1967
*w*62-	" "	-	"	"
*w*78(*K*)-	" "	-	"	"
207-5-*w*62+	K5+ × *w*62-	2:6	Emerson and Yu-Sun	"
*w*BHj+	BHRI × 91-2R+	4:4	UV induced, by Ghikas	Ghikas, 1978
*w*BHj-	" "	4:4	"	"
BB9*w*8+	BBRI- × 9r+	4:4	"	"
BB9*w*8-	" "	4:4	"	"
BB9*w*9+	" "	4:4	"	"
BB9*w*9-	" "	4:4	"	"
BB*w*11+	" "	4:4	"	"
BB*w*11-	" "	4:4	"	"
BB*w*21+	" "	4:4	"	"
BB*w*21-	" "	4:4	"	"
S*w*26.3-	BHR2- × 9r+	4:4	Spontaneous, by Ghikas	"
NG*w*11+	61- × 9r+	4:4	NG induced, by Ghikas	"
NG*w*11-	" "	4:4	"	"
NG*w*12+	" "	4:4	"	"
NG*w*12-	" "	4:4	"	"
NG*w*14.1-	" "	4:4	"	"
NG*w*14.5+	" "	4:4	"	"

Strain	Parental cross	Octad type isolated from	Isolated or induced and isolated by	Reference
NG*w*16.4-	61- × 9r+	4:4	NG induced, by Ghikas	Ghikas, 1978
NG*w*18+				
B*w*3.10-	BBRI- × 25-5R+	4:4	ICR 170 induced by Ghikas	"
B*w*4.2-	" "	4:4	"	"
C*w*1.14-	92- × 9r+	4:4	"	
3*w*IDEC-	2D2+ × EC11*w*78,	3:5	Helmi	Helmi, 1980
2ICR3-	*w*3C1+ × 42-	4:4	"	"
WS*w*8	25-5R+ × 7R7-	4:4	UV induced, by Mafi	Mafi, personal communication
WS*w*18	" "	4:4	"	"
94-1*w*9+	BB9*w*9+ × 92-	2:6	Howell	Present work
202-6*w*9-	" "	2:6	"	"
204-5*w*9+	" "	2:6	"	"
217-2*w*9+	" "	2:6	"	"
73-2*w*9-	" "	2:6	"	"
190-2*w*9-	" "	2:6	"	"
1BB9*w*9-	BB9*w*9+ × 7R7-	4:4	"	"
14-4*w*BHj+	*w*BHj- × 25-5R+	2:6	"	"
15-1*w*BHj-	" "	2:6	"	"
3NG*w*18-	NG*w*18+ × 16P-	4:4	"	"
4*w*62-	207-5-*w*62+ × 7114-	4:4	"	"
5*w*62+	" "	4:4	"	"
7*w*62-	" "	4:4	"	"
8*w*62-	" "	4:4	"	"
10*w*62-	" "	4:4	"	"
12*w*62-	" "	4:4	"	"
NG*w*M1+	25-5R+ × 7R7-	4:4	NG induced, by Howell	"
S*w*M1+	" "	4:4	Spontaneous, by Howell	"
UV*w*M1-	" "	4:4	UV induced, by Howell	"

Strain	Parental cross	Octad type isolated from	Isolated or induced and isolated by	Reference
UV*ω*M2-	25-5R+ × 7R7-	4:4	UV induced, by Howell	Present work
UV*ω*M3+	" "	4:4	"	"
UV*ω*M4-	" "	4:4	"	"
UV*ω*M5-	" "	4:4	"	"
UV*ω*M6-	" "	4:4	"	"
UV*ω*M7+	" "	4:4	"	"
UV*ω*M8+	" "	4:4	"	"
Granular ascospore mutant strains				
1*gr*+	92- × 9r+	4:4	NG induced, by Helmi	Helmi, personal communication
2*gr*2S+	" "	4:4	"	"
3*gr*-	" "	4:4	"	"
3GBIV*gr*-	3r- × 9r+	4:4	UV induced, by Helmi	"
*gr*33+	25-5R+ × 7R7-	4:4	Spontaneous, by Mafi	Mafi, personal communication
*gr*37+	" "	4:4	"	"
*gr*53-	" "	4:4	"	"
BH27*gr*+	BH19-1- × 25-5R+	4:4	Spontaneous, by Howell	Present work
UV*gr*M1+	25-5R+ × 7R7-	4:4	UV induced, by Howell	"
Pink ascospore mutant strain				
UV*p*M1+	25-5R+ × 7R7-	4:4	UV induced, by Howell	Present work

- denotes lack of information

CHEMICALS

The suppliers of chemicals used in this work were as follows:

Aldrich Chemicals Co.: N-methyl-N'nitro-N-nitrosoguanidine (NG)

BDH Analar : trace elements for Vogel's solution; acetone, sucrose, D-L glucose and magnesium sulphate

BDH Chemicals : biotin, pronase, all amino acids and vitamins, monopotassium phosphate and disodium phosphate

Difco : Bacto-agar, Bacto-casamino acids and Bacto-yeast extract

Hopkin and Williams : methyl 4-hydroxybenzoate

Koch-Light Laboratories Ltd.: adenine, thymine, cytosine and guanine; cellulose powder, ethyl methanesulphonate (EMS), potassium dihydrogen orthophosphate, dipotassium hydrogen ortho-phosphate and pronase

Oxoid : chloramphenicol and oxytetracycline

Sigma : DL-p-flurophenylalanine and ethyl methanesulphonate (EMS)

Whatman : cellulose powder

Acridine mustard (ICR 170) was supplied by Dr. H. J. Creech to Dr. B. C. Lamb

GENERAL METHODS

Preparation of media

All media were prepared with distilled water and concentrations, unless otherwise stated, were in weight/volume percentages. Vogel's trace elements solution, phosphate salts plus biotin and magnesium sulphate solution were all kept in separate sterile stocks and renewed approximately every three months. The crossing, complete and minimal media were those of Yu-Sun (1964a) as modified by Wickramaratne (1974), while the stock culture medium was that suggested by Mendenhall (personal communication to Lamb). The detailed composition of these nutrient media was as follows:

Crossing medium:
10 ml phosphate and biotin solution (25g KH_2PO_4, 30g K_2HPO_4 and 1 mg biotin in 480 ml distilled water), 5 ml magnesium sulphate solution (25g $MgSO_4 . 7H_2O$ in 250 ml distilled water), 0.1 ml Vogel's trace elements solution (Vogel, 1956), 3g yeast extract, 10g glucose, 2g cellulose powder, 0.5g casamino acids and 18g Difco bacto-agar in 980 ml distilled water.

Complete medium:
this was of the same composition as crossing medium, but without cellulose powder.

Stock culture medium:
this was of the same composition as crossing medium, but without cellulose powder or casamino acids.

Minimal medium:

this medium, in addition to salts, glucose and bacto-agar at the same concentration as in crossing medium, contained 5g L-asparagine and 0.1mg thiamine hydrochloride per litre.

All the above media were sterilized by autoclaving for 15 minutes at 15 p.s.i. These media, when necessary, were appropriately supplemented to satisfy experimental conditions or growth requirements of the strains under study.

Two ascospore germination media were used, as devised by Helmi (1980). The horse dung extract germination medium was prepared by shaking approximately 30g air-dried horse dung in 1 ℓ distilled water for one hour, then filtering through muslin. The filtrate was centifuged at 17000 r.p.m. for 10 minutes to remove colloidal material. The supernatent was filtered through Whatman No.1 filter paper; 20g Difco bacto-agar was added and the medium autoclaved for 20 minutes at 15 p.s.i. The horse dung used should be fresh; any storage of dung should be short and in a cool place. The final medium can lose its germination power on prolonged storage or prolonged exposure to light. Plain agar germination medium (for certain white ascospores only) was prepared by suspending 20g Difco bacto-agar in 1 ℓ distilled water and autoclaving for 15 minutes at 15 p.s.i.

The spore collecting medium was a 1.5% water agar (Difco bacto-agar) plus 7% (v/v) of the mould inhibitor stock solution, which was a 10% solution of methyl 4-hydroxybenzoate in ethanol (96% or 98%). This inhibitor was to prevent spontaneous germination of ascospores, especially

certain white ascospores, on the collecting lid, but did not inhibit germination after spores were transferred to germination medium (method devised by Lamb, personal communication).

Sterilization of media

This was as described by Wickramaratne (1974) except for solutions of chemicals which were unstable at autoclaving temperatures. Such solutions were filter sterilized by passage through 0.2μ pore sized Metricel membrane filters (supplied by Gallenkamp).

Preparation of ascospore suspensions

The ascospores harvested on a collecting lid were treated with 2-3 ml pronase solution (0.1%) for 15 minutes. The lid was shaken gently to ensure that all ascospores received even pronase treatment. After the gelatinous material clinging to the ascospores had dissolved, the spores were in free suspension which could be taken up into small vials using Pasteur pipettes. Any spores remaining on the collecting lid were resuspended in sterile distilled water and drawn off in the same way.

Inoculation, Crossing techniques, Collection of spores, Method of scoring, Germination of ascospores and Photography

These were described by Wickramaratne (1974), but following Helmi (1980) a slightly modified crossing technique was used. Frequently, aerial mycelium covers the apothecia,

trapping the octads, preventing them from reaching the collecting lid. This mycelium was removed 2-3 days before dehiscence of the octads, by gently scraping the crossing plate with a sterile spatula. For ascospore germination the heat shocks at 50°C were 2 hours for wild-type spores and ½ hour for granular spores (Ghikas, 1978; Helmi, 1980) while they were modified for various white spore mutants ranging from 0 to 4 hours.

Calculations

Calculations were carried out in part with a HP-55 but mainly with a HP-67 Hewlett-Packard programmable calculator, using standard programmes plus those written by Dr. B.C.Lamb.

P A R T O N E

Gene conversion of the

BB9*w*9, *w*BHj and NG*w*18 mutations

Chapter 1. Preliminary evidence for further conversion control systems from earlier studies.

Introduction

In the Pasadena strains of *Ascobolus immersus* several genetic factors are known which control the parameters of gene conversion at various sites in the genome. The first genetic control of conversion to be elucidated was that affecting white ascospore pigmentation mutation *w*62. This system consists of two states of a single factor closely linked to *w*62. Crosses homozygous for either state of the factor give high conversion frequencies of *w*62 (6-11%), while heterozygous crosses show much lower conversion frequencies. This system is now referred to as conversion control factor 1 (*ccf*-1) since it was the first to be discovered in the Pasadena strains by Emerson and Yu-Sun (1967). Secondly, conversion at ascospore pigmentation locus I (loci numbered by Ghikas, 1978), which is unlinked to *w*62, is controlled by at least three separate factors, the different genotypes of which give different locus I conversion frequencies averaging from 0.66% to about 23%. The first of these conversion control factors to be discovered is now termed conversion control factor 2 (*ccf*-2) and consists of three forms - *ccf*-2(*P*), *ccf*-2(*K*) and *ccf*-2(*91*) - which are closely linked to locus I, show incomplete dominance to each other and *cis*/*trans* position effects on locus I conversion (Lamb and Helmi, 1978). The "Super factor" (Helmi and Lamb, 1979) has now been shown to consist of two, unlinked, interacting components, *ccf*-3 and *ccf*-4, and apparently approximately doubles whatever gene conversion frequency at locus I is deter-

mined by the *ccf*-2 factors. *ccf*-3 is unlinked to locus I, while *ccf*-4 is loosely linked to it. Of the two conversion enhancing "alleles" of the "Super" combination — *ccf*-3(*E*) and *ccf*-4(*r*) — *ccf*-3(*E*) is dominant while *ccf*-4(*r*) is recessive, with *ccf*-4(*R*) completely epistatic to *ccf*-3 and *ccf*-3(*e*) recessively epistatic to *ccf*-4 (Helmi and Lamb, 1981). These *ccf* "alleles" have been designated according to their effects on conversion frequencies where *E* implies a dominant enhancer; *e* a recessive reducer; *R* a dominant reducer; *r* a recessive enhancer.

Preliminary evidence for additional conversion control systems and statement of problems to be investigated

Lamb and Ghikas (1979) studied the gene conversion properties of 29 induced (9 UV, 9 NG and 11 ICR-170) and 9 spontaneous white ascospore mutations. Each mutant strain was crossed to three types of derived wild-type strain, i.e. strains carrying *ccf*-2(*P*), *ccf*-2(*K*) or *ccf*-2(*91*). Single mutants often gave very different conversion results in the three types of cross with any or all of the following changes in: percentage of octads among conversion asci showing postmeiotic segregation; percentage with conversion to wild-type among conversion octads; and in total conversion frequency. Six mutations clearly fell into the latter category and thus were prime targets for investigating whether these differences in conversion frequency were under genetic control, and if so, were hitherto unknown genetic control factors responsible. The data obtained by Lamb and Ghikas (1979)

relating to these 6 mutations are shown in Table 2. A preliminary inspection of these data enables them to be divided into the three groups shown in the Table.

Group I data: involving mutant strains BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21

These original mutant strains were UV-induced and isolated by Ghikas (1978) from a cross involving the following derived wild-type strains:

$$\text{BBRI} - \left[ccf\text{-}2(P)\right] \times 9\text{r} + \left[ccf\text{-}2(P)\right]$$

Thus all four mutant strains must have carried *ccf*-2(*P*). From Table 2 it can be seen that all four mutations exhibited a similar conversion spectrum in high conversion frequency (HCF) crosses and a similar spectrum in low conversion frequency (LCF) crosses, while the spectrum differed between HCF and LCF crosses in two ways. Most noticeably, there was far less postmeiotic segregation (5:3 + 3:5) in the LCF crosses, while there was also less conversion to wild-type (+) in these crosses. The latter difference was less pronounced and largely due to the drastically depressed frequency of 5:3 PMS octads in the LCF crosses. The conversion frequencies (considering narrower ratio octads only) were broadly similar in the HCF crosses, ranging from 8.31 to 15.31% and very similar in the LCF crosses, ranging from 1.39 to 2.10%. The four mutations BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 therefore show very similar conversion properties.

TABLE 2: Preliminary evidence for further conversion control systems

Mutant strain	Crossed to		Octad classes (+:*w*),%					*%NRCF	†Relative %PMS	s%Conversion to +	Total octads
			4:4	6:2	2:6	5:3	3:5				
(GROUP I)											
BB9*w*8	61-	*ccf*-2(*P*)	91.27	4.53	0.48	3.23	0.40	8.64	42.01	89.82	4549
	42-	*ccf*-2(*91*)	97.90	0.81	1.16	0.11	0.03	2.10	6.67	43.40	7567
BB9*w*9	92-	*ccf*-2(*P*)	98.24	1.04	0.59	0.10	0.03	1.76	7.39	64.96	7796
	BBR1-	*ccf*-2(*P*)	86.63	6.68	0.50	5.20	0.74	13.12	45.27	90.57	808
	42-	*ccf*-2(*91*)	98.61	0.93	0.35	0.10	0.01	1.39	7.91	74.00	7169
BB*w*11	25-5R+	*ccf*-2(*P*)	88.27	6.23	1.03	3.72	0.70	11.69	37.81	85.16	2422
	9r+	*ccf*-2(*P*)	87.32	6.44	1.61	3.62	0.80	12.48	35.42	80.65	497
	352+	*ccf*-2(*91*)	98.35	1.02	0.56	0.07	-	1.65	4.24	65.96	2844
BB*w*21	BBR1-	*ccf*-2(*P*)	84.69	7.88	1.17	5.36	0.89	15.31	40.82	86.50	1790
	KIII-	*ccf*-2(*K*)	98.45	0.62	0.81	0.09	0.03	1.55	7.74	45.92	6324
	42-	*ccf*-2(*91*)	98.26	0.62	0.83	0.17	0.12	1.82	15.93	45.24	2413

TABLE 2: (continued)

Mutant strain	Crossed to		Octad classes(+:w),%					*%NRCF	†Relative % PMS	s%Conversion to +	Total octads
			4:4	6:2	2:6	5:3	3:5				
(GROUP II)											
*w*BHj	9r+	*ccf*-2(*P*)	92.73	4.31	1.17	0.80	0.86	7.14	23.25	71.56	8867
	KIII-	*ccf*-2(*K*)	94.26	3.82	0.57	-	1.34	5.74	23.34	66.67	1046
	42-	*ccf*-2(*91*)	85.61	8.81	1.87	2.13	1.42	14.23	24.95	76.87	7805
(GROUP III)											
NG*w*18	92-	*ccf*-2(*P*)	95.74	2.36	0.71	0.76	0.38	4.22	27.01	74.07	5753
(LOCUS II)	KIII-	*ccf*-2(*K*)	99.82	0.12	-	0.04	0.02	0.18	11.11	88.89	10260
	42-	*ccf*-2(*91*)	96.67	2.11	0.35	0.59	0.26	3.31	25.68	81.56	8541

* % NRCF (Narrower ratio conversion frequency) $= \frac{(6:2 + 2:6 + 5:3 + 3:5) \times 100}{\text{Total octads}}$

† Relative % PMS (Postmeiotic segregation) $= \frac{(5:3 + 3:5) \times 100}{(6:2 + 2:6 + 5:3 + 3:5)}$

s % Conversion to + $= \frac{(6:2 + 5:3) \times 100}{(6:2 + 2:6 + 5:3 + 3:5)}$

Based on data of Lamb and Ghikas (1979)

A consideration of the data involving mutation BB9*w*9 shows that primary conversion control cannot be exerted by *ccf*-2 since a cross to one *ccf*-2(*P*) carrying strain (92-) shows LCF (1.76%) while a cross to another such strain (BBRI-) shows HCF (13.12%). No such clearcut evidence was available for mutations BB9*w*8, BB*w*11 and BB*w*21 since mutant strains of each were only crossed to one *ccf*-2(*P*) carrying strain. However, none of these mutations responded to *ccf*-2 in the same manner as locus I mutations.

Unfortunately, Ghikas (1978) was unable to map all four mutations in this group with respect to each other, since none of the desired crosses was fertile. It therefore remained possible at the commencement of this work that these four mutations (BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21) were merely re-isolations of the same mutation as they had been isolated from the same cross. They could also be closely linked mutations responding to the same conversion control system; unlinked mutations responding to the same conversion control system or unlinked mutations responding to different conversion control systems in a similar

manner. It was decided to investigate these questions as well as elucidating the conversion control system acting on BB9*w*9 which seemed the primary candidate for study since large scale influences of *ccf*-2 could be ruled out in this case.

Group II data: involving *w*BHj mutant strains

The original *w*BHj mutant strains were UV-induced and isolated by Ghikas (1978) from a cross involving the following derived wild-type strains:

$$\text{BHRI} - \left[ccf\text{-}2(P)\right] \times \text{91-2R} + \left[ccf\text{-}2(91)\right]$$

Thus it was not known whether the original *w*BHj strains carried *ccf*-2(*P*) or *ccf*-2(*91*). From the results shown in Table 2, high and low converting crosses are apparently clearly definable, with conversion frequencies for the two LCF crosses of 7.14 and 5.74% and 14.23% for the HCF cross. Some differences in conversion spectrum also occur between the three crosses. It would further seem that conversion at the *w*BHj site cannot primarily be controlled by *ccf*-2, unless the action of *ccf*-2 at this unlinked site differs from that at locus I: the *w*BHj conversion frequency was higher in the cross to a *ccf*-2(*91*)strain (42-) than it was in the cross to a *ccf*-2(*P*) carrying strain (9r+). This is exactly opposite to the conversion behaviour of locus I mutations and would be so whether both the *w*BHj strains carried *ccf*-2(*P*) or *ccf*-2(*91*). The situation would be more complex if the (+) and (-) mating type *w*BHj strains carried different forms of *ccf*-2. It was resolved to further investigate the conversion behaviour of *w*BHj as

it seemed a good candidate to enable the elucidation of a further genetic control of gene conversion.

Group III data: involving NG*w*18 mutant strains

The original mutant strain NG*w*18+ was NG-induced and isolated by Ghikas (1978) from a cross involving the following derived wild-type strains:

$$61 - \left[ccf\text{-}2(P)\right] \times 9r + \left[ccf\text{-}2(P)\right]$$

Thus the original NG*w*18+ mutant strain must have carried *ccf*-2(*P*). The data displayed in Table 2 show that genetic control of the conversion behaviour of mutation NG*w*18 by *ccf*-2 can be ruled out because crosses of the same mutant strain to *ccf*-2(*P*) and *ccf*-2(*91*) carrying derived wild-types gave similar high conversion frequencies (4.22 and 3.31% respectively) which were about 20-fold higher than in the LCF cross (0.18%). Clear differences in conversion spectrum between the HCF and LCF crosses can also be seen, with reduced PMS in the LCF cross. There is no clearcut pattern in the percentage conversion to wild-type.

Mutation NG*w*18 is part of locus II along with mutation S*w*26 with which it has not been observed to recombine (Ghikas, 1978). Mutation NG*w*16 lies at a distance of 2.5 recombination units and may be regarded as part of locus II (mutations *w*10 and *w*78 show similar recombination frequencies in certain crosses and are both considered part of locus I) although it was not by Ghikas (1978). S*w*26 and NG*w*16 do not show the same conversion behaviour as each other or NG*w*18

(Lamb and Ghikas, 1979). Therefore the NG*w*18 system was selected as a fruitful area of study in the hope of characterizing fresh genetic conversion control systems.

The main objectives of this section of research can be summed up as follows: to determine whether the detected differences in conversion behaviour of unlinked mutations BB9*w*9, *w*BHj and NG*w*18 are due to genetic conversion control factors (at least in part); a full investigation and characterization of the nature and behaviour of the factor(s) involved; the originality or otherwise of these factors; a determination of whether mutations BB9*w*8, BB*w*11 and BB*w*21 respond to the same genetic conversion control system as BB9*w*9 and the linkage relations of these four mutations; a more general investigation of the specificity of any newly characterized control factors. Furthermore, during the course of this work, Helmi and Lamb (1981) elucidated the "Super" genetic conversion control system which, on the basis of limited data, seemed to be specific to ascospore pigmentation locus I. The effect of this system on the mutations under study was also investigated.

In close connection with the above objectives, a search was undertaken for further ascospore colour mutations linked to the white sites under study. The isolation and induction of such mutations would enable a clearer picture of the genetic controls and mechanism of gene conversion at any site to be built up from data of the following type: the action of a given control factor on a set of linked ascospore colour mutations of different mutagenic origins; the frequency and extent of co-conversion; the occurrence of

polarity in conversion and the nature of any such polarity. The results of mutant induction, isolation and mapping are discussed in PART TWO of this work.

Chapter 2: The genetic control of conversion of the BB9*w*9 white ascospore pigmentation mutation

Preliminary experiments

Initially it was important to verify the results obtained by Lamb and Ghikas (1979) concerning the conversion properties of mutation BB9*w*9, shown in Table 2. Thus strain BB9*w*9+ was crossed to derived wild-type strains 92-, BBR1- and 42-. Despite repeated attempts the crosses involving strains BBR1- and 42- were no longer fertile. In an attempt to revive these strains, inocula were allowed to grow through *Ascobolus* complete medium supplemented with 100mg/ℓ chloramphenicol and repeatedly subcultured on this medium to clear our cultures of these strains of any possible bacterial contamination which might have rendered the strains sterile. However, the procedure did not restore any fertility to the strains so treated. Such strains are probably senescent and might thus respond to growth on medium supplemented with ethidium bromide in the manner described for senescent strains of *Podospora anserina* by Belcour and Begel (1981).

Of the three original crosses performed by Lamb and Ghikas (1979) only that involving derived wild-type strain 92- was repeatable. BB9*w*9 mutant strains were therefore crossed to a range of derived wild-type strains in our stock collection with the aim of obtaining crosses showing similar conversion behaviour to the original crosses shown in Table 2. Seven fertile crosses were achieved and the results from these crosses are displayed in Table 3. From this Table it can be seen that the conversion frequencies

TABLE 3: Conversion data from + x BB9*w*9 crosses

Mutant strain	Crossed to	Octad classes (+:*w*),%					%NRCF	Relative % PMS	%Conv. to +	Total octads
		4:4	6:2	2:6	5:3	3:5				
BB9*w*9+	92-	96.82	1.68	0.55	0.68	0.23	3.14	28.98	75.16	4711
BB9*w*9-	93+	96.53	1.42	1.54	0.51	-	3.47	14.70	55.62	777
BB9*w*9+	7R7-	87.91	6.49	0.89	3.17	0.48	11.03	33.09	87.58	4162
BB9*w*9-	25-5R+	89.76	5.21	1.23	3.60	0.09	10.14	36.39	86.97	1055
BB9*w*9+	RS8-1-	84.56	7.87	1.11	5.55	0.91	15.44	41.84	86.92	991
BB9*w*9+	RS17-1-	88.61	5.18	0.62	4.55	1.04	11.39	49.08	85.43	966
BB9*w*9+	RS17-3-	88.27	6.54	0.59	3.81	0.64	11.59	38.40	89.38	4203

of the seven crosses fall into two clearly defined categories. The first two crosses in the Table show much lower conversion frequencies (3.14 and 3.47%) than the remaining five crosses (10.14 to 15.44%). No intermediate conversion frequencies were obtained. The variability among crosses in each category was assessed by the $\Sigma\chi^2$ homogeneity test. The two LCF crosses were highly homogeneous ($\Sigma\chi^2 = 0.10$, $p = 0.70\text{-}0.80$, 1df), while the five HCF crosses were heterogeneous with respect to narrower ratio conversion frequencies ($\Sigma\chi^2 = 17.24$, $p < 0.01$, 4df). However, when the data from cross BB9*w*9+ × RS8-1- are excluded, the remaining four crosses show good homogeneity for conversion frequency ($\Sigma\chi^2 = 1.86$, $p = 0.50\text{-}0.70$, 3df).

These conversion frequency results are in agreement with those obtained by Lamb and Ghikas (1979) with clearly defined LCF and HCF crosses with similar conversion frequencies observed for each category. Furthermore, *ccf*-2 is not implicated in these clearcut differences in conversion frequency since derived wild-type strains carrying the same form of *ccf*-2 — *ccf*-2(*P*) — can give LCF(92-) and HCF (7R7-) when crossed to the same BB9*w*9 mutant strain. This agrees with the original findings of Lamb and Ghikas (1979). The differences in conversion spectrum between LCF and HCF crosses originally observed by these authors were seen in this set of crosses but the differences were not so dramatic. HCF crosses gave conversion spectra more typical of base substitution mutations, i.e. they showed high PMS with a conversion spectrum of type C, according to Leblon (1972 a,b). Proportionately less PMS was observed in LCF crosses, but was

still fairly frequent, especially in cross BB9*w*9+ × 92-. Lamb and Ghikas (1979) were able to classify the LCF crosses they performed into conversion spectrum type A (Leblon, 1972 a,b), supposedly more typical of frameshift mutations. This classification was not possible for cross BB9*w*9+ × 92-, although it had been one of the original crosses performed by Lamb and Ghikas (1979). The second element of a conversion spectrum — percentage conversion to wild-type — also differed between the LCF and HCF crosses, with less conversion to wild-type in the two LCF crosses. The HCF crosses showed very high conversion frequencies to wild-type (85.43 to 89.38%).

These crosses broadly confirmed the existing data on the conversion behaviour of BB9*w*9, providing HCF and LCF crosses for further analysis. The difference in conversion frequency between the two sets of crosses strongly suggests that the conversion behaviour of BB9*w*9 is under genetic control and the system involved cannot be *ccf*-2. At the outset a simple, easily testable model of conversion control was assumed (a closely linked, monogenic system) and this formed the basis upon which the following experimental programmes of crosses were devised.

Monogenic conversion control of BB9*w*9

The data in Table 3 show that when strain BB9*w*9+ was crossed to derived wild-type strains 92- and 7R7- very different conversion frequencies resulted. Since the BB9*w*9+ strain was the same in each case, and if the conversion control of this mutation is monogenic, then strains 92-

and 7R7- must carry different forms of the conversion control factor. Therefore either LCF cross BB9*w*9+ × 92- or HCF cross BB9*w*9+ × 7R7- must be heterozygous for different forms of the conversion control factor. Since gene conversion of the *w*62 mutation in the Pasadena strains of *Ascobolus immersus* is reduced when its closely linked conversion control factor is heterozygous in a cross (Emerson and Yu-Sun, 1967) and the same situation applies to the four *cv* modifiers in the European strains (Girard and Rossignol, 1974), it was felt more likely that LCF cross BB9*w*9+ × 92- might be heterozygous for a closely linked conversion control factor, so this cross was chosen for analysis first. The theoretical basis for this analysis is as follows and is illustrated in Figure 1, where *A* and *B* stand for the two states of the conversion control factor: in a cross doubly heterozygous for a white mutation and a closely linked conversion control factor, analysis of the 6 red (wild-type) spores from 6+:2*w* convertant octads (whether they arise by symmetrical or asymmetrical H-DNA formation) should reveal that 4 spores carry the same form of the control factor as the red-spored parent, while 2 spores will have inherited the control factor originally present in the white-spored parent. The latter 2 spores should therefore show altered conversion behaviour in backcrosses to the white mutant parent, unless certain forms of dominance are involved and the white mutation and its conversion control factor do not usually co-convert.

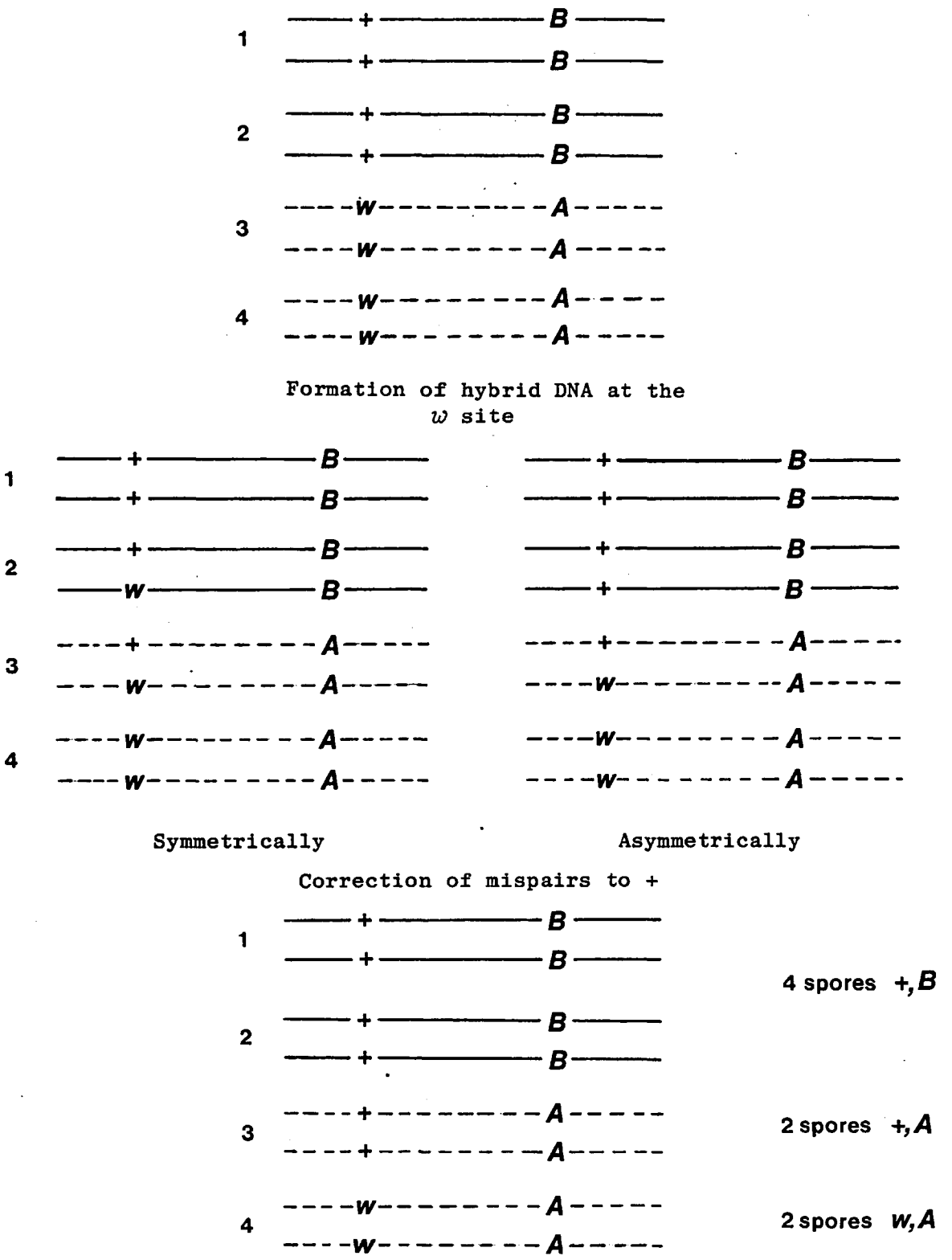


Fig. 1: Origin of wild-type segregants with altered conversion properties in 6+:2ω octads from + × ω crosses, where the conversion control factor is heterozygous, closely linked, but not co-converting with the ω site.

Conversion behaviour of wild-type isolates from 6+:2*w* octads from LCF cross BB9*w*9+ × 92-

Following the programme outlined above, wild-type spores were isolated from 35 6+:2*w* convertant octads. Ascospore germination was poor in this experiment, with only 60 wild-type spores germinating. Of these, 25 isolates gave fertile backcrosses to strain BB9*w*9+ (the remaining 35 isolates were infertile with both BB9*w*9+ and BB9*w*9-, but were probably mostly of (+) mating type since strain BB9*w*9- was now showing only very poor fertility). The conversion data from these 25 crosses are shown in Table 4. 21 isolates showed LCF when backcrossed to BB9*w*9+ and thus their conversion behaviour closely resembled that of the wild-type parent, with a range of conversion frequencies from 1.50 to 5.72%. The remaining 4 isolates showed clearly altered conversion behaviour in these backcrosses, with greatly increased conversion frequencies of 11.91 to 13.51%. These isolates would appear to be recombinant for the white site and its conversion control factor, having arisen by gene conversion at the white site. It is worth noting that these 4 isolates with altered conversion properties arose in pairs in 2 octads (numbers 3 and 5) and these pairs almost certainly represent sister spores. Since ascospore germination was poor and it was necessary to cross all wild-type isolates to the same BB9*w*9+ strain (since the only BB9*w*9- strain available at this time was almost infertile and in any case may not have carried the same form of the conversion control factor as BB9*w*9+) complete analysis of all 6 wild-type

TABLE 4: Conversion data of wild-type spores from 6+:2*w* octads isolated from cross BB9*w*9+ x 92-; isolates were crossed to mutant strain BB9*w*9+

Isolate No. (octad.spore)	Octad classes (+:*w*),%					%NRCF	Relative % PMS	%Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
1.1	98.50	-	0.38	0.75	0.38	1.50	73.33	50.00	266
1.2	96.91	1.93	0.48	0.48	0.19	3.09	21.68	77.99	1035
1.3	95.14	2.92	0.53	1.24	0.09	4.77	27.88	87.21	1131
1.5	97.19	1.53	0.51	0.60	0.17	2.81	27.40	75.80	1174
2.1	97.06	1.41	0.94	0.24	0.35	2.94	20.07	56.12	849
3.1	87.60	7.42	0.68	3.91	0.29	12.30	34.15	92.11	1024
3.2	87.87	7.45	0.43	3.62	0.43	11.91	34.01	92.94	470
4.4	98.20	1.25	0.40	0.05	0.10	1.80	8.33	72.22	2004
4.6	96.47	2.31	0.24	0.84	0.04	3.44	25.58	91.56	4536
5.1	86.04	9.49	0.97	2.60	0.45	13.51	22.58	89.49	4426
5.4	87.13	8.58	0.82	3.06	0.23	12.69	25.93	91.73	2191
5.5	95.83	2.37	0.42	1.15	0.10	4.03	31.02	87.34	2875
6.1	94.78	3.56	0.57	0.89	0.13	5.16	19.76	86.24	1571
6.6	94.28	3.18	1.03	1.43	0.08	5.72	26.40	80.59	1259
7.3	97.41	1.58	0.44	0.51	0.06	2.59	22.01	80.69	1583
7.4	95.85	2.99	0.36	0.80	-	4.15	19.28	91.33	1373
9.1	95.70	2.64	0.83	0.74	-	4.21	17.58	80.29	1210
14.2	96.21	2.78	0.62	0.15	0.23	3.79	10.03	77.31	1294
15.1	97.35	1.13	0.52	0.96	0.04	2.65	37.74	78.87	2304
17.1	97.56	1.37	0.38	0.38	0.31	2.44	28.28	71.72	1311
17.6	96.73	2.34	-	0.47	0.47	3.27	28.75	85.93	214
20.2	95.29	2.69	1.12	0.67	0.22	4.71	18.89	71.34	446
24.2	96.28	0.76	0.59	1.35	1.01	3.72	63.44	56.72	1183
29.3	97.95	1.66	0.15	0.20	-	2.00	10.00	93.00	2046
29.5	95.97	2.32	0.50	1.11	-	3.93	28.24	87.28	993

Isolated with altered conversion frequencies are underlined

21 isolates show low conversion frequency (1.50 - 5.72%NRCF)

4 " " high " " (11.91 - 13.51%NRCF)

X^2 (for expected ratio of 2LCF:1 altered CF among crosses) = 2.64

p = 0.10 -0.20 (1df)

spores in any octad was not possible. The data must therefore be dealt with as originating from random wild-type spores isolated from a population of 6+:2*w* octads. If little or no co-conversion of the BB9*w*9 mutation and its conversion control factor occurs and the preliminary model of conversion control at this site is correct, a ratio of 2LCF:1 altered CF among the crosses shown in Table 4 would be expected (out of 6 wild-type spores, 4 would carry the same *ccf* form as the wild-type parent while 2 would not) unless the control factor genotype of the wild-type isolates is not apparent due to dominance of one form of the *ccf* over the other. In such a situation, all the isolates could show the same conversion behaviour. The observed values of 21:4 do fit the expected 2:1 ratio for LCF:altered CF crosses (χ^2, applying Yates' correction, = 2.64, p = 0.10-0.20, 1df). Despite this statistically acceptable fit, fewer altered conversion segregants were observed than expected. This might have been due to the relatively small number of isolates which gave fertile crosses, or perhaps some co-conversion occurs between the white site and its *ccf*, reducing the number of altered segregants arising by single-site conversion of the white site. If the white site and its conversion control factor were less closely linked, crossing-over unassociated with conversion would actually increase the number of segregants with altered conversion properties.

The results from this analysis do seem to indicate that the LCF cross BB9*w*9+ × 92- is heterozygous for at least one

ccf affecting the conversion of BB9*w*9. This factor may be closely linked to BB9*w*9 since some co-conversion of the two sites is consistent with the data. On this basis, HCF cross BB9*w*9+ × 7R7- should be homozygous for the *ccf* in question and a similar analysis of the conversion properties of wild-type isolates from 6+:2*w* octads should reveal that none exhibit altered conversion behaviour in backcrosses to BB9*w*9.

It should be noted that the data in Table 4 indicate a considerable range in (narrower ratio) conversion frequency, with values of 1.50 to 5.72% among LCF crosses. Even when crosses with a total sample size of less than 1000 octads are excluded, the data are still heterogeneous for conversion frequency ($\Sigma\chi^2 = 85.43$, $p < 0.01$, 14df). Nevertheless, the range of variation between crosses believed to be of the same *ccf* genotype was no greater than between replicates of the same cross. It was hoped to demonstrate this more clearly by selecting 2 crosses at extreme ends of the LCF range and 2 crosses in the mid-range and repeating these crosses with 5 replicates of each. Unfortunately, when this was attempted, the crosses concerned were no longer fertile. However, it does seem that in this experiment at least, the variation in conversion frequency between LCF crosses is due to imperfectly controlled environmental effects, rather than the segregation of minor modifiers of gene conversion, but the latter cannot be ruled out.

The conversion spectrum was also variable among the LCF crosses and no clearcut differences were apparent bet-

ween the LCF segregants and the altered HCF segregants, although the latter did tend to show higher frequencies of conversion to wild-type if not higher frequencies of PMS.

Conversion behaviour of wild-type isolates from 6+:2*w* octads from HCF cross BB9*w*9+ × 7R7-

Wild-type spores were isolated from 26 6+:2*w* octads from HCF cross BB9*w*9+ × 7R7-. 34 wild-type spores germinated, from which 18 fertile crosses resulted. From Table 5 it can be seen that 13 of these isolates gave fertile backcrosses with the BB9*w*9+ parental strain, while only 5 were fertile with BB9*w*9- as a tester. This might have been due to linkage of BB9*w*9 to the mating type locus, but this is most unlikely (and was not suggested by later data) due to the known poor fertility of strain BB9*w*9-. Table 5 shows that all of the crosses were of the HCF type, with conversion frequencies ranging from 9.03 to 15.59%, relatively high PMS frequencies (29.52 to 47.64%) and high frequencies of conversion to wild-type (83.62 to 95.26%). Thus all the wild-type segregants showed broadly the same conversion control properties as the 7R7- wild-type parent. It should also be noticed that the BB9*w*9+ and BB9*w*9- tester strains both behaved in the same way, indicating that both of these strains probably carry the same form of the *ccf* in question. The variation in conversion frequency (from 9.03 to 15.59%) could be due to the segregation of minor modifiers of gene

TABLE 5: Conversion data of wild-type spores from 6+:2*w* octads isolated from cross BB9*w*9+ x 7R7-; isolates were crossed to BB9*w*9+ or BB9*w*9- mutant strains

Isolate No. (octad.spore)	Octad classes (+:*w*), %					% NRCF	Relative % PMS	% Conv. to +	Total Octads
	4:4	6:2	2:6	5:3	3:5				
4.2-	90.22	6.22	0.44	2.40	0.39	9.45	29.52	91.22	3618
4.3-	88.74	5.56	1.20	3.87	0.28	10.91	38.04	86.43	1421
6.1-	88.55	6.22	1.09	3.11	0.54	10.95	33.30	85.13	2219
7.3+	84.87	8.34	0.73	4.46	0.55	14.07	35.58	90.91	3298
8.4-	89.56	5.55	1.27	3.08	0.42	10.32	33.91	83.62	1657
8.5-	89.54	5.15	0.79	3.41	0.87	10.22	41.88	83.76	1262
9.1-	90.18	5.57	0.92	2.69	0.44	9.63	32.54	85.86	2710
9.3+	89.06	5.81	0.56	4.02	0.32	10.70	40.52	91.78	3410
9.5-	90.20	6.24	0.17	2.80	0.28	9.49	32.46	95.26	3573
9.6-	88.62	5.42	0.85	4.41	0.54	11.22	44.12	87.61	1292
14.1-	88.77	5.19	0.59	4.54	0.72	11.03	47.64	88.13	3063
14.3+	84.17	7.41	1.27	5.94	0.97	15.59	44.32	85.63	2995
14.4+	91.55	4.52	0.59	3.05	0.23	8.38	39.09	90.23	3054
14.6+	87.27	6.73	0.46	4.72	0.52	12.43	42.16	92.12	3668
15.6-	86.14	6.74	0.75	5.24	1.12	13.86	45.92	86.50	267
16.3-	90.13	4.75	0.95	3.67	0.38	9.75	41.54	86.36	1580
16.5-	90.97	4.66	0.84	3.17	0.37	9.03	39.19	86.62	1074
16.6+	88.61	6.91	0.81	3.23	0.27	11.21	31.19	90.37	1115

All crosses show high conversion frequency (9.03 - 15.59% NRCF)

conversion frequency in the cross or imperfectly controlled environmental effects.

Taken together, the above two experiments point to the existence of a conversion control factor primarily responsible for determining the degree of gene conversion of mutation BB9*w*9, giving high conversion frequencies (c9-15%) in crosses homozygous for one form of the factor (let this be designated *A*) with reduced conversion frequencies when the factor is heterozygous, at least in *w*, *A* × +, *B* crosses (where *B* is designated as the other form of the factor). The two parental crosses chosen for analysis can now be written:

BB9*w*9 + (*A*) × 92 - (*B*) LCF

BB9*w*9 + (*A*) × 7R7 - (*A*) HCF

From this it can be seen that the two convertant wild-type spores in 6+:2*w* octads from LCF cross BB9*w*9+ × 92- should carry *ccf* form *A*, inherited from the white-spored parent. Such segregants, when backcrossed to BB9*w*9+, would produce a HCF *A* × *A* type of cross. Indeed, in this experiment the segregants showing altered conversion properties in such backcrosses were indeed high converting (see Table 4).

The above assumes that only one *ccf* is responsible for the large differences in conversion frequency which mutation BB9*w*9 can show. The following experiment was carried out to test this more conclusively.

Conversion behaviour of random wild-type spores isolated from cross 25-5R+ × 92-

It was deduced that a cross between derived wild-type strains 25-5R+ and 92- would be heterozygous for the *ccf(s)* responsible for the conversion behaviour of BB9*w*9: wild-type strain 92- has been designated as carrying form *B* of the *ccf*; Table 3 shows that cross BB9*w*9- × 25-5R+ is HCF. Since it is known that BB9*w*9- carries form *A* (like BB9*w*9+), 25-5R+ must also carry form *A* if the above cross is to be HCF. Thus cross 25-5R+ × 92- must be heterozygous for the postulated monogenic conversion control system, but if further genes are responsible for the large scale differences between HCF and LCF crosses, this cross could be heterozygous for those as well. To test this, 200 wild-type spores were isolated randomly from the progeny of cross 25-5R+ × 92- (all octads observed were 8+:0*w*). 56 spores germinated and 23 of these isolates gave fertile crosses with the BB9*w*9+ tester strain. No isolates showed fertility with any of the BB9*w*9- re-isolates then available. The data obtained are shown in Table 6 and Figure 2.

From this Table and Figure it can be seen that of the 23 random isolates which gave fertile crosses with BB9*w*9+, 15 isolates gave LCF crosses (with conversion frequencies of 1.82 to 3.08%) behaving like the 92- parent, while 8 gave HCF crosses (with conversion frequencies of 8.92 to 14.14%) behaving like the 25-5R+ parent. If the conversion control of BB9*w*9 is monogenic, a 1:1 ratio of LCF and HCF crosses should be expected from this type of experiment.

TABLE 6: Conversion data of random wild-type spores isolated from cross 25-5R+ x 92- when crossed to BB9ω9+

Isolate No.	Octad classes (+:ω), %					% NRCF	Relative % PMS	% Conv. to +	Total Octads
	4:4	6:2	2:6	5:3	3:5				
3	96.97	1.57	1.33	0.12	-	3.03	3.96	55.96	826
5	88.36	5.70	0.57	4.77	0.45	11.64	44.85	91.12	4176
6	86.73	6.90	0.38	5.52	0.15	13.27	42.73	95.91	1304
7	85.86	4.55	6.06	2.02	1.52	14.14	25.04	46.43	198
8	97.19	1.66	0.96	0.13	0.06	2.81	6.76	63.70	1564
14	96.96	1.91	0.59	0.42	0.09	3.04	16.78	77.41	4245
16	90.16	5.01	0.80	3.87	0.14	9.84	40.75	90.43	3516
23	98.21	1.12	0.22	0.34	0.11	1.79	25.14	81.56	894
25	97.61	1.39	0.62	0.39	-	2.39	16.32	74.17	1295
27	90.02	5.26	0.63	3.58	0.38	9.98	39.68	89.75	5529
30	96.92	2.02	0.63	0.43	-	3.08	13.96	79.55	2530
31	98.18	1.09	0.36	0.36	-	1.82	19.78	80.11	274
32	98.14	0.70	0.23	0.93	-	1.86	50.00	87.63	429
35	97.56	1.22	0.98	0.24	-	2.44	9.84	59.84	409
36	97.91	1.22	0.70	0.17	-	2.09	8.13	66.51	573
37	90.83	6.09	0.24	2.51	0.08	9.17	28.24	93.78	3709
41	97.52	1.31	0.78	0.33	-	2.48	13.31	66.13	765
44	90.42	6.60	0.43	2.56	-	9.58	26.72	95.62	1409
45	89.33	5.20	0.87	4.39	0.22	10.67	43.20	89.88	1847
48	98.73	1.19	-	0.09	-	1.27	7.09	100.00	2278
50	98.13	1.23	0.30	0.30	0.05	1.87	18.72	81.82	2032
51	97.88	1.36	0.38	0.38	-	2.12	17.92	82.08	1322
56	96.92	1.54	1.03	0.51	-	3.08	16.56	66.56	195

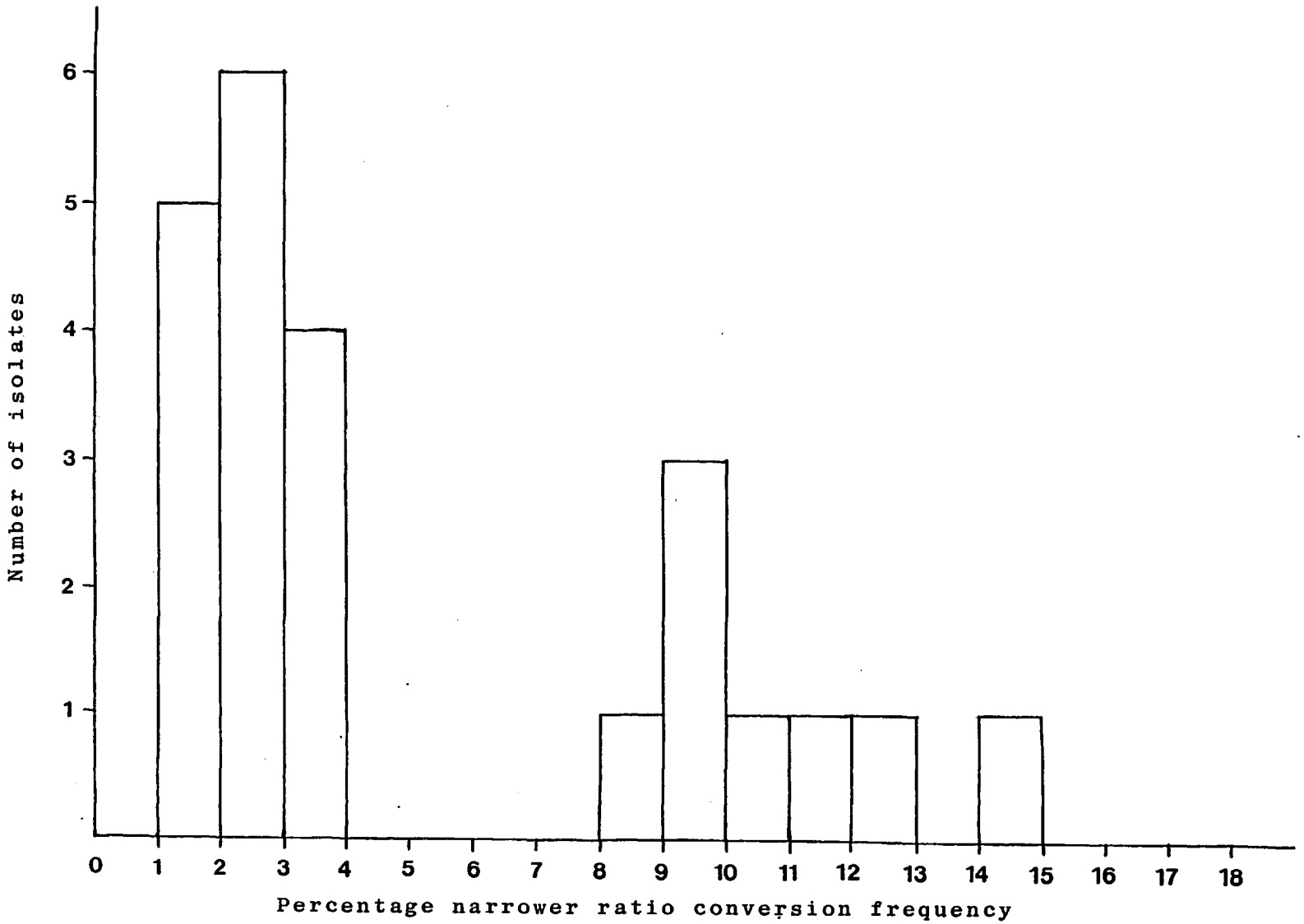


Fig. 2: Narrower ratio conversion frequencies of random wild-type isolates from cross 25-5R+ x 92- when crossed to BB9w9+

The observed values of 15:8 are an acceptable fit to such a 1:1 ratio (χ^2 = 2.13, p = 0.10-0.20, 1df) and so the data from this experiment are perfectly consistent with monogenic conversion control of BB9*w*9. However, the data are also consistent with two pairs of factors interacting, producing two phenotypes either in a 2:1, or more likely, a 3:1 ratio (χ^2 for 2:1 = 0.021, p = 0.80-0.90, 1df; χ^2 for 3:1 = 1.17, p = 0.20-0.30, 1df). Additional experiments should be carried out to distinguish between these possibilities. As a beginning, further random spores should be isolated from cross 25-5R+ × 92- to increase the sample size of crosses investigated. 23 is a relatively small number of random isolates and perhaps double this number would provide a large enough pool of crosses to distinguish between monogenic and di- or oligo-genic control of BB9*w*9 conversion. Figure 2 rules out polygenic control. It should also be remembered that if the conversion control is monogenic and the *ccf* itself undergoes conversion, as reported for *ccf*-2 by Helmi (1980), then a strict 1:1 ratio of HCF:LCF crosses in an experiment such as this could be perturbed. However, it would be unlikely for the perturbation to be large.

In addition, Table 6 shows quite a clear difference in conversion spectrum between the HCF and LCF crosses, with much less PMS in the LCF crosses. Disregarding those crosses with a total sample size of less than 500 octads, the relative frequency of PMS ranged from 3.96 to 25.14% in the LCF crosses. In fact, the latter value was an extreme, all other values being less than 19%. In the

HCF crosses the relative frequency of PMS ranged from 39.68 to 44.85% for all crosses except two, which gave values of 26.72 and 28.24% respectively. Frequencies of conversion to wild-type were lower in the LCF crosses, ranging from 55.96 to 82.08%, with one extreme value of 100%. In the HCF crosses the frequency range was 89.75 to 95.62%. Therefore in agreement with the earlier findings of Lamb and Ghikas (1979) the LCF crosses can broadly be assigned to conversion spectrum class A and the HCF crosses to class C.

In summary, these results point to the existence of at least a single conversion control factor acting on mutation BB9*w*9, with a reduction of gene conversion and PMS when the control factor is heterozygous in a cross. This control factor can be designated conversion control factor 5 or *ccf*-5 (since it was the fifth such factor to be discovered in the Pasadena strains of *Ascobolus immersus*), remembering that it may act on BB9*w*9 in conjunction with other factors.

Linkage relations of BB9*w*9 and *ccf*-5

Initially the assumption was made that the conversion control system acting on BB9*w*9 was monogenic and closely linked since this was the most straightforward hypothesis to test. The results from the experiments already outlined are in agreement with at least one genetic conversion control factor (*ccf*-5) acting on BB9*w*9 and nothing has conflicted with the assumption of close linkage between the *ccf* and the white mutation in question. The actual link-

age relationship between BB9*w*9 and *ccf*-5 was determined by analysis of the LCF cross BB9*w*9+ x 92-. This cross is doubly heterozygous for the BB9*w*9 mutation and the two forms of *ccf*-5:

$$\text{BB9}w9,\ ccf\text{-5}(A),\ + \times +^{\text{BB9}w9},\ ccf\text{-5}(B),\ -$$

Thus if BB9*w*9 and *ccf*-5 are closely linked, most, if not all, wild-type spores randomly isolated from 4+:4*w* non-convertant octads should carry *ccf*-5(*B*) and show conversion behaviour like the wild-type parent, while similarly isolated white spores should mostly carry *ccf*-5(*A*) and behave like the white-spored mutant parent. Any isolates showing altered conversion behaviour in suitable test crosses would be recombinants for the two sites in question.

Conversion behaviour of wild-type spores randomly isolated from 4+:4*w* octads from cross BB9*w*9+ × 92-

300 wild-type spores were randomly isolated from 4+:4*w* octads from cross BB9*w*9+ × 92-. Of these 300 spores 99 germinated and 45 of these isolates gave fertile back-crosses to BB9*w*9+. The results obtained from these crosses are shown in Table 7.

This Table shows that all 45 random wild-type isolates gave LCF when crossed to BB9*w*9+, with a range of conversion frequencies from 1.27 to 5.35%. The latter figure is an extreme value, being derived from a cross that gave an extremely small sample of dehisced octads. Odd high conversion frequencies have often been observed in crosses

TABLE 7: Conversion data of random wild-type isolates from 4+:4*w* octads from cross BB9*w*9+ x 92- when crossed to BB9*w*9+

Isolate No.	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1	97.90	1.31	0.35	0.44	-	2.10	1143
2	97.00	1.78	0.84	0.37	-	3.00	1067
3	98.36	0.88	0.51	0.25	-	1.64	792
10	98.06	1.34	0.24	0.36	-	1.94	823
11	96.79	2.41	0.34	0.46	-	3.21	873
17	98.70	0.87	0.33	0.11	-	1.30	922
21	98.01	0.90	0.72	0.36	-	1.99	554
24	98.02	1.18	0.54	0.27	-	1.98	1864
30	97.00	1.83	0.33	0.83	-	3.00	601
31	97.23	2.05	0.41	0.31	-	2.77	975
32	97.66	1.29	0.89	0.16	-	2.34	1238
33	97.53	1.41	0.71	0.35	-	2.47	1417
34	97.22	1.08	0.77	0.93	-	2.78	648
35	98.42	0.93	0.37	0.28	-	1.58	1078
37	97.92	1.39	0.23	0.46	-	2.08	2598
38	97.84	1.05	0.67	0.33	0.06	2.16	1804
41	97.97	1.02	0.34	0.68	-	2.03	295
49	98.25	1.27	0.36	0.06	0.06	1.75	1659
50	97.70	1.69	0.47	0.07	-	2.30	1477
53	97.81	1.09	0.73	0.36	-	2.19	549
54	97.33	2.05	0.51	0.10	-	2.67	975
57	97.59	1.55	0.43	0.43	-	2.41	1867
58	96.08	2.57	0.58	0.77	-	3.92	1555
59	98.73	0.72	0.18	0.36	-	1.27	1658
61	97.92	0.63	0.72	0.63	0.09	2.08	1108
62	97.53	1.59	0.22	0.66	-	2.47	2264
65	96.92	1.51	0.86	0.65	-	3.08	1395

TABLE 7: (Continued)

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
66	96.83	1.92	0.78	0.48	-	3.17	1671
68	97.69	1.31	0.70	0.30	-	2.31	996
70	98.69	0.87	0.27	0.16	-	1.31	1830
73	98.65	0.97	0.16	0.11	-	1.25	1847
77	97.22	2.14	0.43	0.21	-	2.78	468
78	98.67	0.92	0.29	0.12	-	1.33	1731
79	97.67	1.48	0.17	0.62	-	2.27	1761
81	95.52	2.69	0.90	0.90	-	4.48	335
82	96.14	2.84	0.79	0.16	0.08	3.86	1269
84	96.64	1.21	1.21	0.54	0.40	3.36	744
87	96.57	1.99	0.33	0.77	-	3.09	905
89	97.84	1.62	0.27	0.27	-	2.16	370
90	96.62	2.41	0.24	0.36	0.36	3.38	829
91	97.31	1.54	0.77	0.38	-	2.69	260
92	96.43	2.21	0.32	0.89	-	3.41	1904
94	97.49	2.09	-	0.31	-	2.41	955
96	94.65	3.34	1.67	0.33	-	5.35	299
99	97.13	2.23	-	0.64	-	2.87	314

showing poor fertility (Helmi, 1980). Therefore all the wild-type isolates showed conversion properties like their wild-type parent. No recombinants showing altered, high, conversion properties were observed among the 45 progeny tested.

Conversion behaviour of white mutant spores randomly isolated from 4+:4*w* octads from cross BB9*w*9+ × 92-

50 white mutant spores were randomly isolated from 4+:4*w* non-convertant octads from cross BB9*w*9+ × 92-. 18 of these spores germinated and 8 of these isolates gave fertile crosses with wild-type parental strain 92-. The data obtained from these crosses are displayed in Table 8.

From this Table it can be seen that all 8 random white spore isolates gave LCF when crossed to 92-, with a range of conversion frequencies from 1.06 to 2.31%. Therefore all the white spore isolates exhibited conversion properties like the white mutant parent. No recombinants showing altered conversion properties were observed among the 8 progeny tested. The experiment described below shows that any recombinants would have given *ccf*-5(*B*) × *ccf*-5(*B*) crosses which are high converting.

Combining the data from the above two experiments it can be seen that no progeny among the 53 tested showed recombination between BB9*w*9 and *ccf*-5. This confirms that these two sites are indeed closely linked. It is worth noting that not all 4+:4*w* octads analysed in an experiment such as this need be true non-convertant octads. In the

TABLE 8: Conversion data of random white spore isolates from 4+:4w octads from cross BB9w9+ x 92- when crossed to 92-

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1	98.49	0.93	-	0.58	-	1.51	860
4	98.94	0.70	-	0.30	0.06	1.06	1986
7	98.52	0.66	0.49	0.33	-	1.48	607
9	97.49	1.21	0.39	0.70	0.21	2.51	1208
10	97.92	1.18	0.38	0.44	0.08	2.08	570
13	97.69	1.27	0.51	0.35	0.18	2.31	1008
16	98.08	1.16	0.21	0.31	0.24	1.92	1321
17	97.98	1.21	0.28	0.42	0.11	2.02	847

absence of other suitable scorable markers, some could be correction 4:4s or aberrant 4:4s, which unless co-conversion of the *w* site and its *ccf* usually occur, would yield some recombinants for these sites. Aberrant 4:4s are not necessarily rare as Paquette (1978) pointed out that such asci comprised more than 40% of postmeiotic segregations of the *b2*A4 mutation in the European strains of *Ascobolus immersus*. However, in the Pasadena strains, Ghikas and Lamb (1977) reported that the frequency of ab4:4 asci for the locus I mutation *w*78 was only 0.08% in both LCF and HCF crosses. The results obtained in this experiment indicate that correction and aberrant 4:4 octads cannot be frequent unless their occurrence is masked by co-conversion of BB9*w*9 and *ccf*-5 for which there is circumstantial evidence.

The experiments described already in this Chapter have furnished data on the conversion behaviour (frequency and spectrum) of mutation BB9*w*9 in two types of cross:

1) *w*, *ccf*-5(*A*) × + , *ccf*-5(*B*) LCF

2) *w*, *ccf*-5(*A*) × + , *ccf*-5(*A*) HCF

It was therefore necessary to determine the conversion behaviour of BB9*w*9 in two remaining types of cross:

3) *w*, *ccf*-5(*B*) × + , *ccf*-5(*B*)

4) *w*, *ccf*-5(*B*) × + , *ccf*-5(*A*)

Conversion behaviour of BB9*w*9 in *ccf*-5(*B*) × *ccf*-5(*B*) crosses

Before the conversion behaviour of BB9*w*9 in *ccf*-5(*B*) × *ccf*-5(*B*) crosses could be determined, suitable *ccf*-5(*B*) carrying derived wild-type tester strains of both mating types had to be made available. Strain 92- was designated as one of the tester strains since it was of the required *ccf*-5 genotype and perfectly fertile. However, it was necessary to construct a *ccf*-5(*B*) wild-type strain of (+) mating type.

Isolation of *ccf*-5(*B*) derived wild-type strains of (+) mating type

From the experiment designed to test the linkage relations of BB9*w*9 and *ccf*-5, wild-type spores had been randomly isolated from 4+:4*w* octads from cross BB9*w*9+ $\left[ccf\text{-}5(A)\right]$ × 92- $\left[ccf\text{-}5(B)\right]$. 99 isolates were available of which 45 had given fertile backcrosses to BB9*w*9+. All of these (-) mating type derived wild-types carried *ccf*-5(*B*). Of the remaining 54 isolates, 10 were selected and crossed to a BB9*w*9- $\left[ccf\text{-}5(A)\right]$ re-isolate known to show good fertility in other crosses. Altogether 7 fertile crosses resulted and as can be seen from Table 9, all crosses were low converting as expected, so all the wild-type segregants must have carried *ccf*-5(*B*). The cross involving segregant number 19 was the most fertile and this isolate was selected as a suitable wild-type *ccf*-5(*B*) tester of (+) mating type, and designated as strain 92-19+.

TABLE 9: Conversion data of random wild-type isolates from 4+:4*w* octads from cross BB9*w*9+ x 92- when crossed to 1BB9*w*9-

Isolate No.	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
4	96.68	2.82	0.20	0.30	-	3.32	601
19	97.95	1.65	0.15	0.20	0.05	2.05	1999
40	98.21	1.19	0.30	0.30	-	1.79	1271
52	97.05	1.95	0.41	0.49	-	2.95	843
69	97.45	1.83	0.12	0.55	0.05	2.55	951
85	97.82	1.69	0.18	0.31	-	2.18	745
98	96.94	1.98	0.36	0.52	0.20	3.06	1009

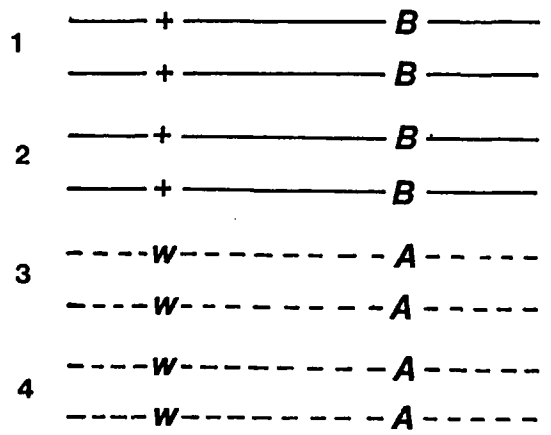
Conversion behaviour of white spores isolated from 2+:6*w* octads from cross BB9*w*9+ × 92-

No BB9*w*9 strains known to carry *ccf*-5(*B*) were available so a direct test of the conversion behaviour of mutation BB9*w*9 in *ccf*-5(*B*) × *ccf*-5(*B*) crosses was not possible. First it was necessary to obtain the required BB9*w*9 strains. This was achieved by isolating white spores from 2+:6*w* octads from the LCF cross BB9*w*9+ × 92-, which can be written:

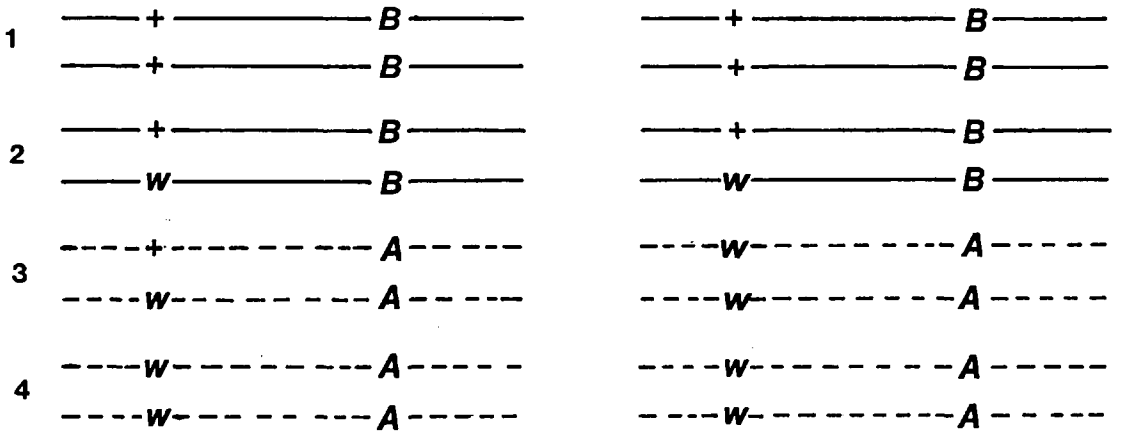
w, *ccf*-5(*A*),+ × +, *ccf*-5(*B*),-

From Figure 3 it can be seen that analysis of the 6 white spores from 2+:6*w* convertant octads (whether they arise by symmetrical or asymmetrical H-DNA formation) should reveal that 4 spores carry *ccf*-5(*A*) and give LCF crosses with *ccf*-5(*B*) wild-type strains, while 2 spores should carry *ccf*-5(*B*) and hence show altered conversion characteristics in crosses to the same tester strains. This will only be observed in all such octads if no co-conversion of the two closely linked sites occurs.

Initially white spore germination in this experiment was extremely poor, but was later improved by the establishment of optimum germination conditions for BB9*w*9 mutant spores (see PART TWO of this thesis). Altogether, white spores were isolated from 254 2+:6*w* octads of which 98 germinated and 47 of these isolates gave fertile crosses with *ccf*-5(*B*) derived wild type strains. The data obtained from these crosses can be seen in Table 10.



Formation of hybrid DNA at the w site



Symmetrically Asymmetrically

Correction of mispairs to w

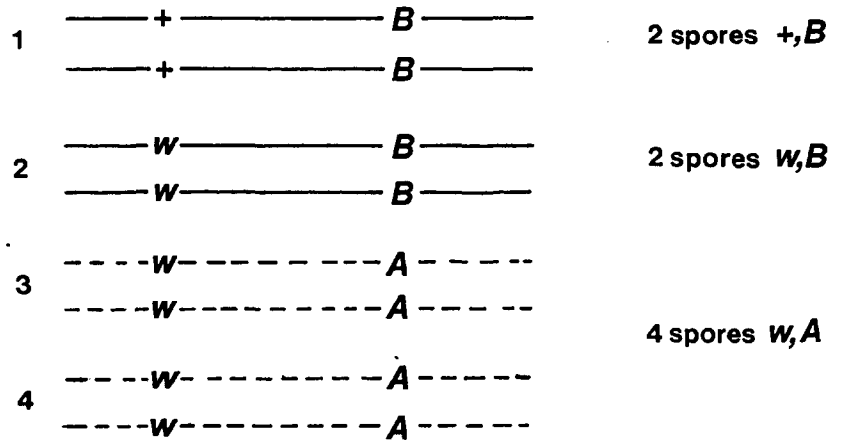


Fig. 3: Origin of white mutant segregants with altered conversion properties in 2+:6w octads from a + × w cross, where the conversion control factor is heterozygous, closely linked, but not co-converting with the w site.

TABLE 10: Conversion data of white spore isolates from 2+:6*w* octads from cross BB9*w*9+ x 92- when crossed to *ccf*-5(*B*) carrying wild-type strains 92- or 92-19+.

Isolate No. (octad.spore)	Octad classes (+:*w*),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
11.6-	96.81	1.87	0.70	0.47	0.16	3.19	19.75	73.35	1286
73.2-	97.95	1.65	0.15	0.20	0.05	2.05	12.20	90.24	1999
88.1+	97.21	1.76	0.74	0.29	-	2.79	10.39	73.48	680
91.2+	98.36	0.91	0.55	0.18	-	1.64	10.98	66.46	550
94.1+	88.39	6.41	2.67	1.79	0.66	11.54	21.23	71.06	2730
108.2-	97.64	1.45	0.76	0.08	0.08	2.36	6.78	64.83	1312
110.2-	95.67	2.89	0.87	0.58	-	4.33	13.39	80.14	693
122.2-	97.89	1.58	0.35	0.18	-	2.11	8.53	83.41	569
131.1-	98.59	1.18	-	0.24	-	1.41	17.02	100.00	676
171.1+	97.71	1.60	0.48	0.16	0.05	2.29	9.17	76.86	1879
185.6+	98.83	0.70	0.47	-	-	1.17	0.00	59.83	426
186.3+	97.55	1.84	0.17	0.45	-	2.45	18.37	93.47	1795
187.4+	98.06	1.14	0.34	0.46	-	1.94	23.71	82.47	1752
190.2-	98.21	1.19	0.30	0.30	-	1.79	16.76	83.24	671
190.3+	97.73	1.71	0.05	0.51	-	2.27	22.47	97.80	2160
201.4-	98.52	0.99	0.08	0.33	0.08	1.48	27.70	89.19	2433
202.6-	92.14	5.05	1.05	1.67	0.10	7.86	22.52	85.50	2098
203.2-	98.67	0.84	0.23	0.27	-	1.33	20.30	83.46	2624
204.5+	90.22	6.21	1.71	1.47	0.23	9.62	17.67	79.83	1289
205.1-	97.24	0.69	0.69	1.38	-	2.76	50.00	75.00	145
205.3+	98.76	0.41	0.58	0.25	-	1.24	20.16	53.25	1210
205.6-	98.61	1.16	-	0.23	-	1.39	13.61	100.00	2589
206.5-	98.52	1.24	-	0.20	0.03	1.48	15.54	97.30	2973
208.6-	97.27	1.54	0.27	0.85	0.08	2.73	34.07	87.55	2602
209.4+	97.55	1.35	0.43	0.58	0.10	2.45	27.76	78.78	2078
210.6+	97.55	1.60	0.33	0.53	-	2.45	21.63	86.94	2444

TABLE 10 (continued)

Isolate No. (octad.spore)	Octad classes (+:w),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
213.1-	98.10	1.19	0.08	0.60	-	1.87	32.09	95.72	3693
217.1+	97.27	1.46	0.58	0.68	-	2.73	24.91	78.39	1027
217.2-	91.65	5.34	1.10	1.71	0.21	8.35	22.99	84.43	4383
217.4-	98.77	0.62	0.12	0.49	-	1.23	39.84	90.24	813
217.5-	98.35	1.14	0.12	0.27	0.12	1.65	23.64	85.45	2549
218.6+	95.79	2.61	0.93	0.67	-	4.21	15.91	77.91	1187
221.1-	98.87	0.90	0.05	0.19	-	1.13	16.81	96.46	2121
221.2-	96.94	2.25	0.36	0.45	-	3.06	14.71	88.24	1112
224.3-	97.25	1.57	0.39	0.78	-	2.75	28.36	85.45	255
224.4-	98.20	1.07	0.53	0.20	-	1.80	11.11	70.56	1502
227.3+	97.85	1.18	0.63	0.34	-	2.15	15.81	70.70	2452
227.6+	97.77	1.26	0.43	0.44	0.10	2.23	24.22	76.23	1865
237.4+	98.64	0.68	0.45	0.23	-	1.36	16.91	66.91	441
248.2-	98.60	0.78	0.08	0.54	-	1.40	38.57	94.29	1289
248.4+	98.09	1.56	0.17	0.09	0.09	1.91	9.42	86.39	1152
248.6+	96.41	1.79	-	1.79	-	3.59	50.00	100.00	390
250.6+	98.33	1.23	0.16	0.27	-	1.67	16.17	89.82	3662
251.1-	98.70	0.78	0.35	0.17	-	1.30	13.08	73.08	1156
252.2-	98.06	1.32	0.53	0.09	-	1.94	4.64	72.68	1135
252.3+	95.43	2.65	1.03	0.74	0.15	4.57	19.47	74.18	679
253.1+	97.41	1.69	0.54	0.36	-	2.59	13.90	79.15	1660

Isolates with altered conversion frequencies are underlined

43 crosses show low conversion frequency (1.13 - 4.57% NRCF)
4 " " high " " (7.86 - 11.54% NRCF)

X^2 (for expected ratio of 2LCF: 1 altered CF among crosses) = 11.94
$p < 0.01$ (1df)

Due to poor ascospore germination, complete analysis of the 6 white spores in any octad was impossible. Thus the data must be treated as originating from white spores randomly isolated from a population of 2+:6*w* octads. In this case a ratio of 2:1 for LCF: altered CF crosses would be expected. From Table 10 it can be seen that of the 47 white spore isolates, 43 gave LCF crosses with *ccf*-5(*B*) wild-type testers (with conversion frequencies of 1.13 to 4.57%) while 4 isolates showed altered, elevated conversion frequencies (of 7.86 to 11.54%) in similar crosses. These data do not fit the expected 2:1 ratio (χ^2, applying Yates' correction = 11.94, $p < 0.01$, 1df). Nevertheless, it would still seem that the 4 isolates showing altered, enhanced conversion frequencies have arisen by conversion at the BB9*w*9 site in 2+:6*w* octads and therefore carry *ccf*-5(*B*). In this case it can be deduced that BB9*w*9 shows a high conversion frequency in *ccf*-5(*B*) × *ccf*-5(*B*) crosses. In fact, the conversion frequency is very similar to that observed in *ccf*-5(*A*) homozygous crosses, ranging from 7.86 to 11.54% in this experiment. However, the conversion spectrum does not resemble that of *ccf*-5(*A*) homozygous crosses, appearing more similar to that occurring in +,*ccf*-5(*B*) × *w*, *ccf*-5(*A*) LCF crosses, i.e., PMS is relatively infrequent ranging from 17.67 to 22.99% (see Table 10).

It is important to consider why far fewer segregants with altered conversion properties were detected than was expected. Of the 4 altered segregants, 2 were of (+) mating type and 2 of (-). Thus such segregants were

detectable by crossing to both of the derived wild-type tester strains used in this experiment, ruling out a specific effect by one of the testers such that no *ccf*-5(*B*) white segregants could be detected using that particular tester strain. A second, simple explanation would involve frequent co-conversion of BB9*w*9 and *ccf*-5. Experiments already described have suggested some co-conversion of these sites, although not to the extent necessary to explain these results. This in itself does not rule out co-conversion as the cause of the paucity of altered conversion segregants in this experiment. It is worth noting that in previous experiments it was always high converting segregants (in appropriate test crosses) that were rarer than expected, although not significantly so. This would of necessity be so, since wild-type or white mutant segregants with altered conversion properties can only be isolated from crosses heterozygous for the closely linked *ccf*-5, which are low converting. Thus, if progeny from such a cross are crossed to the appropriate parental strain as a tester, parental-type segregants will give LCF crosses and recombinants show HCF. However, the results from this experiment again raise the possibility that a second conversion control factor may be acting on BB9*w*9 and interacting with *ccf*-5. In certain crosses the genotypes of the second *ccf* would act to suppress the conversion-enhancing combinations of *ccf*-5. It might be revealing to repeat this experiment with *ccf*-5(*A*) derived wild-type tester strains to see whether any inconsistencies are revealed. If only

ccf-5 is involved in BB9*w*9 conversion control, all isolates which previously gave LCF crosses should now give HCF. If some did not, evidence for a second *ccf* able to reduce BB9*w*9 conversion would be strong.

Conversion behaviour of BB9*w*9 in +, *ccf*-5(*A*) × *w*, *ccf*-5(*B*) crosses

It has been shown that mutation BB9*w*9 shows a low conversion frequency and reduced postmeiotic segregation in +, *ccf*-5(*B*) × *w*, *ccf*-5(*A*) crosses (compared with +, *ccf*-5(*A*) × *w*, *ccf*-5(*A*) crosses) i.e. with *ccf*-5(*A*) in *cis* to the mutation. It does not necessarily follow from this that BB9*w*9 would show the same conversion behaviour in similar heterozygous +, *ccf*-5(*A*) × *w*, *ccf*-5(*B*) crosses, but with *ccf*-5(*A*) in *trans* to the mutation. The *A* and *B* forms of *ccf*-5 could show *cis/trans* position effects on BB9*w*9 conversion, either in terms of conversion frequency, spectrum or both. Such *cis/trans* position effects on the conversion of locus I mutations by the closely linked *ccf*-2 factors were reported by Lamb and Helmi (1978). It was thus endeavoured to determine the conversion behaviour of BB9*w*9 in crosses of the +, *ccf*-5(*A*) × *w*, *ccf*-5(*B*) type.

From the previous experiment 4 BB9*w*9 isolates were available known to carry *ccf*-5(*B*). Two were of (+) mating type and two of (-). These mutant isolates were crossed to derived wild-type strains 7R7- and 25-5R+ respectively, both shown to carry *ccf*-5(*A*). Three of

TABLE 11: Conversion data from +, *ccf*-5(*A*) x BB9*w*9, *ccf*-5(*B*) crosses. The derived wild-type tester strains were 25-5R+ and 7R7−.

Isolate No. (octad.spore)	Octad classes (+:*w*),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
94.1+	97.79	1.15	0.66	0.30	0.10	2.21	18.10	65.61	6023
204.5+	98.40	1.02	0.28	0.25	0.03	1.58	17.72	80.38	3616
217.2−	98.06	0.98	0.48	0.44	0.03	1.94	24.23	73.20	3150

the four crosses were fertile and the conversion data obtained from these are shown in Table 11.

This Table shows that +, *ccf*-5(*A*) × *w*, ccf-5(*B*) crosses are low converting with relatively infrequent postmeiotic segregation and conversion overwhelmingly to wild-type, but not so overwhelmingly as in the *ccf*-5(*A*) homozygous crosses. In all these respects the conversion behaviour of BB9*w*9 is closely similar to that seen in +, *ccf*-5(*B*) × *w*, *ccf*-5(*A*) crosses, with no noticeable *cis/trans* position effects.

Originality of *ccf*-5

ccf-5 can be shown to be a truly original conversion control factor and not in any way associated with the four hitherto known *ccfs* in the Pasadena strains of *Ascobolus immersus* :

ccf-1 determines the conversion behaviour of closely linked white ascospore pigmentation mutation *w*62 in a way broadly similar to the action of *ccf*-5 on BB9*w*9, which is closely linked to *ccf*-5. However, mutations BB9*w*9 and *w*62 were mapped in this work and shown to be unlinked (R.F. = 50.35%) and thus *ccf*-1 and *ccf*-5 must be quite separate, distinct factors. *ccf*-2 is closely linked to locus I and indeed it was suggested by Helmi (1980) that it might actually lie within that locus. Ghikas (1978) found that BB9*w*9 was unlinked to locus I mutation *w*78 and so *ccf*-5 cannot be confused with *ccf*-2. Furthermore, the data of Lamb and Ghikas (1979) presented in Table 2 of this work showed that BB9*w*9 could show high or low conver-

sion frequency when crossed to different derived wild-type strains carrying the same *ccf*-2 factor. Helmi (1980) reported that *ccf*-3 is linked to the mating type locus, while in this work BB9*w*9 and hence *ccf*-5 showed no signs of linkage to the mating type. The situation is not so unambiguous for *ccf*-4 which is reported as being loosely linked to locus I (Helmi, 1980). As already mentioned, BB9*w*9 (and therefore *ccf*-5) is unlinked to locus I mutation *w*78. It cannot conclusively be deduced from this that *ccf*-4 and *ccf*-5 are unlinked and therefore of necessity distinct, although it is likely since the modes of action of the two factors appear quite distinct. To conclusively determine that the two factors are distinct, 5 different "Super" derived wild-type strains (of genotype *ccf*-3(*E*), *ccf*-4(*r*)) were crossed to the same BB9*w*9 mutant strain. All 5 crosses were fertile and the results obtained are shown in Table 12.

This Table shows that different strains of the same *ccf*-4 genotype can give high or low conversion frequency when crossed to the same BB9*w*9 mutant strain. Two crosses in the Table are HCF while three are LCF. These results clearly indicate the non-identity of *ccf*-4 and *ccf*-5.

Thus, in summary, it can be concluded that *ccf*-5 is a truly novel factor in the Pasadena strains of *Ascobolus immersus*. It is quite distinct from the four previously known conversion control factors.

TABLE 12: Conversion data of BB9*w*9+ when crossed to various "Super" derived wild-type strains

Super strain	Octad classes (+:*w*),%					%NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
659	98.86	0.22	0.10	0.65	0.17	1.14	2912
474	89.41	7.31	0.57	2.63	-	10.51	1410
391	91.80	4.85	0.36	2.71	0.17	8.09	1802
7114	98.39	1.15	0.26	0.20	-	1.61	3471
1116	98.12	1.19	0.50	0.20	-	1.88	1093

TABLE 13: Conversion behaviour of BB9*w*8+, BB9*w*9+, BB*w*11+ and BB*w*21+ when crossed to derived wild-type strain 92- (*ccf*-5(*B*))

Mutant strain	Octad classes (+:*w*),%					%NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
BB9*w*8+	97.94	1.52	0.48	0.35	0.06	2.06	3615
BB9*w*9+	96.82	1.68	0.55	0.68	0.23	3.14	4711
BB*w*11+	98.90	0.62	0.26	0.21	0.02	1.10	5792
BB*w*21+	98.26	0.93	0.81	-	-	1.74	864

Conversion behaviour of mutations BB9*w*8, BB*w*11 and BB*w*21 in response to *ccf*-5

In the Preliminaries to this Chapter, based on the data of Lamb and Ghikas (1979), it was stated that the four mutations BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 all exhibited a similar conversion spectrum in HCF crosses and a similar spectrum in LCF crosses, while the spectrum differed between the two types of cross, with less postmeiotic segregation (see Table 2) in the LCF crosses. Furthermore, the conversion frequencies were broadly similar in HCF crosses (8.31-15.31%) and very similar in LCF crosses (1.39-2.10%). The four mutations therefore exhibited very similar conversion properties. They were all UV-induced and isolated by Ghikas (1978) from the same parental cross involving derived wild-type strains BBR1- and 9r+, each carrying *ccf*-2(*P*). Ghikas (1978) was unable to map the four mutations with respect to each other due to infertile crosses. Overall, this situation suggested four possibilities:

1. The four mutations are merely re-isolations of the same mutation.
2. " " " are closely linked, responding to the same *ccf*(*s*)
3. " " " are unlinked, responding to the same *ccf*(*s*)
4. " " " are unlinked, responding to different *ccfs* in a similar manner.

In this section the aim is to distinguish between these possibilities. Obviously a determination of the linkage

relations of the 4 mutations should be an initial starting point and could rule out two of the possibilities at once and should have been simple. This was not so and indirect means had to be resorted to. These experiments will be described later.

Conversion behaviour of BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 strains when crossed to the same derived wild-type strain

Lamb and Ghikas (1979) were unable to cross isolates of all four mutant strains to the same derived wild-type strain. However, this was possible in this work. The derived wild-type strain used was 92-, known to carry *ccf*-5(*B*). The conversion data obtained from these crosses are shown in Table 13.

From this Table it can be seen that all four mutant strains (BB9*w*8+, BB9*w*9+, BB*w*11+ and BB*w*21+) gave low conversion frequencies when crossed to 92-. If at least one mutant strain had shown a high conversion frequency, either this strain was not responding to *ccf*-5 or a second *ccf* was acting to reduce gene conversion in that cross. This experiment was a useful introduction but something far more rigorous was necessary to determine whether all four mutations in question respond to *ccf*-5.

Conversion behaviour of BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 strains when crossed to random wild-type isolates from the cross 25-5R+ × 92-

23 random wild-type isolates were available from the cross 25-5R+ (*ccf*-5(*A*)) × 92-(*ccf*-5(*B*)). Of these isolates 15 were thought to carry *ccf*-5(*B*) and 8 *ccf*-5(*A*), by virtue of the data obtained when they were crossed to BB9*w*9+. From these 23 isolates, 10 were selected and crossed to strains BB9*w*8+, BB*w*11+ and BB*w*21+. These crosses were performed in order to show whether a particular isolate, giving, say, LCF with BB9*w*9+ would also give LCF with all the other three mutant strains, or whether it would give HCF with some of the other mutants, or vice-versa. The data so obtained from these crosses are given in Table 14, including that from the original crosses involving BB9*w*9+.

From this Table it can be seen that when any random wild-type isolate from cross 25-5R+ × 92- gave HCF when crossed to BB9*w*9+, it also gave HCF when crossed to BB9*w*8+, BB*w*11+ and BB*w*21+ mutant strains. Similarly, it can be seen that if a wild-type isolate gave LCF when crossed to BB9*w*9+, it also gave LCF when crossed to the other three mutant strains (not all isolates gave fertile crosses with all four mutant strains). Additionally, the conversion spectra were similar within any group of four crosses to one wild-type isolate. This was particularly true of HCF crosses, where PMS was frequent. It was less frequent in all LCF crosses, with anomalous results of zero PMS or 40% PMS in crosses where very small sample sizes are being considered. In Table 14 only

TABLE 14: Conversion data of random wild-type spores isolated from cross 25-5R+ x 92-; isolates were crossed to BB9*w*8+, BB9*w*9+, BB*w*11+ and BB*w*21+ mutant strains

Isolate No.	Crossed to	Octad classes (+:*w*),%					%NRCF	Relative % PMS	Total octads
		4:4	6:2	2:6	5:3	3:5			
3	BB9*w*9+	96.97	1.57	1.33	0.12	-	3.03	3.96	826
	BB*w*21+	97.47	1.48	0.96	-	0.09	2.53	3.55	1146
5	BB9*w*8+	93.05	3.57	0.94	2.44	-	6.95	35.10	532
	BB9*w*9+	88.36	5.70	1.33	4.77	0.45	11.49	45.43	4176
	BB*w*11+	89.11	5.69	1.14	3.90	0.16	10.89	37.28	615
	BB*w*21+	84.23	6.98	2.22	5.61	0.95	15.77	41.60	945
7	BB9*w*9+	85.86	4.55	6.06	2.02	1.52	14.14	25.04	198
	BB*w*21+	84.78	10.87	2.17	2.17	-	15.22	14.26	46
8	BB9*w*8+	100.00	-	-	-	-	0.00	0.00	121
	BB9*w*9+	97.19	1.66	0.96	0.13	0.06	2.81	6.76	1564
	BB*w*11+	98.40	0.64	0.32	0.64	-	1.60	40.00	313
	BB*w*21+	97.67	2.33	-	-	-	2.33	0.00	257
14	BB9*w*8+	97.92	0.89	0.97	0.07	0.15	2.08	10.57	1343
	BB9*w*9+	96.96	1.91	0.59	0.42	0.09	3.04	16.77	4245
	BB*w*11+	98.36	0.98	0.59	-	0.07	1.64	4.27	1526
	BB*w*21+	98.30	1.14	0.47	0.09	-	1.70	5.29	1057
16	BB9*w*8+	91.11	5.21	0.80	2.62	0.16	8.80	31.60	3126
	BB9*w*9+	90.16	5.01	0.80	3.87	0.14	9.81	40.88	3516
	BB*w*11+	89.94	4.74	1.33	3.61	0.38	10.06	39.66	527
	BB*w*21+	87.21	5.81	2.33	3.49	1.16	12.79	36.36	86
23	BB9*w*8+	98.02	1.25	0.51	0.22	-	1.98	11.11	1361
	BB9*w*9+	98.21	1.12	0.22	0.34	0.11	1.79	25.14	894
	BB*w*11+	98.29	0.79	0.66	0.13	-	1.57	8.28	762
	BB*w*21+	96.47	3.14	0.39	-	-	3.53	0.00	255
25	BB9*w*8+	98.32	-	0.68	-	-	0.68	0.00	293
	BB9*w*9+	97.61	1.39	0.62	0.39	-	2.39	16.32	1295
	BB*w*11+	99.68	0.32	-	-	-	0.32	0.00	314
27	BB9*w*8+	89.71	4.79	1.86	3.39	0.26	10.29	35.47	3072
	BB9*w*9+	90.02	5.26	0.63	3.58	0.38	9.86	40.16	5529
	BB*w*11+	89.53	5.18	1.46	3.15	0.56	10.36	35.81	888
	BB*w*21+	88.18	7.03	1.87	2.54	0.34	11.78	24.45	2673
30	BB9*w*9+	96.92	2.02	0.63	0.43	-	3.08	13.96	2530
	BB*w*11+	99.21	0.79	-	-	-	0.79	0.00	382
	BB*w*21+	100.00	-	-	-	-	0.00	0.00	55

the relative frequency of PMS component of conversion spectrum is dealt with since the degree of conversion to wild-type is far less diagnostic of HCF and LCF crosses. However, in terms of PMS, the conversion spectrum clearly differed between HCF and LCF crosses.

Thus it seems almost certain that mutations BB9*w*8, BB*w*11 and BB*w*21 respond to the same conversion control system as BB9*w*9, but this experiment does not resolve whether the four mutations are unlinked, linked or are even re-isolations of the same mutation.

Linkage relations of BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21

Initially the intercrosses between the original strains carrying these mutations, performed without success by Ghikas (1978), were repeated, again unsuccessfully. Following this, strains mutant for BB9*w*8, BB*w*11 and BB*w*21 were re-isolated. Altogether 15 strains of each were obtained. Many re-isolates of BB9*w*9 of known mating type were already available. All 15 isolates of BB9*w*8, of BB*w*11 and of BB*w*21 were individually crossed to fertile tester strains of BB9*w*9 of both mating types. None of the crosses was fertile. These crosses were repeated, using dilute dung medium (instead of *Ascobolus* crossing medium) and also crossing medium to which liquid dung medium was added 10 days after inoculation. Neither modified medium produced any fertile crosses. At this stage it was decided to try two alternative methods of bulk crossing, which do not yield so much information but might show greater success.

The bulk crosses

1. 15 isolates each of BB9*w*8, BB*w*11 and BB*w*21 were available and 15 similar isolates of BB9*w*9 of both mating types were obtained from the stock collection. Altogether 10 bulk crossing plates were set up, with 3 replicates of each:

1	BB9*w*8	×	BB9*w*9	
2	"	×	BB*w*11	
3	"	×	BB*w*21	Intercrosses and selfcrosses combined
4	BB9*w*9	×	BB*w*11	
5	"	×	BB*w*21	
6	BB*w*11	×	BB*w*21	
7	BB9*w*8	×	BB9*w*8	
8	BB9*w*9	×	BB9*w*9	Self crosses only
9	BB*w*11	×	BB*w*11	
10	BB*w*21	×	BB*w*21	

For each intercross plate a single inoculum from each of the 15 isolates of each of the two mutant strains was placed randomly on the crossing plate, making 30 inocula in all. On such a crossing plate, e.g. BB9*w*8 × BB9*w*9, three types of cross are possible ; BB9*w*8 × BB9*w*8 and BB9*w*9 × BB9*w*9 self crosses and more importantly, BB9*w*8 × BB9*w*9 intercrosses. If a mixture of all three types of cross occur, most octads produced would be 0:8s. However, if the two mutations are <u>not</u> homoalleles, then intercrosses should be able to produce 4:4 and 2:6 octads. Such a bulk cross could at least demonstrate that the mutations concerned are not homoalleles, which may or may not be re-isolations of the same mutation.

For the self crosses, only the 15 inocula from the 15 isolates of a particular mutant were present on any one crossing plate. These self crosses were set up to provide material for the second method of bulk crossing attempted. All of these bulk crosses were infertile.

2. This method of bulk crossing involved inoculating germinating ascospores on the same sort of bulk crossing plates as described above. In this way many more isolates would be available to participate in the bulk crossing. Unfortunately, the task was complicated in that no fertile $w \times w$ crosses had been produced for any of the four mutants, even by the earlier bulk crossing method. Ascospore suspensions can easily be prepared from $w \times w$ crosses, consisting only of white spores. Such a suspension can directly be sown on germination medium. Since this was not possible, $+ \times w$ crosses were performed for each of the four mutants and white spores were manually isolated and transferred to a spore suspension. This suspension was sown on plates of dung germination medium at a density of about 200 spores per plate. Under appropriate conditions (5 hours heatshock at 50°C - see Part Two) 40-50% of the spores germinated, and these germlings were used to set up the 6 Intercross type of bulk cross listed above. Each plate contained about 50 germlings for each of the mutants under test. No fertile bulk crosses resulted.

This lack of fertile $w \times w$ self- or inter-crosses was quite amazing. One possible explanation suggests itself. Suppose that all four white mutations really are re-isolations

of the same mutation. Perhaps the BB9*w*9 mutation shows pleiotropic effects such that in addition to determining spore colour, it can also confer sterility - or at least very reduced fertility - in crosses homozygous for it. Alternatively, the sterility factor might be closely linked to BB9*w*9. If the latter hypothesis were true, then it should be possible to separate the *w* site and the sterility factor by conversion at the *w* site. With this idea in mind, white mutant spores with altered conversion characteristics isolated from 2+:6*w* octads from cross BB9$_w$9+× 92- were used in further attempts to get fertile crosses of at least BB9*w*9 to BB9*w*8, BB*w*11 and BB*w*21. BB9*w*9 isolates number 94.1+, 202.6- 204.5+ and 217.2- (see Table 10) were used in a further form of the bulk cross. These BB9*w*9 convertant isolates were inoculated on to 3 sets of plates and the 15 isolates of each of BB9*w*8, BB*w*11 and BB*w*21 were inoculated on to one set of plates each. There were 3 replicates in each set. Again no fertile crosses resulted. Thus if a recessive sterility factor does exist in these mutant strains, it may have co-converted with BB9*w*9 or it may be a pleiotropic effect of BB9*w*9 itself. Alternative explanations are possible. Perhaps several genes each reducing fertility quantitatively and recessively could be involved. If the strains used are highly inbred and homozygous, then sterility could result, but the strains are not inbred. Senescence can be ruled out since most of the mutant strains used show good fertility with derived wild-type strains, even when the same strains have been used over a number

of years, e.g. BB9*w*9+ and 92-.

In summary, all direct methods of mapping BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 failed due to cross infertility. One less direct method of mapping these mutations remained to be tried.

Linkage relations of BB9*w*8, BB*w*11 and BB*w*21 to *ccf*-5

The crosses and data described indicate that mutations BB9*w*8, BB*w*11 and BB*w*21 respond to *ccf*-5 in the same way as BB9*w*9, to which *ccf*-5 is closely linked. Therefore experiments were carried out to determine the linkage relations of BB9*w*8, BB*w*11 and BB*w*21 to *ccf*-5. (+) mating type strains of all these 3 mutations gave LCF crosses with derived wild-type strain 92-(*ccf*-5(*B*)). These crosses must therefore have been heterozygous for the *A/B* forms of *ccf*-5. For each of the three crosses, wild-type spores were randomly isolated from 4+:4*w* octads and backcrossed to the white mutant parent. About 20 wild-type isolates were obtained for each of the three crosses and each was backcrossed to its white mutant parent. 11 isolates gave fertile crosses with the BB9*w*8+ parental strain and 9 isolates showed fertility with the BB*w*21+ parental strain. The results so obtained are displayed in Tables 15 and 16 respectively. None of the random wild-types isolated from cross BB*w*11+ × 92- showed fertility with the BB*w*11+ parental strain.

TABLE 15: Conversion data of random wild-type isolates from 4+:4w octads from cross BB9w8+ x 92- when backcrossed to BB9w8+

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
11	99.27	0.49	-	0.24	-	0.73	411
12	98.48	0.51	0.25	0.76	-	1.52	394
14	98.68	0.83	0.26	0.16	0.07	1.32	1384
15	98.61	0.93	0.46	-	-	1.39	216
17	98.12	1.57	-	0.31	-	1.88	619
20	96.30	2.22	-	0.74	0.74	3.70	235
24	97.98	1.52	0.18	0.22	0.10	2.02	1006
27	99.14	0.48	0.16	0.16	0.05	0.86	1859
29	98.36	1.20	0.20	0.24	-	1.64	824
30	98.34	1.15	0.35	0.16	-	1.66	1235
32	97.99	1.38	0.50	-	0.13	2.01	797

TABLE 16: Conversion data of random wild-type isolates from 4+:4w octads from cross BBw21+ x 92- when backcrossed to BBw21+

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
3	98.22	1.12	0.41	0.20	0.05	1.78	1899
5	96.13	3.00	0.23	0.65	-	3.87	1155
8	98.74	0.88	0.23	0.15	-	1.26	605
13	98.46	1.02	0.32	0.20	-	1.54	839
14	99.24	0.61	0.09	0.05	-	0.76	2118
17	98.89	0.53	0.34	0.23	-	1.11	2621
18	98.19	1.08	-	0.72	-	1.81	477
21	98.93	0.72	-	0.35	-	1.07	349
27	99.45	0.40	0.08	0.08	-	0.55	1265

Although the number of crosses is small, it can be seen from Table 15 that all 11 crosses were LCF, i.e. the wild-type isolates carried *ccf*-5(*B*) like the derived wild-type parental strain 92-. No recombinants were observed among the 11 isolates tested, indicating that BB9*w*8 is linked to *ccf*-5.

Similarly in Table 16 it can be seen that all 9 isolates gave LCF crosses with the BB*w*21+ parental tester strain. No recombinants were observed. Thus BB*w*21 appears linked to *ccf*-5.

Summary

Taking the results from all the above experiments together it is clear that all four mutations BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 respond similarly to *ccf*-5. BB9*w*9 is certainly closely linked to *ccf*-5 and BB9*w*8 and BB*w*21 must be linked to it too. It seems likely that further experiments will reveal that BB*w*11 and *ccf*-5 are also linked. Thus it would appear that the four mutations in question are either closely linked or are re-isolations of the same mutation. Circumstantial evidence points to the latter. The mutations were all UV-induced from the same parental strains. It is not on record whether they were all induced and isolated in the same experiment but that is probable (Ghikas, personal communication). Furthermore, all four mutations show the same optimum germination conditions (4 hours heat shock at 50°C on dung germination

medium) which are unusual for white ascospore mutations in our stocks. This suggests that all four mutations confer the same spore wall phenotype as optimum germination conditions seem related most to the spore wall phenotype rather than its pigmentation. Therefore mutations BB9*w*8, BB*w*11 and BB*w*21 are probably re-isolations of mutation BB9*w*9, but this has not been shown conclusively.

Specificity of *ccf*-5

The clearcut effects of *ccf*-5 on the conversion behaviour of mutation BB9*w*9 have been well documented, but it was not known whether the action of *ccf*-5 is specific to BB9*w*9 (and closely linked mutations if BB9*w*8, BB*w*11 and BB*w*21 are not homoalleles) or extends to other parts of the genome. It has already been ascertained that *ccf*-5 has no apparent effects on the conversion of locus I mutations since strains carrying *ccf*-5(*A*) and *ccf*-5(*B*) both give high conversion frequencies when crossed to such mutations if the wild-type strains concerned carry *ccf*-2(*P*) (data of Lamb and Ghikas (1979) and further data obtained in this work). In this experiment derived wild-type tester strains carrying *ccf*-5(*A*) and *ccf*-5(*B*) were crossed to all mutants of loci II, III and IV and mutants *w*BHj and NG*w*14 which are unlinked to these three loci (mapping and locus numbering by Ghikas, 1978). The behaviour of mutant strains *w*BHj and NG*w*18 was particularly sought since their conversion control systems were also to be investigated in this work. The derived wild-type tester strains used were

25-5R+ and 7R7-, each carrying *ccf*-5(*A*), and 92-19+ and 92-, each carrying *ccf*-5(*B*). Not all of the crosses were fertile, such that for several mutants data from pairs of crosses to *ccf*-5(*A*) and *ccf*-5(*B*) wild-types were not available. However, pre-existing data were able to fill in the gaps here. It has been shown that mutations BB9*w*8, BB*w*11 and BB*w*21 respond in the same way to *ccf*-5 as does BB9*w*9. This applies whether the four mutations are closely linked or are homoalleles. Applying this information to the data in Table 2, it was deduced that strains BBR1- and 61- carry *ccf*-5(*A*) while strains 352+, 42- and KIII- carry *ccf*-5(*B*). Knowing this, crosses of the mutant strains under study to *ccf*-5(*A*) and *ccf*-5(*B*) wild-types can be found in the work of Lamb and Ghikas (1979). These crosses are included in Table 17 along with those performed in this work. As a result, there are conversion data for each mutant under study (bar two) when crossed to at least one *ccf*-5(*A*) and one *ccf*-5(*B*) wild-type strain. More importantly, for most mutations there are conversion data involving the same mutant strain when crossed to the two types of derived wild-type.

locus II

NG*w*18. *ccf*-5 does not seem to be implicated in the difference between HCF and LCF crosses involving mutation NG*w*18. Derived wild-type strains carrying *ccf*-5(*A*) and *ccf*-5(*B*) both gave LCF crosses to the same NG*w*18 strain.

TABLE 17: Conversion data of locus II, III, IV and unlinked mutations in crosses to *ccf*-5(*A*) and *ccf*-5(*B*) derived wild-type strains

Mutant	Crossed to	Octad classes (+:*w*),%					% NRCF	Total octads
		4:4	6:2	2:6	5:3	3:5		
LOCUS II	7R7- (*A*)	99.81	-	0.05	0.14	-	0.19	3664
	92- (*B*)	96.76	1.90	0.56	0.59	0.19	3.24	5888
NG*w*18	42- " *	96.67	2.11	0.35	0.59	0.26	3.30	8541
	KIII- "*	99.82	0.12	-	0.04	0.02	0.18	10260
	92-19+(*B*)	99.86	0.03	-	0.11	-	0.14	3698
S*w*26	92- "*	99.60	0.20	0.07	0.12	0.02	0.40	6000
	42- "*	99.60	0.01	0.04	0.20	0.15	0.40	8060
	25-5R+(*A*)	98.30	1.14	0.19	0.38	-	1.70	1057
	92-19+(*B*)	98.11	-	-	1.89	-	1.89	475
NG*w*16	92- "*	95.98	0.67	0.20	2.41	0.75	4.02	2535
	42- "*	94.23	1.50	0.30	3.15	0.78	5.73	5270
	KIII- "*	95.35	1.35	0.18	2.65	0.25	4.43	3999
LOCUS III								
	9r+ (*A*)*	99.84	0.07	0.02	0.05	0.02	0.16	10934
B*w*4.2	92-19+(*B*)	99.91	-	0.03	0.06	-	0.09	3252
	42- "*	98.52	0.56	0.09	0.71	-	1.37	4458
	9r+ (*A*)*	99.68	0.05	0.08	0.19	-	0.32	7524
B*w*3.10	92-19+(*B*)	99.87	0.06	-	0.06	-	0.13	3182
	42- "*	99.73	0.04	-	0.19	-	0.23	4841
	9r+ (*A*)*	99.34	0.15	0.35	0.16	-	0.66	6862
C*w*1.14	25-5R+ "	99.50	0.08	0.34	0.06	0.02	0.50	6232
	92-19+(*B*)	99.35	0.08	0.52	0.04	0.01	0.65	7517

TABLE 17: (continued)

Mutant	Crossed to	Octad classes (+:w),% 4:4	6:2	2:6	5:3	3:5	% NRCF	Total octads
LOCUS IV								
	7R7- (*A*)	99.75	0.06	0.06	0.06	0.06	0.25	1600
	92- (*B*)*	99.41	0.08	0.16	0.08	0.27	0.59	5092
NG*w*11	KIII- "*	99.57	0.04	0.13	0.11	0.16	0.43	5522
	42- "*	99.55	0.10	0.17	0.03	0.15	0.45	5952
	92-19+ "	99.44	0.24	-	0.31	-	0.56	2880
NW*w*12	92- (*B*)*	99.71	0.06	0.15	0.03	0.05	0.29	10010
	42- " *	99.74	0.04	0.04	0.07	0.05	0.19	8347
UNLINKED MUTATIONS								
	7R7- (*A*)	80.41	9.48	3.59	2.90	2.45	18.42	4685
*w*BHj	42- (*B*)*	85.61	8.81	1.87	2.13	1.42	14.23	7805
	KIII- " *	94.26	3.82	0.57	-	1.34	5.74	1046
	25-5R+(*A*)	92.47	3.49	1.39	1.25	1.00	7.12	13560
	25-5R+(*A*)	99.70	-	0.15	0.05	0.10	0.30	1969
NG*w*14	92-19+(*B*)	100.00	-	-	-	-	0.00	1091
	42- (*B*)*	99.72	0.08	0.06	0.09	0.05	0.28	6487

* Data of Lamb and Ghikas (1979)

(*A*) denotes *ccf*-5(*A*) strain

(*B*) denotes *ccf*-5(*B*) strain

Additionally, two other crosses involving *ccf*-5(*B*) strains were HCF.

S*w*26. Unfortunately this mutant only gave fertile crosses with *ccf*-5(*B*) wild-types. This mutation has always shown a low conversion frequency in crosses to all wild-type strains undertaken. It does not recombine with NG*w*18 and may be a deletion.

NG*w*16. *ccf*-5(*A*) and *ccf*-5(*B*) wild-types gave very similar conversion frequencies with this mutant. These frequencies were rather higher in the remaining three crosses involving *ccf*-5(*B*) wild-types, but the NG*w*16 strain was not the same. Ghikas (1978) did not classify NG*w*16 as part of locus II as it mapped 2.5 recombination units from NG*w*18, but in some crosses *w*10 and *w*78, both classified as locus I mutations, show similar recombination frequencies. Thus, in this work, mutation NG*w*16 is considered as part of locus II.

These data show that *ccf*-5 is not involved in locus II conversion control. This is particularly interesting in the case of NG*w*18 as its conversion behaviour is investigated later in this thesis.

locus III

B*w*4.2. Small differences in the conversion frequency of this mutation were observed by Lamb and Ghikas (1979) and Helmi (1980), which may or may not be due to genetic causes. *ccf*-5 would not seem to be implicated since

ccf-5(*B*) strains can give "LCF" and "HCF" in crosses to mutant strains, but not unfortunately the same one.

B*w*3.10 and C*w*1.14. Both of these mutations show low conversion frequency in crosses to both *ccf*-5(*A*) and *ccf*-5(*B*) strains, although the frequencies are slightly higher in the case of C*w*1.14.

There is no evidence from this data that *ccf*-5 affects locus III conversion frequencies in any way.

locus IV

NG*w*11 and NG*w*12. Both mutations show low conversion frequencies in both types of cross and there is no evidence for any really detectable effects of *ccf*-5. Small variations in conversion frequency do occur for NG*w*11, but since the number of convertant octads was so small, this is most probably due to the effect of sampling on small numbers.

*w*BHj

This mutation can show high conversion frequencies when the same strain is crossed to *ccf*-5(*A*) and *ccf*-5(*B*) wild-types. Furthermore, crosses of the same strain to two *ccf*-5(*B*) carrying derived wild-types gave both high and low conversion frequencies. This shows that *ccf*-5 is not responsible for the large differences in conversion frequency which can be exhibited by the *w*BHj mutation.

NG*w*14

The data provide no evidence for the action of *ccf*-5 on NG*w*14.

Summary

This experiment and earlier data provide no evidence for the action of *ccf*-5 on mutations of loci I, II, III and IV and unlinked mutations *w*BHj and NG*w*14. As far as the data go, it would appear that the action of *ccf*-5 is specific to BB9*w*9. It would be most interesting to induce mutations closely linked to BB9*w*9 of different mutagenic origins and determine the action of *ccf*-5 on them. This specificity of *ccf*-5 to the BB9*w*9 site is not surprising in view of the mode of action of the *ccf*, which will be discussed in Chapter 5.

Origin of the two *ccf*-5 factors

All our laboratory strains of *Ascobolus immersus* have been derived from the original two isolates of the Pasadena strains, taken from a dung sample by Dr. C. Yu-Sun and Professor S. Emerson in 1963. These original wild-type isolates were designated P5- and K5+. Thus any genetic conversion control factors with different forms which have since been discovered could have originated in 3 ways:

1. They could have been present as a polymorphism in the original P5- and K5+ strains.
2. One form may have since arisen from the other by mutation.

3. One form may have since arisen from the other by various recombinational processes, such as unequal crossing-over, etc.

The obvious way to distinguish between these possibilities for *ccf*-5 was to cross BB9*w*9 strains of known *ccf*-5 genotype to the original P5- and K5+ wild-type strains to determine whether one strain carried *ccf*-5(*A*) and the other *ccf*-5(*B*). If both apparently carried the same factor, then either a second *ccf* is involved in controlling BB9*w*9 conversion, or the *ccf*-5 factors arose later in the ancestry of the Pasadena strains. Further crosses would be necessary to distinguish between these possibilities.

However, for many years all subcultures of the original P5- and K5+ strains had been completely sterile so it seemed that direct crosses of BB9*w*9 to the seminal strains would not be possible. Nevertheless, Dr. Lamb had retained 1 lyophilised culture each of P5- and K5+, originally made by Professor Emerson in 1966. These cultures proved vegetatively revivable and after repeated attempts, a fertile replicate of cross BB9*w*9+ × P5- was obtained, but unfortunately no fertile crosses involving strain K5+ resulted. Cross BB9*w*9+ × P5- proved to be high converting as the data in Table 18 show. Since strain BB9*w*9 carries *ccf*-5(*A*) it would seem that original wild-type strain P5- must also carry *ccf*-5(*A*): the conversion data are typical of *A* × *A* rather than *A* × *B* crosses with high conversion frequency, overwhelmingly to wild-type and frequent PMS.

Simply because original wild-type strain P5- carries *ccf*-5(*A*) one cannot deduce that the other original strain K5+ carries *ccf*-5(*B*). Since no crosses to the latter strain were fertile, it was resolved to consider pre-existing data involving crosses of BB9*w*9 strains (or BB9*w*8, BB*w*11 and BB*w*21 since they behave in the same way) to early descendants of K5+ and if such crosses had not been attempted, to perform them. The data of Lamb and Ghikas (1979) included a cross of BB*w*21+ × KIII-. It can be seen from the pedigree diagram in Fig.4 that derived wild-type strain KIII- is a very early descendent of K5+ in terms of generations if not years.

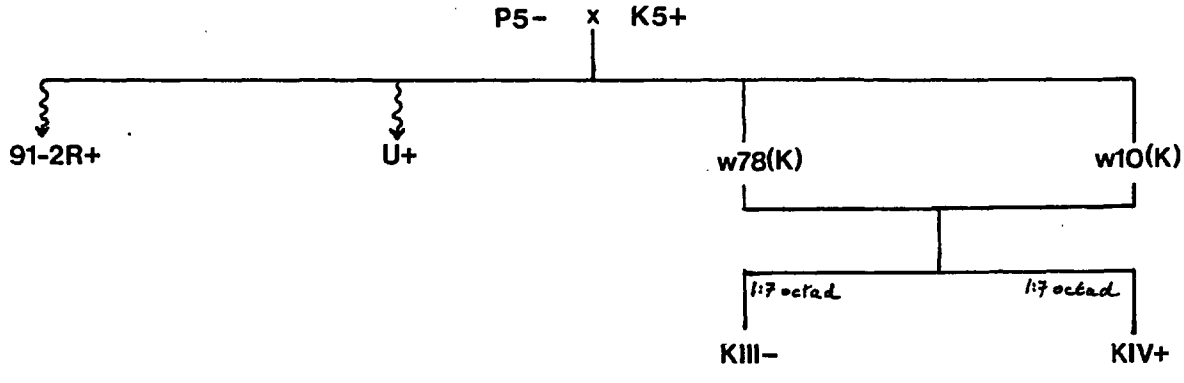


Fig.4: Ancestry of derived wild-type strain KIII-

(K): strain carries *ccf*-2(*K*)

Wavy line: details unknown.

Thus KIII- is only a second generation descendent of K5+, having been isolated from a 1:7 octad from a cross between closely linked locus I mutations *w*10 and *w*78, which were both isolated from cross P5- × K5+. The conversion data from cross BB*w*21+ × KIII-, displayed in Table 18, show that this cross is low converting for BB*w*21 with conversion not strongly to wild-type and infrequent PMS, i.e. typical of a

+, *ccf*-5(*B*) × *w*, *ccf*-5(*A*) cross, indicating that strain KIII- carries *ccf*-5(*B*), since BB*w*21+ carries *ccf*-5(*A*). Thus *ccf*-5(*B*) existed in the Pasadena strains in the second generation arising from original wild-type strains P5- and K5+. This means that it is very likely that K5+ carried *ccf*-5(*B*) and so in all probability the two forms of *ccf*-5 were present in the two original Pasadena strains as a polymorphism. The same has been shown to be true of the two forms of *ccf*-1 and the *P* and *K* forms of *ccf*-2 by Emerson and Yu-Sun (1967). Helmi (1980) attempted to trace the ancestry of the two forms each of *ccf*-3 and *ccf*-4 to the original P5- and K5+ strains, postulating that they too existed as polymorphisms in the original strains. Confirmatory tests of his predicted genotypes have yet to be carried out.

TABLE 18: Conversion data from crosses BB9*w*9+ × P5- and BB*w*21+ × KIII-

Cross	Octad classes (+:*w*), %					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
BB9*w*9+ P5-	90.97	5.12	0.64	3.07	0.13	8.96	35.71	91.40	1562
BB*w*21+ KIII-*	98.45	0.62	0.81	0.09	0.03	1.55	8.16	45.81	6324

* Data of Lamb and Ghikas (1979)

Discussion

The above experiments have demonstrated that the conversion behaviour of mutation BB9*w*9 is largely under genetic control with only small variations in conversion frequency being attributable to imperfectly controlled environmental factors. The factor primarily responsible for BB9*w*9 conversion control is novel, having been shown to be distinct from the hitherto known *ccfs* 1, 2, 3 and 4 and was designated *ccf*-5. Close linkage of *ccf*-5 to BB9*w*9 has been established, while so far two alternative forms of *ccf*-5 - *A* and *B* - have been discovered in the Pasadena strains. Wild-type and white mutant strains carrying either *ccf*-5(*A*) or *ccf*-5(*B*) were isolated by analysing 4+:4*w*, 6+:2*w* or 2+:6*w* octads from + × *w* crosses heterozygous for the two forms of *ccf*-5. The isolation of such strains made possible a whole cycle of + × *w* crosses with all combinations of the *ccf*-5 factors:

+, *ccf*-5(*A*) × *w*, *ccf*-5(*A*)
+, *ccf*-5(*A*) × *w*, *ccf*-5(*B*)
+, *ccf*-5(*B*) × *w*, *ccf*-5(*A*)
+, *ccf*-5(*B*) × *w*, *ccf*-5(*B*)

As has been seen, scoring dehisced octads from + × *w* crosses has enabled conversion frequencies - both to wild-type and to mutant - and the relative frequencies of PMS to be studied, which is an advantage over systems which detect only prototrophs in heteroallelic crosses of auxotrophs.

The overall results for each of the above types of cross are summarized in Table 19. The results for each type of cross were based on pooling data from many crosses of that

TABLE 19: Conversion data from + × BB9*w*9 crosses with different combinations of *ccf*-5 factors.

ccf-5 factor in		Octad classes (+:*w*) Frequency as % of total octads					Total octads	%NRCF ± S.E.	Octad classes (+:*w*) Relative frequency %				Relative frequency of PMS, %	*Some conversion parameters Direction of conversion ratio			Efficiency of correction ratio		
+	BB9*w*9	4:4	6:2	2:6	5:3	3:5			6:2	2:6	5:3	3:5		a	b	c	d	e	f
A	A	88.65	6.25	0.70	3.69	0.44	81517	11.08±0.11	56.40	6.29	33.36	3.95	37.31	8.93	8.39	8.80	1.06	4.41	1.68
A	B	97.61	1.50	0.40	0.43	0.04	142685	2.37±0.04	63.31	16.90	18.11	1.68	19.79	3.75	10.75	4.08	0.35	9.09	4.04
B	A	98.03	1.07	0.51	0.32	0.06	12789	1.96±0.12	54.58	25.90	16.33	3.19	19.52	2.10	5.33	2.27	0.39	9.32	4.16
B	B	90.72	5.67	1.57	1.70	0.30	10500	9.24±0.28	61.34	17.01	18.35	3.30	21.65	3.61	5.67	3.79	0.64	8.24	3.62

* a-d : direction of conversion ratio (conversion to +/conversion to *w*)

a = value for detected conversion classes with meiotic segretation = 6:2/2:6

b = value for detected conversion classes with postmeiotic segregation = 5:3/3:5

c = overall value for dual hybrid chromatid models = 2(6:2) + 5:3/2(2:6) + 3:5

d = overall value for single hybrid chromatid models = (6:2)(3:5)/(2:6)(5:3)

e-f : efficiency of correction ratio (detected correction events/detected non-corrections)

e = dual hybrid chromatid models = 2(6:2 + 2:6) + 5:3 + 3:5/5:3 + 3:5

f = single hybrid chromatid models = 6:2 + 2:6/5:3 + 3:5

type. These crosses were either replicates of the same cross or different individual crosses, but identical with respect to BB9*w*9 and *ccf*-5 genotypes. No crosses which gave a total sample of less than 1000 dehisced octads were included. The variability among crosses of the same type was tested by the $\Sigma\chi^2$ homogeneity test, applying this to narrower ratio conversion frequency data. The data from three types of cross were heterogeneous, with only the data from *B* × *A* crosses showing homogeneity ($\Sigma\chi^2$ = 4.70, p = 0.05-0.10, 2df), but only 3 crosses were of this category, compared with 28 *A* × *A* crosses and 66 *A* × *B* crosses. There were 4 *B* × *B* crosses. However, certainly among *A* × *B* LCF crosses it was shown that there was as much variation in conversion frequency between replicates of the same cross as there was between different crosses. This suggested that imperfectly controlled environmental factors were at least partially responsible for the heterogeneity among data from crosses of the same type. This, of course, does not rule out effects of genetic background on conversion frequencies at the BB9*w*9 site, but makes them more difficult to quantify.

Considering the data in Table 19, it can be seen that the conversion frequency of BB9*w*9 is high in crosses homozygous for either form of the closely linked *ccf*-5 and low in crosses heterozygous for these factors. There is a slight difference in conversion frequency between the two types of HCF cross which is nonetheless highly significant ($\Sigma\chi^2$ = 33.42, p < 0.01, 1df). The mean conversion frequencies were 11.08% for the *A* × *A* crosses (28 in all)

and 9.24% for the *B* × *B* crosses (4 in all). This difference in conversion frequency may have a genetic basis, most likely involving *ccf*-5 or other minor modifiers of gene conversion. Overall, it can be said that the conversion frequency of BB9*w*9 is reduced about 5-fold in crosses heterozygous for the two forms of *ccf*-5 compared with crosses homozygous for either form. Secondly, considering conversion spectrum, the relative frequency of PMS is about twice as high in *A* × *A* crosses as it is in all the other three types of cross. So, PMS is not only depressed in *ccf*-5 heterozygous LCF crosses, but also in *ccf*-5(*B*) homozygous HCF crosses. Thus the mechanism by which gene conversion of BB9*w*9 is reduced by *ccf*-5 cannot intrinsically be involved with the relative changes in PMS. The situation cannot directly be compared with that appertaining to the *cv* modifiers of gene conversion in the European strains of *Ascobolus immersus* (Girard and Rossignol, 1974) since for any of the four modifiers a whole cycle of crosses (i.e. *A* × *A*, *A* × *B*, *B* × *A* and *B* × *B*) was not performed for any single mutant in the latter work. Therefore, although it is known that *cv2* may reduce gene conversion and PMS of a given mutation several hundred-fold (and PMS may be totally suppressed) when heterozygous as opposed to *A* × *A* homozygous in a cross, the frequencies of conversion and PMS are not known for the same mutation in *B* × *B* homozygous crosses.

The data also clearly show that the conversion spectrum of a particular mutation cannot solely be determined by the molecular nature of that mutation, as postulated by Leblon (1972 a,b) and supported by the work of Yu-Sun et al.(1977). Mutation BB9*w*9 showed a conversion spectrum of type C in

A × *A* crosses and a broadly A-type spectrum in the three other types of cross. As has already been stated, these conversion spectrum differences could be due to the action of *ccf*-5 or other minor modifiers of gene conversion. These results are in agreement with the findings of Lamb and Ghikas (1979). It had been hoped to determine the molecular nature of mutation BB9*w*9 by reversion studies, but this was not possible due to the complete lack of fertile *w* × *w* crosses involving this mutation. Considering conversion spectra in more detail, the relative frequencies of each of the narrower ratio conversion classes show relatively good agreement for the *A* × *B*, *B* × *A* and *B* × *B* crosses. The main differences in conversion spectrum between these crosses and the *A* × *A* crosses are that 5:3 PMS octads are rarer and 2:6 octads more frequent in the former three types of cross, i.e. where *ccf*-5(*B*) is present. This might be an attribute of the *ccf*-5 system itself, but other explanations might be possible. Interactions of the *ccf*-5 factors with other minor modifiers of conversion behaviour could be involved. Can a case be advanced for a role being played by a heterozygous cryptic mutation (terminology of Lamb, 1975) close to BB9*w*9 such that when heterozygous in a cross it can co-convert with the latter mutation, modifying its conversion spectrum (Leblon and Rossignol, 1973)? Since such co-conversion of a cryptic and a known mutation can increase, but not decrease the correction frequency of the known mutation, crosses involving the known mutation which showed the highest frequency of PMS would be without the influence of nearby cryptic heterozygosity, or at least

such heterozygosity would be at a minimum (Lamb and Ghikas, 1979), i.e. in this case *A* × *A* crosses would be homozygous for the cryptic mutation or its "wild-type", while all or most of the *A* × *B* *B* × *A* and *B* × *B* crosses would be heterozygous for the cryptic mutation. This situation could not easily be maintained by the system described, since BB9*w*9 and the nearby cryptic mutation would normally have to co-convert if the cryptic mutation were to be able to influence the conversion behaviour of BB9*w*9. This would lead to some of the *A* × *A* crosses in this work being heterozygous for the cryptic mutation, while some crosses in the other three categories would be homozygous. Thus cryptic heterozygosity close to BB9*w*9 cannot readily explain the conversion spectrum difference between *A* × *A* and the remaining three types of cross, but if such heterozygosity does occur in some crosses, it could help explain heterogeneity among conversion spectra from the same type of cross.

In some of the data accumulated, the conversion spectrum was rather variable in LCF crosses, both in terms of the relative frequency of PMS and percentage conversion to wild-type. As stated above, incompletely controlled environmental factors could be responsible for this, minor modifiers of conversion behaviour may be involved, or co-conversion of a nearby cryptic mutation in some crosses could be the cause. It is worth mentioning that greater variability in these frequencies (and % NRCF) might be expected in LCF crosses simply because smaller numbers of convertant octads are involved and small numbers are subject

to greater random sampling variation. A second factor is that a background level of apparent PMS octads - particularly 5:3s - may be phenocopies (and these are frequent at locus '*46*' in the European strains of *Ascobolus immersus* according to Gajewski et al. 1968) and this value may be variable due to environmental conditions. Such variability would be more pronounced in LCF than HCF crosses if the background level of phenocopy 5:3 octads is similar in both types of cross. In Chapter 4 of this thesis it is reported that 5:3 phenocopies are very frequent (probably variable in number) in LCF crosses involving locus II mutation NG*ω*18. This phenomenon could also explain the apparent difference in the relative frequency of PMS for cross BB9*ω*9+ × 92- when performed by Lamb and Ghikas (1979) and in this work.

An additional source of heterogeneity in conversion spectra for *A* × *A* HCF crosses could arise because the 5:3/6:2 ratio is not constant throughout the period of octad dehiscence from a cross. In this work the ratio was observed to decrease with time. Similar decreases with time of dehiscence were also observed for the 3:5/2:6 ratio, but this was more variable. Thus for all crosses performed, octads were scored throughout the period that a cross was fertile. Changes in the 5:3/6:2 ratio with time of dehiscence were first reported by Helmi (1980) for mutation BB*ω*11, which is probably a re-isolate of BB9*ω*9.

The conversion parameters a, b, c, d, e and f shown in Table 19 enable further information to be gained from the conversion data of BB9*ω*9 in each type of cross. These estimates were defined by Wickramaratne and Lamb (1978) and

the definitions are given in Table 19.

This analysis assumes that *ccf*-5 not only controls the overall conversion frequency, but also the differences in conversion spectrum between the four categories of cross. On this basis the results from the estimates show that factors *ccf*-5(*A*) and *ccf*-5(*B*) affect the direction of conversion ratio and the efficiency of correction ratio, both with formulae for dual hybrid chromatid and single hybrid chromatid models of gene conversion. In all cases except one, the differences in parameter values largely occur between *A* × *A* and the remaining three types of cross. *A* × *A* crosses showed a greater ratio of conversion to wild-type in meiotic segregation octads and in all conversion octads combined on both single and dual H-DNA models; conversely, such crosses also showed a lower efficiency of correction ratio on both types of model. The only exception to this division between *A* × *A* and all other types of cross occurred for the direction of conversion ratio in PMS octads where *A* × *A* and *A* × *B* crosses showed a similar bias in conversion to wild-type. This might be a genuine genetic feature or could be due to variability in the frequency of apparent 3:5 octads which were rare in all types of cross and rarer than usual in most *A* × *B* crosses.

The data obtained in these experiments and summarized in Table 19 show that the two forms of *ccf*-5 do not show any appreciable *cis/trans* position effects on BB9*w*9 conversion, either in terms of conversion frequency, spectrum or both. Slight differences in conversion spectrum are apparent but these are probably due to trivial reasons largely unconnected with *ccf*-5. This is unlike the three forms of *ccf*-2 which show clear *cis/trans* position effects on the conversion behaviour of closely linked locus I mutations (Lamb and Helmi, 1978).

The experiments performed in this work have accumulated considerable circumstantial evidence for some co-conversion of mutation BB9*w*9 and *ccf*-5. No direct evidence has been gained since poor ascospore germination - particularly of BB9*w*9 white mutant spores early in this work - and the unavailability of suitable tester strains of both mating types at the time the experiments were performed, meant that full analysis of 6+:2*w* and 2+:6*w* convertant octads was not possible. Such an analysis would have unambiguously demonstrated the occurrence or otherwise of co-conversion. Nevertheless, it seems extremely likely that it does occur, particularly as BB9*w*9 and *ccf*-5 have been shown to be very closely linked, but the frequency cannot be quantified from the data in this thesis.

The overall data on BB9*w*9 conversion behaviour clearly point to primary conversion control (particularly of % NRCF) being exerted by *ccf*-5. The data are inconsistent with polygenic control of BB9*w*9 conversion, but do not rule out

the existence of a second *ccf* which may interact with *ccf*-5 such that some crosses which are genotypically high converting with respect to *ccf*-5 become low converting. There is no positive direct evidence for such a second factor but the exploratory experiments for the detection of such a factor already described should definitely be undertaken.

It is quite clear that mutations BB9*w*8, BB*w*11 and BB*w*21 respond to *ccf*-5 in the same way as does BB9*w*9. Although these mutations could not be mapped directly, it was shown that BB9*w*8 and BB*w*21 are closely linked to *ccf*-5. The same is most probably true of BB*w*11. This leaves the possibilities that the four mutations BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 are closely linked or are actually re-isolations of the same mutation. It seems most probable that the latter is true since all four mutations were UV-induced from the same parental cross, probably in the same experiment (Ghikas, personal communication). Furthermore, the fact that no fertile selfcrosses of these mutations (this mutation) were obtained despite all the procedures attempted does indeed seem to imply that BB9*w*9 may have a pleiotropic effect as a recessive sterility factor. Failing this, it would seem that such a factor must be closely linked to BB9*w*9.

Experiments were also undertaken which determined that *ccf*-5 has no effect on the gene conversion behaviour of mutations in loci II, III and IV and unlinked mutations *w*BHj and NG*w*14. Other data already indicated that *ccf*-5 could have no effect on locus I mutations. It is probable that even if further mutations were included in a subsequent

experiment, *ccf*-5 would have no effect on their conversion properties either. Its action seems limited to its own vicinity, although the induction and isolation of other mutations in that vicinity is of great interest for further work. This specificity of *ccf*-5 to closely linked sites and its action in reducing gene conversion frequencies of such a site in heterozygous crosses, are strongly indicative of the nature and mode of action of *ccf*-5, topics which are discussed fully in Chapter 5.

What of the possible origins of the two forms of *ccf*-5? It has been shown that *ccf*-5(*A*) was present in the original P5- wild-type strain isolated in 1963. Due to infertility of the other original wild-type strain K5+, it was not possible to demonstrate that this strain carried *ccf*-5(*B*), although this is most probable since this was shown to be present in derived wild-type strain KIII- - a second generation derivative of P5- and K5+. Thus it would seem that the two forms of *ccf*-5 were present as a polymorphism in the two original isolates of the Pasadena strains. This is also true of the two forms of *ccf*-1 and the *P* and *K* forms of *ccf*-1 (Emerson and Yu-Sun, 1967). It would seem probable that in natural populations of *Ascobolus immersus* in the Pasadena area, further polymorphic variants of *ccf*-5 occur. The *ss* recombination modulator gene in *Neurospora crassa* (which also reduces intragenic recombination at a closely linked site in heterozygous crosses) has three known polymorphic variants in wild and laboratory populations of the organism (Catcheside, 1981). It is also possible that variants of *ccf*-5(*A*) or (*B*) may have arisen by recombinational events in our laboratory stocks.

The (*91*) form of *ccf*-2 is presumed to have arisen in such a way (Helmi, 1980). If such further variants of *ccf*-5 occur in our laboratory stains, they might be detected in further studies.

Chapter 3. The genetic control of conversion of the *w*BHj white ascospore pigmentation mutation.

Preliminary experiments

Initially it was important to verify the results obtained by Lamb and Ghikas (1979) concerning the conversion properties of mutation *w*BHj, shown in Table 2. Thus strain *w*BHj+ was crossed to derived wild-type strains KIII- and 42- while strain *w*BHj- was crossed to 9r+. Despite repeated attempts, these crosses were no longer fertile. As an example, 30 replicates of the cross *w*BHj+ × 42- were performed. Revival of the derived wild-type strains by allowing inocula of each to grow through *Ascobolus* complete medium supplemented with 100 mg/ℓ chloramphenicol and repeatedly sub-culturing on this medium was unsuccessful. Such a method was attempted to clear our cultures of these strains of any possible bacterial contamination which might have rendered the strains sterile.

As a result of this inability to repeat the original crosses of Lamb and Ghikas (1979), *w*BHj mutant strains were crossed to a range of derived wild-type strains in our stock collection with the aim of obtaining crosses showing similar conversion behaviour to the original crosses shown in Table 2. One of the derived wild-type strains used was 25-5R+ since this was a direct descendent of 9r+, one of the strains used by Lamb and Ghikas (1979). Another derived wild-type strain used was KIV+ since this was derived from the same parental cross as KIII-, one of the original strains used by Lamb and Ghikas (1979). The 4P-,

- 5P-, 6P-, 7P-, 9P-, 11P-, 16P-, 17P-, 18P- and 24P- derived wild-type strains all originated from the cross 25-5R+ × 7R7-, while 1D2+ is a "Super" strain. 14 fertile crosses were achieved and the data from these crosses are displayed in Table 20, along with data from the 3 original crosses performed by Lamb and Ghikas (1979). From this Table it can be seen that the conversion frequencies of the 17 crosses fall into two broad categories. The first 8 crosses in the Table show lower conversion frequencies (5.74 to 8.86%NRCF) than the remaining 9 crosses (11.70 to 18.42%NRCF). In the latter HCF category, apart from one cross giving 11.70%NRCF, no other crosses gave a convsion frequency of less than 13.97%. The variability among crosses in each category was assessed by the $\Sigma\chi^2$ homogeneity test. Both the LCF ($\Sigma\chi^2 = 80.20$, $p < 0.01$, 7df) and the HCF ($\Sigma\chi^2 = 93.11$, $p < 0.01$, 8df) groups of crosses were heterogeneous with respect to narrower ratio conversion frequencies. However, replicates of the same cross also showed heterogeneity in the case of LCF cross *w*BHj- × 25-5R+. 7 replicates of this cross were performed all giving sample sizes of over 1000 octads. Among these replicates the range of conversion frequencies was 5.41 to 8.85% ($\Sigma\chi^2 = 24.58$, $p < 0.01$, 6df). Therefore the conversion frequency heterogeneity among crosses of the same group, i.e. LCF or HCF is probably largely due to imperfectly controlled environmental effects, but the rôle of genetic background cannot be ruled out, particularly among the HCF crosses which appeared more highly variable with respect to conversion frequency.

These conversion frequency results are broadly in agreement with those obtained by Lamb and Ghikas (1979) with the crosses performed being assignable to LCF and HCF groups, but with some considerable spread of frequencies in the HCF group. Furthermore, *ccf*-2 is not implicated in these differences in conversion frequency since all the derived wild-type strains used (with the

TABLE 20: Conversion data from +x *w*BHj crosses

Mutant strain	Crossed to	Octad classes (+:*w*),%					%NRCF	Relative % PMS	% Conversion to +	Total octads
		4:4	6:2	2:6	5:3	3:5				
*w*BHj-	9r+*	92.73	4.31	1.17	0.80	0.86	7.14	23.25	71.56	8867
"	25-5R+	92.47	3.49	1.39	1.25	1.00	7.12	31.60	66.57	13560
*w*BHj+	6P-	91.76	4.12	2.25	1.00	0.75	8.11	21.58	63.13	801
"	9P-	91.08	4.92	1.85	1.23	0.86	8.86	23.59	69.41	1625
"	16P-	90.65	5.69	1.45	0.96	0.58	8.68	17.74	76.61	1037
"	18P-	91.04	5.09	1.17	1.57	0.91	8.75	28.34	76.11	2298
"	KIII-*	94.26	3.82	0.57	-	1.34	5.74	23.34	66.67	1046
*w*BHj-	KIV+	93.05	3.67	0.79	1.29	0.99	6.75	33.78	73.48	1007
*w*BHj+	42-*	85.61	8.81	1.87	2.13	1.42	14.23	24.95	76.87	7805
"	7R7-	80.41	9.48	3.59	2.90	2.45	18.42	29.04	67.21	4685
"	4P-	82.81	9.46	2.48	2.44	1.53	16.90	23.49	70.41	1047
"	5P-	83.33	8.97	3.65	2.17	1.78	16.57	23.84	67.23	1014
"	7P-	88.12	6.84	2.11	1.67	1.08	11.70	23.50	72.74	3889
"	11P-	84.72	8.54	3.06	1.58	1.79	14.96	22.53	67.65	949
"	17P-	85.79	8.57	2.06	2.11	1.23	13.97	23.91	76.45	4132
"	24P-	83.58	8.82	2.45	1.47	2.94	15.68	28.13	65.63	408
*w*BHj-	1 D2+	84.35	8.69	2.65	2.40	1.42	15.16	25.20	73.15	4458

* Data of Lamb and Ghikas (1979)

exception of KIII-, KIV+ and 42-) carried the same form of *ccf*-2 - *ccf*-2(*P*). It would also seem that the *ccf*-3 and *ccf*-4 "Super" factors are not involved, since "Super" and "non-Super" wild-types both give HCF when crossed to *w*BHj mutant strains.

No significant differences in conversion spectrum are discernible between the LCF and HCF crosses. Considering the relative frequency of PMS, this varied from 17.74 to 33.78% in the LCF crosses, while the range was 22.53 to 29.04% in the HCF crosses, i.e. PMS was relatively frequent in both types of cross. Additionally, the direction of conversion was predominantly to wild-type in both categories of cross, ranging from 63.13 to 76.61% in the LCF crosses and 65.63 to 76.87% in the HCF crosses. Thus in agreement with the original work of Lamb and Ghikas (1979) the conversion spectrum of mutation *w*BHj can be classified as type C according to the criteria of Leblon (1972 a,b). This conversion spectrum type has been deemed typical of base substitution mutations by the latter author. In the original crosses performed by Lamb and Ghikas (1979) the conversion spectrum of cross *w*BHj+ × KIII- appeared rather different from that of the other 2 original crosses or any subsequent crosses, i.e. 5:3 octads were absent, 2:6 octads less and 3:5 octads more common than in other crosses. It was impossible to obtain a fertile repeat of this cross so that the difference in conversion spectrum could not be investigated further. The cross involving strain KIV+ (derived from the same cross as KIII-) showed a perfectly usual conversion spectrum in close agreement with all other

crosses. The original "deviant" conversion spectrum might have been due to a random sampling effect (particularly if only 1 collecting lid was harvestable for this cross) or could have had genetic causes.

Thus these crosses broadly confirmed the existing data on the conversion behaviour of *w*BHj, providing LCF and HCF crosses for further analysis. The difference in conversion frequency between the two sets of crosses strongly suggests that the conversion behaviour of *w*BHj is under genetic control and the system involved cannot be *ccf*-2. Neither can it be *ccf*-5 as it was shown in Chapter 2 that the latter factor has no effect on *w*BHj conversion behaviour. From the data in Table 20 it appears that at least one conversion control factor is determining the conversion frequency of *w*BHj. At the outset a simple, easily testable model of conversion control was assumed (a closely linked, monogenic system) and this formed the basis upon which the following experimental programmes of crosses were devised.

Monogenic conversion control of *w*BHj

The data in Table 20 show that when strain *w*BHj- was crossed to derived wild-type strains 25-5R+ and 1D2+ very different conversion frequencies resulted. Since the *w*BHj strain was the same in each case, and if the conversion control of this mutation is monogenic, then strains 25-5R+ and 1D2+ must carry different forms of the conversion control factor. Therefore either LCF cross *w*BHj- × 25-5R+ or HCF cross *w*BHj- × 1D2+ must be heterozygous for differ-

ent forms of the conversion control factor. Due to the fact that gene conversion of the *w*62 and BB9*w*9 mutations in the Pasadena strains of *Ascobolus immersus* is reduced when their respective closely linked conversion control factors are heterozygous in a cross (Emerson and Yu-Sun, 1967; present work) and the same situation applies to the four *cv* modifiers in the European strains (Girard and Rossignol, 1974), it was felt more likely that LCF cross *w*BHj- × 25-5R+ might be heterozygous for a closely linked conversion control factor, so this cross was chosen for analysis. The theoretical basis for this analysis was explained in Chapter 2 and illustrated in Figure 1 (page 123) but a brief resumé is given here: analysis of the 6 wild-type spores from 6+:2*w* convertant octads (whether they arise by symmetrical or asymmetrical H-DNA formation) should reveal that 4 spores carry the same form of the control factor as the wild-type parent, while 2 spores will have inherited the control factor originally present in the white mutant parent. The latter 2 spores should show altered conversion behaviour in backcrosses to the white mutant parent, unless certain forms of dominance are involved or the white mutation and its conversion control factor frequently co-convert.

Conversion behaviour of wild-type isolates from 6+:2*w* octads from LCF cross *w*BHj- × 25-5R+

Following the programme outlined above, wild-type spores were isolated from 40 6+:2*w* convertant octads. Germination was poor, but in 4 octads all 6 wild-type spores germinated. These wild-type isolates were backcrossed to the *w*BHj- parental strain and also to *w*BHj+ as

a white mutant tester strain of (+) mating type. In this way it was hoped to discover whether *w*BHj- and *w*BHj+ showed consistent conversion properties and hence carried the same form of the suspected conversion control factor, if monogenic. Not all of the wild-type isolates gave fertile crosses, but 19 did and the conversion data from these crosses are shown in Table 21.

From this Table it can be seen that at least 1 isolate showed altered conversion behaviour in each of the 4 octads analysed, with 2 wild-type isolates showing altered conversion behaviour in octads 11 and 19. Considering the conversion data from each octad in turn, in octad 8, 4 wild-type isolates gave fertile crosses of which 3 were LCF (%NRCF ranged from 6.61 to 8.67%) while 1 isolate gave an altered, enhanced conversion frequency of 17.11% and was hence classifiable as HCF, despite the rather small sample size scored. In octad 9, 4 of the 6 wild-type isolates again gave fertile crosses. 3 of these crosses can be classified as LCF, although the conversion frequencies (9.97 to 10.51%) were rather higher than those for other LCF crosses in this experiment or for those crosses shown in Table 20. 1 isolate gave an altered, enhanced conversion frequency of 14.63%. In octad 11, 5 of the 6 possible crosses were fertile. 3 were definitely LCF, with conversion frequencies of 7.84 to 8.57%. 2 isolates showed enhanced conversion frequencies of 12.10 and 12.92% respectively. Since these 2 isolates are of the same mating type and the altered conversion frequencies agree so well in value, the isolates are almost certainly sister spores, being derived from mitosis of the same meiotic

TABLE 21: Conversion data of wild-type isolates from 6+:2w octads from cross wBHj- x25-5R+ when crossed to wBHj+ or wBHj-

Isolate No. (octad.spore)	Octad classes (+:w),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
8.1+	91.19	4.32	1.88	1.36	1.02	8.67	27.45	65.51	1759
8.2-	92.79	3.90	1.20	0.90	0.60	6.61	22.69	72.62	333
8.4-	82.47	10.52	3.09	1.24	2.27	17.11	20.51	68.73	485
8.6+	91.74	4.10	1.60	0.83	1.32	7.85	27.39	62.80	1440
9.1+	89.79	4.38	2.27	1.62	1.70	9.97	33.30	60.18	1234
9.2+	84.99	7.06	3.24	1.91	2.42	14.63	29.60	61.31	1572
9.3+	89.27	6.78	1.23	0.86	1.37	10.23	21.80	74.68	811
9.5-	89.30	5.57	1.39	1.84	1.71	10.51	33.78	70.50	1579
11.1+	91.11	4.81	1.38	1.09	1.20	8.57	26.72	68.84	2746
11.2+	91.90	4.80	1.08	1.29	0.75	7.92	25.76	76.89	2789
11.3-	86.57	6.70	2.62	1.58	2.01	12.92	27.79	64.09	5956
11.4-	87.54	6.65	2.18	1.45	1.81	12.10	26.94	66.94	5863
11.6+	92.07	4.70	1.24	0.96	0.93	7.84	24.11	72.19	3317
19.1-	84.23	8.95	1.84	2.27	2.26	15.25	29.70	73.57	1363
19.2+	93.49	3.17	0.78	1.56	0.89	6.40	38.28	73.91	1797
19.3+	91.19	3.95	1.54	1.32	1.95	8.76	37.33	60.16	2202
19.4-	86.45	7.62	2.95	1.19	1.35	13.10	19.39	67.25	3624
19.5-	91.87	3.16	1.70	1.09	1.82	7.77	37.45	54.70	824
19.6-	91.19	3.46	1.57	2.20	0.94	8.18	38.39	69.19	318

Isolates with altered conversion frequencies are underlined

product. This is exactly in agreement with what is predicted by theoretical analysis. Furthermore, in this octad, large sample sizes of dehisced octads were scorable for each cross. Finally, in octad 19, all 6 wild-type isolates gave fertile crosses. Exactly as predicted, 4 isolates gave LCF crosses (conversion frequencies ranged from 6.40 to 8.18%), while 2 isolates, again of the same mating type gave altered, enhanced conversion frequencies of 13.10 and 15.25% respectively. In this octad the agreement in conversion frequency between the 2 isolates showing altered conversion frequencies was not so close, being over 2%. Since these isolates, almost certainly having arisen by conversion at the *w*BHj site, must be sister spores, this variation in conversion frequency can be attributed to environmental effects.

The results from this analysis, although based on few octads, do seem to indicate that the LCF cross *w*BHj- × 25-5R+ is heterozygous for at least one *ccf* affecting the conversion of *w*BHj. Some direct evidence is available from this experiment on the linkage relations of this factor and *w*BHj: if the *ccf* were very closely linked to *w*BHj it might be expected (depending on its mode of action) to show some or even frequent co-conversion with *w*BHj, resulting in some octads revealing no wild-type isolates with altered conversion properties. Isolates with altered conversion properties were recoverable in all 4 octads, so co-conversion, if it does occur, cannot be extremely frequent. This might be connected with the mode of action

of the putative *ccf* or could indicate that linkage between the two sites is not that tight. Additionally, if the two sites were loosely linked, or unlinked, then crossing-over between the sites, unassociated with conversion, could produce 4 wild-type isolates with altered conversion properties per octad. In no octads were more than 2 such isolates recovered. Thus if a single conversion control factor is involved, it is likely to be linked to *w*BHj, but the linkage may not be tight.

The data from this experiment also reveal that white mutant tester strains *w*BHj- and *w*BHj+ both show the same conversion properties. In octad 19, *w*BHj- gave LCF crosses with spores number 2 and 3, while *w*BHj+ gave similar LCF crosses with spores number 5 and 6. Furthermore, segregants with altered conversion properties were also detectable in HCF crosses involving *w*BHj- and *w*BHj+ strains: isolate 9.2+ was detected with *w*BHj- as the tester strain, while isolates 8.4-, 11.3-, 11.4-, 19.1- and 19.4- were detected with *w*BHj+ as the tester strain. From this it can be concluded that both original mutant strains carry the same form of the *ccf* under examination.

A brief consideration of the conversion spectrum shows that this was broadly similar in the LCF crosses and also in the altered, HCF crosses. In the first case, the relative frequency of PMS varied from 21.80 to 38.28% and the frequency of conversion to wild-type from 54.70 to 76.89%. In the latter case the frequency ranges were 19.39 to 29.70% and 61.31 to 73.57%.

The above experiment therefore points to the existence

of a conversion control factor responsible for determining the frequency of gene conversion of mutation *w*BHj, giving "low" conversion frequencies (c6-9%) in crosses heterozygous for two forms of this factor. If the two forms of this factor are designated *A* and *B*, then the parental cross chosen for analysis can now be written:

*w*BHj- (*A*) × 25-5R+ (*B*) LCF

If only a single conversion control factor is responsible for the basic conversion frequency differences between LCF and HCF crosses involving mutation *w*BHj, then all the HCF crosses shown in Table 20 must be homozygous for the *A* form of this factor (since *w*BHj- and *w*BHj+ strains appear to carry the same form of the *ccf*). Thus an HCF cross such as *w*BHj- × 1D2+ can be written:

*w*BHj- (*A*) × 1D2+ (*A*) HCF

From this it can be seen that the 2 convertant wild-type spores in 6+:2*w* octads from LCF cross *w*BHj- × 25-5R+ should carry *ccf* form *A*, inherited from the white mutant parent. Such segregants, when backcrossed to *w*BHj+/-, would produce a HCF *A* × *A* type of cross. Indeed, in this experiment the segregants showing altered conversion properties in such backcrosses were indeed high converting. Due to lack of time, it was not possible to carry out a confirmatory analysis of wild-type isolates from 6+:2*w* octads from an *A* × *A* HCF cross. Such an analysis should

reveal that none of the 6 wild-type spores per octad shows altered conversion properties in backcrosses to *w*BHj+/-. Additionally, such an analysis might reveal the existence of other modifiers of gene conversion frequencies (or spectra) if they were segregating in the parental cross. In this context, it is worth noting that in the above experiment the LCF isolates in octad 9 showed slightly higher conversion frequencies than usual for LCF crosses. This difference might have been due to imperfectly controlled environmental conditions or minor genetic modifiers of gene conversion frequencies.

The above assumes that only one *ccf* is responsible for the relatively large differences in conversion frequency which mutation *w*BHj can show. The following experiment was carried out to test this more conclusively.

Conversion behaviour of random wild-type spores isolated from cross BH19-1- × 25-5R+

A cross was performed between derived wild-type strains heterozygous for the *ccf(s)* responsible for the conversion behaviour of *w*BHj: from the last experiment isolate 19.1- (Table 21) was selected as a derived wild-type strain carrying the *A* form of the *ccf* in question. This strain was designated BH19-1- and was crossed to derived wild-type strain 25-5R+, designated as carrying form *B* of the *ccf*. Thus cross BH19-1- × 25-5R+ was heterozygous for the postulated monogenic conversion control system, but if further genes are involved, this cross could be heterozygous for those as well. To test this 350 wild-type spores were

isolated randomly from the progeny of cross BH19-1- × 25-5R+ (all octads observed were 8+:0*w*). 48 spores germinated and these isolates were crossed to white mutant strains *w*BHj- and *w*BHj+. Altogether 30 fertile crosses resulted. The data obtained are shown in Table 22 and Figure 5.

From this Table and Figure it can be seen that of the 30 random isolates which gave fertile crosses with *w*BHj+/-, 16 isolates gave LCF crosses (with conversion frequencies of 6.05 to 8.93%) behaving like the 25-5R+ parent, while 14 gave HCF crosses (with conversion frequencies of 11.15 to 14.80%). However, the separation of the two classes was not that large and while the isolates which gave LCF crosses showed conversion frequencies similar to that shown by parental strain 25-5R+ in a cross to *w*BHj- (7.12%) and a similar range of values as that shown by different replicates of the latter cross (5.41 to 8.85%), the situation was not so clear cut with regard to the HCF crosses. All of the conversion frequencies in these crosses were <u>below</u> those shown by parental strain BH19-1- when crossed to *w*BHj+ (15.25%). The sister spore of BH19-1 gave a lower conversion frequency of 13.10% when crossed to *w*BHj+ (Table 21). These values might be regarded as indicative of the range of conversion frequencies which a given genotype can exhibit in slightly varying environmental conditions. With this in mind the data in Table 22 concerning HCF crosses can be examined more closely. Of the 14 broadly HCF crosses, 6 involved tester strain *w*BHj+ and 8 tester strain *w*BHj-. Considering the 6 crosses involving *w*BHj+, with only one exception the range of conversion

TABLE 22: Conversion data of random wild-type spores isolated from cross BH19-1- x 25-5R+ when crossed to *w*BHj+ or *w*BHj-

Isolate No.	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1-	92.01	4.55	1.75	0.95	0.60	7.86	4506
3-	86.60	7.50	2.75	1.50	1.27	13.02	4402
4+	86.70	7.75	3.07	1.75	0.59	13.15	684
5-	90.88	4.98	1.84	1.07	1.03	8.93	2610
6+	86.35	7.91	3.06	1.55	0.99	13.50	2125
7-	92.04	3.90	2.42	0.70	0.89	7.92	4588
9-	91.16	5.02	2.23	0.61	0.81	8.67	3449
10+	87.80	8.21	2.30	0.75	0.56	11.82	1607
11+	93.20	3.21	1.72	1.27	0.45	6.65	1339
13-	86.48	7.36	3.31	1.41	1.17	13.26	4104
14+	87.17	6.30	2.91	1.45	2.18	12.83	413
15-	91.32	4.34	2.02	1.03	1.14	8.53	1935
17+	93.17	3.87	1.32	0.66	0.74	6.58	1215
18+	90.88	6.01	1.34	0.85	0.57	8.77	1414
19+	88.72	6.03	2.67	1.34	1.12	11.15	2322
22-	92.26	4.29	1.53	1.02	0.82	7.65	4520
24-	91.80	4.76	1.52	0.96	0.77	8.01	4681
26-	88.64	6.09	2.31	1.64	1.10	11.15	2808
28+	93.75	3.49	1.26	0.73	0.56	6.05	3010
29+	87.97	6.72	2.28	1.54	1.30	11.84	1621
30+	87.49	7.26	2.34	1.52	1.07	12.19	1583
31-	85.48	7.91	3.36	1.74	1.25	14.27	1605

TABLE 22: (continued)

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
33+	91.33	5.73	1.82	0.77	0.28	8.60	1431
35-	85.00	8.57	2.82	1.85	1.56	14.80	1027
37+	92.00	4.06	1.54	1.75	0.66	8.00	912
38+	88.21	6.69	2.23	1.49	1.12	11.52	1883
39+	91.54	4.17	2.65	0.88	0.76	8.46	1583
41-	91.70	4.86	1.62	1.01	0.81	8.30	494
43-	84.99	9.29	2.21	1.59	1.44	14.53	3531
45-	92.66	4.23	1.56	1.04	0.22	7.04	2315

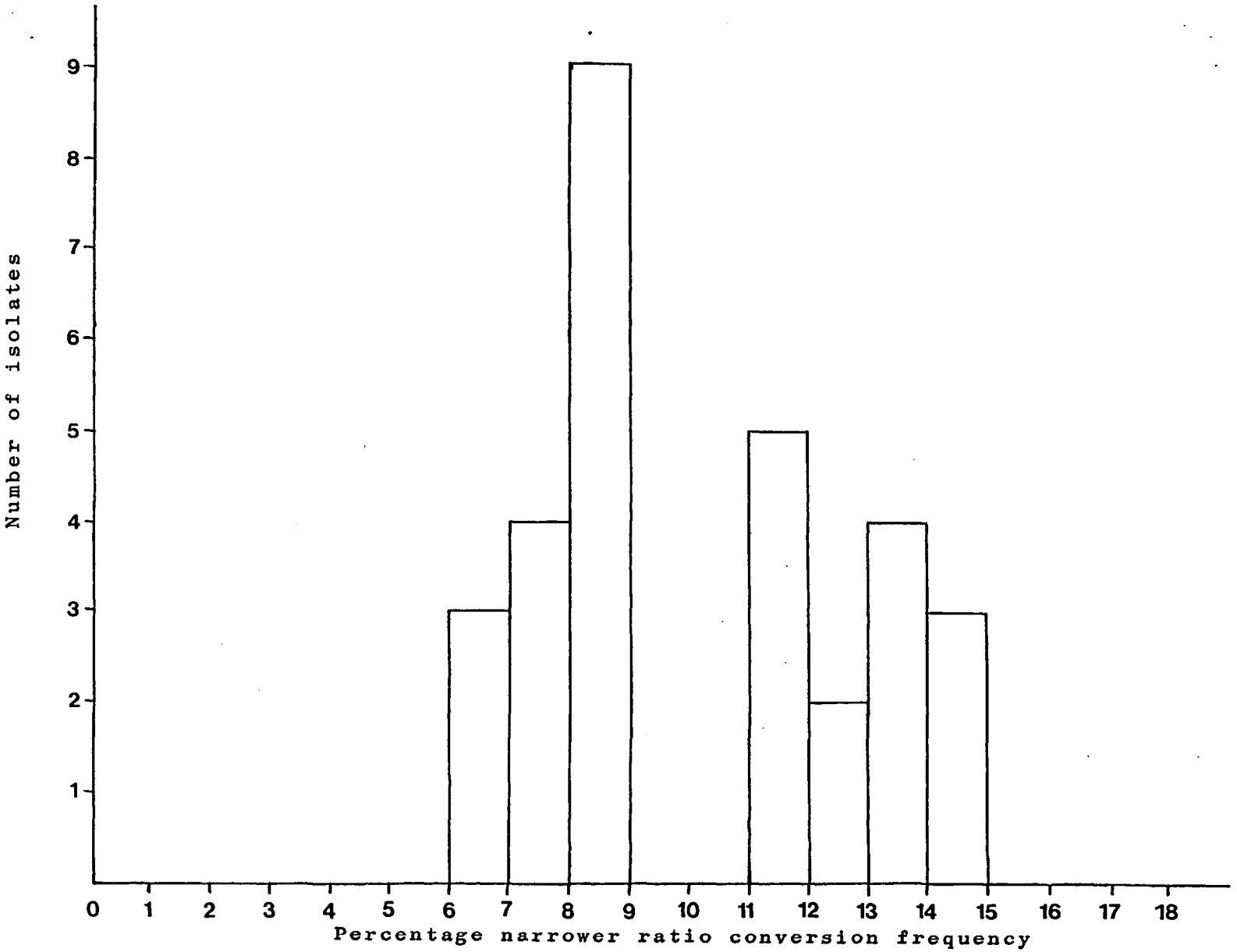


Fig. 5: Narrower ratio conversion frequencies of random wild-type isolates from cross BH19-1- × 25-5R+ when crossed to *w*BHj+ or *w*BHj-

frequencies was 13.02 to 14.80%, i.e. between the values shown by sister spores 19.1- (BH19-1-) and 19.4- when crossed to *ω*BHj+. Considering the 8 crosses involving *ω*BHj- as a tester strain, with two exceptions the range of conversion frequencies was 11.15 to 12.83%, i.e. below the values shown by sister spores 19.1- and 19.4-. Thus there is some slight evidence for an effect of the tester strain used on conversion frequency detected, but the effect would only be slight. It should be noted from Table 20 that strain *ω*BHj- is capable of giving higher conversion frequencies than c 13%. Unfortunately only one such HCF cross involving strain *ω*BHj- is shown in this Table (to 1D2+) and this gave a conversion frequency of 15.16%. In Table 21, isolate 9.2+ gave 14.63% NRCF when crossed to *ω*BHj-. Furthermore, no such effect of the tester strain is discernible among the LCF crosses.

Nevertheless, the difference in conversion frequencies between LCF and HCF crosses, although not great, does seem to have a genuine genetic basis. The data for many of the crosses in Table 22 come from several replicates of the same cross. In all cases agreement between the replicates in terms of conversion frequency was good. Furthermore, in all cases all replicates either showed HCF or LCF. In no cases did replicate conversion frequencies straddle both groups. The ratio of 16:14 very closely fits the expected 1:1 ratio of LCF:HCF crosses arising from this type of experiment if the conversion frequency differences under study are subject to monogenic control (χ^2 = 0.13, p = 0.70-0.80, 1df). Conversion spectra have not been considered in this experiment as they do not differ between LCF and HCF crosses.

In summary, these results do point to the existence of at least a single conversion control factor acting on mutation *w*BHj with a reduction in gene conversion (but no changes in conversion spectrum) when the control factor is heterozygous in a cross. This factor can be designated conversion control factor 6 or *ccf*-6, since it was the sixth such factor to be discovered in the Pasadena strains of *Ascobolus immersus*. The action of this control factor is not drastic, generally reducing conversion frequencies by 50% or less when heterozygous in a cross. It also seems to be subject to the action of minor modifiers of *w*BHj gene conversion and imperfectly controlled environmental conditions. It is difficult to quantify both of these elements. In the cross BH19-1- × 25-5R+ it might be instructive to carry out full octad analysis of the 8+:0*w* octads, which under very carefully controlled conditions might reveal evidence for minor modifiers of gene conversion segregating in the cross. The possible effect of the genetic background of the tester strains used could only be detected or eliminated by using different *w*BHj tester strains of the same mating type.

Linkage relations of *w*BHj and *ccf*-6

Initially the assumption was made that the conversion control system acting on *w*BHj was monogenic and closely linked to *w*BHj since this was the most straightforward hypothesis to test. The results from the experiments already outlined are in agreement with one major genetic conversion control factor (*ccf*-6) acting on *w*BHj and nothing

has conflicted with the assumption of close linkage between the *ccf* and the white mutation in question. The actual linkage relationship between *w*BHj and *ccf*-6 was determined by analysis of the LCF cross *w*BHj- × 25-5R+. This cross is doubly heterozygous for the *w*BHj mutation and the two forms of *ccf*-6:

$$w\text{BHj},\ ccf\text{-}6(A),\ -\ \times\ +\ \overset{w\text{BHj}}{}\ ccf\text{-}6(B),\ +$$

Thus if *w*BHj and *ccf*-6 are closely linked, most if not all wild-type spores randomly isolated from 4+:4*w* non-convertant octads should carry *ccf*-6(*B*) and show conversion behaviour like the wild-type parent, while similarly isolated white spores should mostly carry *ccf*-6(*A*) and behave like the white mutant parent. Any isolates showing altered conversion behaviour in suitable test crosses would be recombinants for the two sites in question. In this experiment, the linkage relationship between *w*BHj and *ccf*-6 was determined solely by the conversion behaviour of wild-type spores randomly isolated from 4+:4*w* octads from a cross heterozygous for both sites in question.

Conversion behaviour of wild-type spores randomly isolated from 4+:4*w* octads from cross *w*BHj- × 25-5R+ when crossed to *w*BHj+ or *w*BHj-

300 wild-type spores were randomly isolated from 4+:4*w* octads from cross *w*BHj- × 25-5R+. Of these 300 spores, 116 germinated and 71 of these isolates gave fertile crosses with *w*BHj+ and *w*BHj- as tester strains. The results obtained from these crosses are shown in Table 23.

TABLE 23: Conversion data of random wild-type isolates from 4+:4*w* octads from cross *w*BHj- x 25-5R+ when crossed to *w*BHj+ or *w*BHj-

Isolate No.	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1+	92.85	3.81	0.24	0.95	-	7.14	210
2-	91.70	3.68	1.40	1.38	1.74	8.16	1495
3-	90.83	4.40	1.80	1.15	1.44	8.80	1386
4+	92.53	3.69	1.29	1.11	1.20	7.29	1084
5+	91.37	3.97	1.12	1.86	1.37	8.32	1611
6-	85.55	6.64	3.18	1.93	2.33	14.08	2485
11-	88.67	5.72	2.36	1.31	1.67	11.07	8720
12+	90.88	4.10	1.67	1.40	1.46	8.63	1854
13-	92.25	3.69	1.68	1.03	1.08	7.48	1844
14+	90.78	4.01	1.00	1.19	2.28	8.49	1096
15-	92.45	4.18	1.68	0.81	0.73	7.40	8938
16+	93.17	3.03	1.77	1.14	0.88	6.83	791
17+	94.23	3.00	1.15	0.92	0.69	5.77	433
18+	88.60	6.10	1.51	1.61	1.87	11.10	1982
19+	89.03	5.71	2.16	1.62	1.08	10.58	1295
20+	95.45	1.24	1.65	0.83	0.83	4.55	242
21+	91.70	4.04	2.24	1.35	0.67	8.30	446
23-	91.17	3.74	1.19	1.36	2.38	8.66	589
24-	93.55	2.05	2.35	0.88	1.17	6.45	341
25+	92.81	4.19	2.10	0.60	0.30	7.19	334
26-	92.51	2.72	1.79	1.87	1.02	7.40	1175
28+	93.39	4.26	0.75	0.75	0.53	6.29	938
30+	91.09	3.67	1.05	2.94	1.26	8.91	954
32-	92.77	3.21	2.41	0.40	1.07	7.10	747

TABLE 23: (continued)

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
33+	91.41	3.91	1.88	1.59	0.96	8.49	2073
35-	93.42	3.76	1.18	0.89	0.71	6.53	2127
37-	93.00	3.17	1.83	0.50	1.33	6.83	600
42-	93.43	3.90	0.95	0.66	0.95	6.47	1051
45-	91.86	2.79	1.40	1.63	2.33	8.14	430
46+	93.19	3.88	1.11	0.89	0.72	6.58	1806
48+	90.96	4.77	1.28	1.38	1.38	8.80	2976
49+	92.31	4.87	1.26	1.10	0.31	7.54	637
50-	91.99	4.32	1.00	1.87	0.77	7.96	2198
51+	87.01	7.21	1.75	1.75	1.75	12.45	916
52+	92.98	3.17	1.02	1.36	1.36	6.91	883
54+	91.70	4.56	0.97	1.12	1.16	7.81	2061
55+	91.62	4.64	1.59	0.68	0.79	7.70	883
56-	92.43	3.54	1.89	0.91	1.07	7.41	1215
57+	90.09	4.72	0.47	2.36	0.47	8.02	212
60-	91.39	4.17	1.39	1.39	1.11	8.05	360
61+	92.69	5.77	0.77	-	0.77	7.31	260
62+	91.57	4.08	0.54	2.72	0.54	7.89	368
63+	93.16	2.66	1.14	0.76	1.14	5.70	263
65-	91.29	5.50	1.29	0.92	0.92	8.63	1309
70+	93.98	2.68	0.33	1.34	1.00	5.36	299
74-	93.06	2.70	1.03	1.54	1.54	6.81	778
75+	91.60	4.27	1.65	1.50	0.90	8.32	1334
76+	93.64	4.24	0.61	0.91	0.61	6.36	330
77+	93.97	2.92	0.57	1.13	1.41	6.03	1061

TABLE 23: (continued)

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
78–	90.35	5.41	1.63	1.27	1.17	9.49	3068
81+	91.38	5.38	0.61	1.28	1.10	8.38	1636
82+	92.21	4.42	1.04	1.04	1.30	7.79	385
83+	91.53	4.74	1.17	1.40	1.17	8.47	1287
84–	91.18	4.90	1.40	0.84	1.68	8.82	714
85+	94.50	2.89	0.87	1.01	0.72	5.50	691
87–	91.01	5.29	1.85	0.80	1.01	8.95	2760
88+	90.85	5.60	0.84	1.49	0.93	8.87	1071
90–	91.80	5.37	1.17	0.94	0.59	7.97	427
92–	92.65	4.70	1.17	0.88	0.59	7.35	340
94–	91.09	4.73	1.91	1.18	0.81	8.64	1100
96–	89.66	5.78	2.12	1.33	0.88	10.12	5791
97–	92.31	3.80	1.63	1.55	0.70	7.69	1288
100+	92.19	3.07	1.18	1.89	0.47	6.62	423
101+	92.10	3.92	1.59	1.06	1.00	7.56	1507
102+	95.41	2.75	0.92	0.46	0.46	4.59	218
105–	90.53	5.59	1.26	1.51	0.89	9.24	4046
108+	91.84	4.34	0.97	1.42	1.29	8.03	1545
110–	91.38	4.74	2.16	0.86	0.86	8.62	232
111–	91.34	5.19	0.87	1.73	0.87	8.66	231
113–	92.23	4.20	1.47	0.42	1.05	7.14	476
116–	91.77	4.11	2.22	0.95	0.79	8.07	632

___ indicates recombinant isolate for *w*BHj and *ccf*-6 sites

--- indicates isolate showing intermediate conversion frequency

Of the 71 crosses shown in this Table, 63 can be classified as LCF with a range of conversion frequencies from 4.55 to 8.95%, although the few values of less than 6% are derived from very small sample sizes. Therefore these 63 isolates, when crossed to *w*BHj+/- tester strains showed conversion properties like their wild-type parent, i.e. they were non-recombinants for *w*BHj and *ccf*-6. 4 isolates broadly gave HCF when crossed to the same *w*BHj tester strains, with a range of conversion frequencies from 11.07% to 14.08%, i.e. they appeared to be recombinant for the *w*BHj and *ccf*-6 sites. This range of high conversion frequencies closely parallels that observed in the last experiment. A remaining 4 isolates gave intermediate conversion frequencies of 9.24 to 10.58% and so were not immediately classifiable. No apparent effect of the tester strain used was apparent, since 3 of these isolates were of (-) mating type and 1 of (+). A more likely origin of these intermediate conversion frequencies would be environmental effects and/or minor modifiers of gene conversion frequency.

Since this experiment did not produce a clearcut result, it was decided to cross the 4 isolates with apparently intermediate conversion frequencies to *w*BHj strains carrying *ccf*-6(*B*). It will be shown in the experiments described below that *B* × *B* crosses are high converting, while *A* × *B* crosses (with *A* both in *cis* and *trans* to *w*BHj) are low converting. Thus if the 4 indeterminate isolates are really non-recombinants and carry *ccf*-6(*B*) they should give HCF when crossed to *w*BHj strains carrying

ccf-6(*B*), while if the isolates were recombinants carrying *ccf*-6(*A*) they would give LCF crosses. The 4 apparently recombinant isolates were also included in this experiment as a confirmatory test of their recombinant nature. Furthermore, 1 clearly non-recombinant isolate (number 54) was included as an additional control along with isolate number 87, which gave the highest %NRCF among the LCF crosses.

Conversion behaviour of wild-type spores randomly isolated from 4+:4*w* octads from cross *w*BHj- × 25-5R+ when crossed to 14-4*w*BHj+ or 15-1*w*BHj-

Altogether 10 isolates from the previous experiment were crossed to *w*BHj strains carrying *ccf*-6(*B*). The strains used were 14-4*w*BHj+ and 15-1*w*BHj- (they are isolates number 14.4+ and 15.1- shown in Table 26. Their isolation is described in the next experiment). The conversion results obtained are shown in Table 24.

From this Table it can be seen that all isolates tested gave unambiguously clear LCF or HCF crosses, with a range of conversion frequencies of 5.52 to 7.79% among the LCF crosses and a range of 11.25 to 17.83% among the HCF crosses. Three replicates of each cross were performed but not all were fertile. However, where 3 replicates of any cross were fertile, all replicates were either LCF or HCF with no intermediate values. The results from this experiment are best considered alongside the results obtained with the same 10 isolates when crossed to *ccf*-6(*A*) tester strains. Accordingly, the conversion frequencies from both types of cross are displayed in Table 25.

TABLE 24: Conversion data of random wild-type isolates from 4+:4*w* octads from cross *w*BHj- x 25-5R+ when crossed to 14-4*w*BHj+ or 15-1*w*BHj-

Isolate No.	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
6-	92.14	4.41	1.61	0.95	0.83	7.79	5917
11-	94.23	3.50	1.07	0.59	0.36	5.52	1685
18+	93.57	3.21	0.83	1.19	0.83	6.06	1089
19+	92.34	3.98	1.49	1.09	0.90	7.46	2011
51-	93.87	3.57	1.23	0.78	0.45	6.02	897
54+	88.39	6.81	1.89	1.57	0.99	11.25	4658
78-	86.45	7.76	2.72	1.55	1.24	13.28	4444
87-	86.28	7.54	2.81	1.66	1.49	13.50	6867
96-	85.82	8.12	2.67	1.77	1.41	13.98	5874
105-	81.80	12.92	2.14	1.57	1.20	17.83	1912

TABLE 25: Conversion data of random wild-type isolates from 4+:4*w* octads from cross *w*BHj- x 25-5R+ when crossed to *w*BHj strains carrying *ccf*-6(*A*) and *ccf*-6(*B*)

Isolate No.	Crossed to *w*BHj, *ccf*-6(*A*) strains			Crossed to *w*BHj, *ccf*-6(*B*) strains		
	Total octads	Total narrower ratio conversion octads	%NRCF	Total octads	Total narrower ratio conversion octads	%NRCF
6-	2485	350	14.08 H	5917	461	7.79 L
11-	8720	965	11.07 H	1685	93	5.52 L
18+	1982	220	11.10 H	1089	66	6.06 L
19+	1295	137	10.58 H	2011	150	7.46 L
51-	916	114	12.45 H	897	54	6.02 L
54+	2061	161	7.81 L	4658	524	11.25 H
78-	3068	291	9.49 L	4444	590	13.28 H
87-	2760	247	8.95 L	6867	927	13.50 H
96-	5791	586	10.12 L	5874	821	13.98 H
105-	4046	374	9.24 L	1912	341	17.83 H

L denotes a LCF cross

H denotes a HCF cross

From Table 25 it can be seen that the 4 suspected recombinant isolates (numbers 6, 11, 18 and 51) had their recombinant nature confirmed by the experiment just described. All 4 gave HCF crosses with *w*BHj *ccf*-6(*A*) strains (11.07 to 14.08%NRCF) and LCF crosses with *ccf*-6(*B*) strains (5.52 to 7.79%NRCF). Thus the isolates concerned must have carried *ccf*-6(*A*). For each isolate there was an approximately 2-fold difference in conversion frequency between each type of cross.

Isolates 54 and 87 which gave LCF crosses to *ccf*-6(*A*) testers (7.81 and 8.95% respectively) showed HCF in crosses to *ccf*-6(*B*) testers (11.25 and 13.50% respectively) i.e. these isolates must have carried *ccf*-6(*B*) and be non-recombinant for the two sites in question.

Finally, the results for the 4 initially non-classifiable isolates can be considered:

Isolate 19: this showed the least clearcut difference in conversion frequencies between the two crosses (10.58 and 7.46%). However, it is probably a recombinant, carrying *ccf*-6(*A*).

Isolate 78: this gave a conversion frequency of 9.49% when crossed to a *ccf*-6(*A*) tester and 13.28% when crossed to a *ccf*-6(*B*) tester. This indicates that the isolate probably carries *ccf*-6(*B*) and is non-recombinant for *w*BHj and *ccf*-6.

Isolate 96: the respective conversion frequencies here were 10.12 and 13.98% and so this isolate would also appear to be non-recombinant.

Isolate 105: this isolate gave 9.24% NRCF when crossed to a *ccf*-6(*A*) tester and 17.83% NRCF when crossed to a *ccf*-6(*B*) tester. This isolate clearly carries *ccf*-6(*B*) and is non-recombinant.

Thus considering the data from the above two experiments combined, it would appear that out of 71 wild-type progeny randomly isolated from 4+:4*w* octads from the cross *w*BHj- × 25-5R+, heterozygous for both *w*BHj and *ccf*-6, 66 progeny were parentals with respect to the latter two sites, while 5 were recombinants. This would correspond to a recombination frequency of 7.04% between *w*BHj and *ccf*-6. These recombinants, depending on the mode of action of *ccf*-6, could have arisen in a number of ways other than by crossing-over between the two sites. For example, not all 4+:4*w* octads need be genuine non-convertant octads. Some could be aberrant 4+:4*w* or correction 4+:4*w* octads, which could yield progeny recombinant for the two sites in question, providing they are not so close that co-conversion is frequent. As mentioned in Chapter 2, Paquette (1978) pointed out that ab 4+:4*w* octads are not necessarily rare and comprise more than 40% of postmeiotic segregations at the *b2* A4 mutation in the European strains of *Ascobolus immersus*. However, in the Pasadena strains, Ghikas and Lamb (1977) reported that the frequency of ab 4+:4*w* octads for the locus I mutation *w*78 was only 0.08% in both LCF and HCF crosses. Some 4+:4*w*s may also be false clusters, i.e. comprised of ascospores dehisced from more than one ascus and clustered by chance to give an apparently normal 4+:4*w* octad. Such false clusters are usually rare, but

their frequency was not ascertained in this work. Thus the estimate of recombination frequency (7.04%) obtained from these experiments is best regarded as an upper limit. *w*BHj and *ccf*-6 are therefore quite closely linked, but not so closely linked as *ccf*-2 and locus I mutations (Lamb and Helmi, 1978; Wickramaratne and Lamb, 1978) and *ccf*-5 and BB9*w*9 (present work). The data from these experiments also show that the action of *ccf*-6 on *w*BHj conversion is not so profound as that of the other *ccfs* described in *Ascobolus immersus* (both Pasadena and European strains) on their respective sites. It seems highly probably that, at least in the strains used in this work, other minor modifiers of *w*BHj conversion are segregating which can cause some 'spreading' of conversion frequencies of both LCF and HCF crosses. Furthermore, the system also seems subject to non-genetic effects which are difficult to identify and quantify. This fact may be connected with the possible mechanisms by which *ccf*-6 affects *w*BHj conversion. Nevertheless, there is clear evidence for a conversion control factor fairly closely linked to *w*BHj and influencing its conversion behaviour. No ambiguous results were obtained in the two experiments described above.

The experiments described in this Chapter have furnished data on the conversion behaviour (both frequency and spectrum) of mutation *w*BHj in two types of cross:

1) *w*, *ccf*-6(*A*) × +, *ccf*-6(*B*) LCF

2) *w*, *ccf*-6(*A*) × +, *ccf*-6(*A*) HCF

The experiments described below determined the conversion behaviour of *w*BHj in the two remaining types of cross, the

results of which have been mentioned in the analysis above:

3) *w*, *ccf*-6(*B*) × +, *ccf*-6(*B*)

4) *w*, *ccf*-6(*B*) × +, *ccf*-6(*A*)

Conversion behaviour of *w*BHj in *ccf*-6(*B*) × *ccf*-6(*B*) crosses

Before the conversion behaviour of *w*BHj in *ccf*-6(*B*) homozygous crosses could be determined, it was necessary to select derived wild-type strains of both mating types, known to carry *ccf*-6(*B*), to act as tester strains. The strains chosen were 25-5R+ (originally designated as carrying *ccf*-6(*B*)) and 18P-. From Table 20 it can be seen that cross *w*BHj+ × 18P- is LCF. Since mutant strains *w*BHj+ and *w*BHj- have both been shown to carry *ccf*-6(*A*), derived wild-type strain 18P- must carry *ccf*-6(*B*).

Conversion behaviour of white spores isolated from 2+:6*w* octads from cross *w*BHj- × 25-5R+

No *w*BHj strains known to carry *ccf*-6(*B*) were available so a direct test of the conversion behaviour of mutation *w*BHj in *ccf*-6(*B*) homozygous crosses was not possible. First it was necessary to obtain the necessary *w*BHj strains via gene conversion. This was achieved by isolating white spores from 2+:6*w* octads from the LCF cross *w*BHj- × 25-5R+, which can be written:

w, *ccf*-6(*A*) × +, *ccf*-6(*B*)

The method of analysis of 2+:6*w* octads from such a cross was described in Chapter 2 and illustrated in Fig.3 (page 145)

and so only a brief resumé is given here: analysis of the 6 white spores from 2+:6*w* convertant octads (whether they arise by symmetrical or asymmetrical H-DNA formation) should reveal that 4 spores carry *ccf*-6(*A*) and give LCF crosses with *ccf*-6(*B*) wild-type strains, while 2 spores should carry *ccf*-6(*B*) and hence show altered conversion characteristics in crosses to the same tester strains. It should be remembered that *w*BHj and *ccf*-6 are not that tightly linked and that any crossing-over between the two sites unassociated with conversion could give 4 white spores with altered conversion characteristics in some octads.

Altogether white spores were isolated from 100 2+:6*w* octads, but at this stage *w*BHj ascospore germination was poor and only 30 spores germinated. 21 of these isolates gave fertile crosses with the 25-5R+ and 18P- derived wild-type strains. The data obtained from these crosses can be seen in Table 26.

Due to poor ascospore germination, complete analysis of the 6 white spores in any octad was impossible, although in octad 14, 5 of the 6 white spores germinated and all gave fertile crosses. Thus the data must be treated as originating from white spores randomly isolated from a population of 2+:6*w* octads. In this case a ratio of 2:1 for LCF: altered CF among the crosses would be expected. From Table 26 it can be seen that of the 21 white spore isolates, 16 gave LCF crosses with *ccf*-6(*B*) wild-type tester strains (with conversion frequencies of 5.00 to 8.39%) while 5 isolates showed altered, elevated conversion frequencies (of 11.18 to 14.45%) in similar crosses. These data closely

TABLE 26: Conversion data of white spore isolates from 2+:6*w* octads from cross *w*BHj- x 25-5R+ when crossed to *ccf*-6(*B*) carrying wild-type strains 25-5R+ or 18P-

Isolate No. (octad.spore)	Octad classes (+:*w*),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
10.3+	93.12	4.36	1.16	0.84	0.42	6.78	18.58	76.70	1904
10.4-	93.32	4.58	1.11	0.37	0.62	6.68	14.82	74.10	808
13.1-	91.61	4.55	2.10	0.35	1.40	8.39	20.86	58.40	286
14.1+	88.03	6.96	2.14	1.78	0.92	11.79	22.90	74.13	1638
14.2-	92.65	3.93	0.87	1.53	1.02	7.35	34.69	74.29	1375
14.3-	94.18	3.06	0.76	1.00	0.94	5.76	33.68	70.49	1702
14.4+	85.30	8.50	2.38	2.38	1.19	14.45	24.71	75.29	1177
14.5+	92.13	4.84	1.31	1.07	0.66	7.87	21.98	75.10	1220
15.1-	86.08	8.99	2.46	1.48	0.74	13.67	16.24	76.59	812
15.2+	94.05	3.18	0.97	1.18	0.51	5.85	28.89	74.53	1949
15.3+	93.83	3.66	1.03	0.77	0.58	6.04	22.35	73.34	1557
15.4+	93.78	3.54	0.75	0.96	0.86	6.11	29.79	73.65	933
18.1+	93.79	3.41	1.36	0.95	0.48	6.21	23.03	70.21	1466
18.3+	93.68	3.54	1.69	0.31	0.62	6.17	15.07	62.40	649
19.1+	94.01	3.90	0.70	1.00	0.40	5.99	23.37	81.80	1001
19.2+	88.60	7.73	1.55	1.33	0.57	11.18	16.99	81.04	2262
19.3+	88.29	7.81	1.80	1.35	0.75	11.71	17.93	78.22	666
19.5-	94.21	3.23	0.90	1.05	0.53	5.71	27.67	74.96	1331
20.3-	94.79	2.29	1.25	0.42	1.04	5.00	29.20	54.20	480
20.4+	94.47	2.86	0.95	0.38	1.34	5.53	31.10	58.59	524
20.6+	92.89	4.11	1.46	0.63	0.59	6.79	17.97	69.81	2533

Isolates with altered conversion frequencies are underlined

16 crosses show low conversion frequency (5.00 - 8.39% NRCF)
5 " " high " " (11.18 - 14.45% NRCF)

X^2 (for expected ratio of 2LCF: 1 altered CF among crosses) = 0.48

p = 0.30 - 0.50 (1df)

fit the expected 2:1 ratio (χ^2, applying Yates' correction = 0.48, p = 0.30-0.50, 1df). Altogether, it is almost certain that the isolates showing altered, elevated conversion frequencies arose by conversion of the *w*BHj site and thus carry *ccf*-6(*B*). In this case it can be deduced that *w*BHj shows a high conversion frequency in *ccf*-6(B) homozygous crosses. In fact, the conversion frequency is very similar to that observed in *ccf*-6(*A*) homozygous crosses, ranging from 11.18 to 14.45% in this experiment. It is worth noting that in octads 14 and 19 in each of which 2 isolates showing altered conversion properties were detected, both isolates were of the same mating type, as one would expect if they were sister spores. In octad 19, the agreement between the two elevated conversion frequencies is very good, the values being 11.18 and 11.71% ($\Sigma\chi^2$ = 0.13, p = 0.70-0.80, 1df). The agreement is less good in octad 14, where the 2 elevated frequencies are 11.79 and and 14.45% ($\Sigma\chi^2$ = 4.35, p = 0.02-0.05, 1df). This difference in conversion frequencies is thus just significant, perhaps being due to the relatively small sample sizes scored for both crosses and also environmental effects on conversion frequency in this system. In no octads were more than 2 isolates with altered conversion frequencies detected.

Table 26 also contains data on the conversion spectra of all the crosses performed. In the LCF *A* × *B* crosses, the relative frequency of PMS ranged from 14.82 to 34.69% while the frequency of conversion to wild-type ranged from 54.20 to 81.80%, i.e. quite a spread of values for both parameters of conversion spectra. In the case of the HCF

B × *B* crosses, the respective values were 16.24 to 24.71% and 74.13 to 81.04%. Thus although the range in value for both parameters was much smaller (probably due to the smaller number of such *B* × *B* crosses) the actual values fell within the ranges observed for the *A* × *B* crosses. It would therefore appear that there are no differences in conversion spectrum between the two types of cross and additionally no difference between *A* × *A*, *A* × *B* and *B* × *B* crosses.

In summary it can be said that *ccf*-6(*B*) homozygous crosses are high converting for *w*BHj, with conversion frequencies closely similar to those observed in *ccf*-6(*A*) homozygous crosses. The conversion spectrum is broadly similar in all three categories of cross investigated so far.

Conversion behaviour of *w*BHj in +, *ccf*-6(*A*) × *w*, *ccf*-6(*B*) crosses

It has been shown that mutation *w*BHj shows a low conversion frequency in +, *ccf*-6(*B*) × *w*, *ccf*-6(*A*) crosses (compared with *ccf*-6(*A*) and *ccf*-6(*B*) homozygous crosses), i.e., with *ccf*-6(*A*) in *cis* to the mutation. It does not necessarily follow from this that *w*BHj would show the same conversion behaviour in similar heterozygous +, *ccf*-6(*A*) × *w*, *ccf*-6(*B*) crosses, but with *ccf*-6(*A*) in *trans* to the mutation. Such *cis*/*trans* position effects on the conversion of locus I mutations by the closely linked *ccf*-2 factors were reported by Lamb and Helmi (1978) but no such *cis*/*trans* position effects of the *ccf*-5 factors on BB9*w*9

conversion were reported in this work. *Cis/trans* position effects are much more likely in the case of closely linked conversion control factors since it is difficult to see how unlinked factors could affect H-DNA formation or mismatch correction by virtue of their position with respect to the site concerned. *Cis/trans* position effects on a mutation's conversion behaviour can occur in terms of conversion frequency, conversion spectrum, or both. In the case of *w*BHj and *ccf*-6 it was thought that *cis/trans* position effects on conversion spectrum were most unlikely since the conversion spectrum was already known to be similar in +, *A* × *w*, *A*; +, *B* × *w*, *A* and +, *B* × *w*, *B* crosses. However, such effects were still thought possible in terms of *w*BHj conversion frequency. Thus it was endeavoured to determine the conversion behaviour of *w*BHj in crosses of the +, *ccf*-6(*A*) × *w*, *ccf*-6(*B*) type.

With this objective in mind, *w*BHj strains carrying *ccf*-6(*B*) and derived wild-type strains carrying *ccf*-6(*A*) were selected from previous experiments. From Table 26 isolates number 14.4+ and 19.2+ (from 2+:6*w* octads) were selected as *w*BHj, *ccf*-6(*B*) strains and designated 14-4*w*BHj+ and 19-2*w*BHj+ respectively. Three derived wild-type strains carrying *ccf*-6(*A*) were selected: isolate number 6 (from a 4+:4*w* octad) from Table 23 and isolates number 11.4- and 19.4- from Table 21 (from 6+:2*w* octads). these isolates were designated RM6-1-, RM11-4- and RM19-4- respectively. All of the isolates - both white mutant and wild-type - had been derived from LCF cross *w*BHj- × 25-5R+. The two white mutant strains were each crossed to all three of the derived wild-type strains and the results obtained

TABLE 27: Conversion data from +, *ccf*-6(*A*) x *w*BHj, *ccf*-6(*B*) crosses

Mutant strain	Crossed to	Octad classes (+:*w*),%					%NRCF	Relative % PMS	%Conv. to +	Total octads
		4:4	6:2	2:6	5:3	3:5				
14-4*w*BHj+	RM6-1-	93.08	3.48	1.69	0.91	0.65	6.73	23.18	65.23	5378
"	RM11-4-	92.11	4.13	1.42	1.13	1.07	7.75	28.39	67.87	9608
"	RM19-4-	94.20	3.25	1.16	0.46	0.74	5.61	21.39	66.13	2584
19-2*w*BHj+	RM6-1-	92.68	4.07	1.44	0.86	0.70	7.08	22.03	69.63	5821
"	RM11-4-	91.89	4.21	1.53	1.15	1.14	8.03	28.52	66.75	7474
"	RM19-4-	92.60	3.67	1.30	0.97	1.12	7.06	29.60	65.72	5783

from these crosses are shown in Table 27.

From Table 27 it can be seen that all six crosses were fertile and large sample sizes of octads were scorable - each cross had three replicates. All six crosses showed low conversion frequencies of 5.61 to 8.03%. There was little variability between replicates of the same cross. The mean % NRCF was 7.29% which compares very closely with the mean value of 7.68% for all the +, *ccf*-6(*B*) × *w*, *ccf*-6 (*A*) crosses performed in this work (see Discussion and Table 30). There is thus no evidence for *cis/trans* position effects of the two *ccf*-6 factors on *w*BHj conversion. It can also be seen from Table 27 that all six crosses gave very closely similar conversion spectra with the frequency of PMS ranging from 21.39 to 29.60% and the frequency of conversion to wild-type ranging from 65.23 to 69.63%. These spectra are similar to those seen in +, *ccf*-6(*B*) × *w*, *ccf*-6(*A*) crosses (and indeed to those of +, *ccf*-6(*A*) × *w*, *ccf*-6(*A*) and +, *ccf*-6(*B*) × *w*, *ccf*-6(*B*) crosses), with no evidence of any *cis/trans* position effects. This latter finding had been expected.

Originality of *ccf*-6

ccf-6 can be shown to be a truly original conversion control factor and not in any way associated with the five hitherto known *ccfs* in the Pasadena strains of *Ascobolus immersus:*

ccf-1 determines the conversion behaviour of closely linked white ascospore pigmentation mutation *w*62 in a way broadly

similar to the action of *ccf*-6 on *w*BHj. Prior to this work the linkage relations of *w*62 and *w*BHj were not known. They were accordingly mapped and found to be unlinked (R.F. = 49.70%). Thus *ccf*-1 and *ccf*-6 must be quite separate, distinct factors. *ccf*-2 is closely linked to locus I (Wickramaratne and Lamb, 1978; Lamb and Helmi, 1978) and it was even suggested by Helmi (1980) that *ccf*-2 might lie within locus I. In this work locus I mutation *w*78 was shown to be unlinked to *w*BHj (R.F. = 48.90%) and so *ccf*-6 cannot be confused with *ccf*-2. Furthermore, the data presented in Table 20 of this work indicate that the same *w*BHj strains can show LCF or HCF when crossed to derived wild-type strains carrying the same form of *ccf*-2 - *ccf*-2(*P*). Helmi (1980) reported that *ccf*-3 is linked to the mating type locus, while in this work *w*BHj and hence *ccf*-6 showed no signs of linkage to the mating type (the best data come from Table 23: of 71 random wild-type isolates from cross *w*BHj- × 25-5R+, 39 are of (+) mating type and 32 of (-), giving a R.F. of 45.07% between *w*BHj and the mating type locus). *ccf*-4 is reported as being loosely linked to locus I (Helmi, 1980) while *w*BHj and hence *ccf*-6 is unlinked to this locus. From this it cannot conclusively be deduced that *ccf*-4 and *ccf*-6 are unlinked. *ccf*-4 is one of the two factors (along with *ccf*-3) which make up the "Super" conversion control system. The data in Table 20 show that "Super" and "non-Super" wild-type strains can both give HCF in crosses to *w*BHj strains, while the data to be considered in Chapter 6 show that "Super" wild-type strains can also give LCF crosses with *w*BHj strains. Overall, the data

do not conclusively show that *ccf*-4 and *ccf*-6 are distinct (since the *w*BHj strain in the above crosses was not the same in each) but this seems highly probable, particularly since the modes of action of *ccf*-4 and *ccf*-6 are different (*ccf*-4 is a dominant reducer of gene conversion at loosely linked locus I sites). Confirmatory crosses of different "Super" strains to the same *w*BHj strain are needed. *ccf*-5 and *ccf*-6 must be completely distinct factors since in this work it was found that mutations BB9*w*9 and *w*BHj are either very loosely linked or unlinked (R.F. = 44.10%). Both *ccfs* are closely linked to the respective mutation whose conversion behaviour they modify. Furthermore it was shown in Chapter 2 of this work that mutation *w*BHj can show high conversion frequencies when the same strain is crossed to derived wild-type strains carrying both forms of *ccf*-5. Also crosses of the same *w*BHj strain to derived wild-types carrying the same *ccf*-5 factor can be both LCF and HCF.

Thus in summary it can be concluded that *ccf*-6 is a truly novel factor in the Pasadena strains of *Ascobolus immersus*. It has been shown conclusively that it is distinct from *ccfs* 1, 2, 3 and 5 and is almost certainly distinct from *ccf*-4.

Specificity of *ccf*-6

The effects of *ccf*-6 on the conversion behaviour of mutation *w*BHj have been well documented, but it was not known whether the action of *ccf*-6 is specific to *w*BHj or extends to other parts of the genome. From the data already obtained it can be ascertained that *ccf*-6 has no

apparent effects on the conversion of locus I mutations since strains carrying *ccf*-6(*A*) and *ccf*-6(*B*) both give high conversion frequencies when crossed to such mutations if the wild-type strains carry *ccf*-2(*P*): all the 'P' strains in Table 20 carry *ccf*-2(*P*) while some carry *ccf*-6(*A*) and some *ccf*-6(*B*).

In this experiment derived wild-type tester strains carrying *ccf*-6(*A*) and *ccf*-6(*B*) were crossed to all mutants of loci II, III and IV plus BB9*w*9 and NG*w*14 mutant strains. The latter two mutations are unlinked to the three loci. The behaviour of mutant strains BB9*w*9 and NG*w*18 was particularly sought since the conversion behaviour of BB9*w*9 was investigated in Chapter 2 of this thesis and an investigation of NG*w*18 conversion behaviour is to form the basis of the next Chapter. The derived wild-type strains used were 7R7- and RM9-2 + (isolate 9.2+, Table 21), each carrying *ccf*-6(*B*). From Table 20 it was deduced that strain 42- carries *ccf*-6(*A*) and strain 9r+ *ccf*-6(*B*). Knowing this, crosses of the mutant strains under study to *ccf*-6(*A*) and *ccf*-6(*B*) wild-types can be found in the work of Lamb and Ghikas (1979). 7 of these crosses are included in Table 28, along with those performed in this work. As a result, conversion data are obtainable for each mutant under study (except NG*w*12) when crossed to at least one *ccf*-6(*A*) and one *ccf*-6(*B*) wild-type strain. More importantly, for each mutation except B*w*3.10 (and NG*w*12) there are conversion data involving the same mutant strain when crossed to the two types of derived wild-type.

TABLE 28: Conversion data of locus II, III, IV and unlinked mutations in crosses to *ccf*-6(*A*) and *ccf*-6(*B*) derived wild-type strains

Mutant	Crossed to	Octad classes (+:*w*),%					% NRCF	Total octads
		4:4	6:2	2:6	5:3	3:5		
LOCUS II								
	7R7- (*A*)	99.81	-	0.05	0.14	-	0.19	3664
NG*w*18	42- " *	96.67	2.11	0.35	0.59	0.26	3.30	8541
	16P- (*B*)	99.79	0.07	0.04	0.11	-	0.21	2855
	42- (*A*)*	99.60	0.01	0.04	0.20	0.15	0.40	8060
S*w*26	RM9-2+ "	100.00	-	-	-	-	0.00	640
	RM11-2+(*B*)	99.78	-	0.11	0.11	-	0.22	894
	RM9-2+ (*A*)	94.95	0.60	0.12	3.13	0.96	4.81	831
NG*w*16	RM11-2+(*B*)	95.99	0.88	0.25	1.88	1.00	4.01	799
	25-5R+ "	98.30	1.14	0.19	0.38	-	1.70	1057
LOCUS III								
	RM9-2+ (*A*)	99.11	-	0.30	-	0.60	0.89	336
B*w*4.2	RM11-2+(*B*)	99.57	-	-	0.22	0.22	0.43	462
	9r+ "*	99.84	0.07	0.02	0.05	0.02	0.16	10934
B*w*3.10	42- (*A*)*	99.73	0.04	-	0.19	-	0.23	4841
	9r+ (*B*)*	99.68	0.05	0.08	0.19	-	0.32	7524
	RM9-2+ (*A*)	99.52	-	0.24	-	-	0.24	826
C*w*1.14	RM11-2+(*B*)	98.68	0.08	0.77	0.23	0.23	1.32	1288
	25-5R+ (*B*)	99.50	0.08	0.34	0.06	0.02	0.50	6232

TABLE 28: (continued)

Mutant	Crossed to		Octad classes (+:*w*),% 4:4	6:2	2:6	5:3	3:5	% NRCF	Total octads
LOCUS IV									
	7R7-	(*A*)	99.75	0.06	0.06	0.06	0.06	0.25	1600
NG*w*11	42-	"*	99.55	0.10	0.17	0.03	0.15	0.45	5952
	18P-	(*B*)	99.66	0.06	0.06	0.06	0.17	0.34	1733
UNLINKED MUTATIONS									
	42-	(*A*)*	98.61	0.93	0.35	0.10	0.01	1.39	7169
BB9*w*9	7R7-	"	87.91	6.49	0.89	3.17	0.48	11.03	4162
	18P-	(*B*)	89.76	5.21	1.23	3.60	0.09	10.14	1649
	RM9-2+	(*A*)	99.71	-	0.10	0.10	0.10	0.29	1019
NG*w*14	RM11-2+	(*B*)	99.46	0.36	-	-	0.18	0.54	558
	25-5R+	"	99.70	-	0.15	0.05	0.10	0.30	1969
	18P-	"	99.80	0.08	-	0.08	0.03	0.20	3552

* Data of Lamb and Ghikas (1979)

(*A*) denotes *ccf*-6(*A*) strain

(*B*) denotes *ccf*-6(*B*) strain

locus II

NG*w*18. *ccf*-6 does not seem to be implicated in the difference between HCF and LCF crosses involving mutation NG*w*18. Derived wild-type strains carrying *ccf*-6(*A*) can give both LCF and HCF crosses to the same NG*w*18 mutant strain. There is an approximately 15-fold difference in conversion frequency between the two types of cross in this data. Furthermore, derived wild-type strains carrying *ccf*-6(*A*) or *ccf*-6(*B*) can both give LCF crosses to the same NG*w*18 strain.

S*w*26. All three fertile crosses involving this mutation were low converting - this mutation has always shown a low conversion frequency in crosses to all wild-type strains undertaken, ranging from 0.14 to 0.40%. It is unlikely that the slight differences observed in S*w*26 conversion frequency in this work were due to the action of *ccf*-6, despite the fact that the *ccf*-6(*A*)+ cross showed zero conversion frequency and the *ccf*-6(*B*)+ cross showed a conversion frequency of 0.22%. The crosses concerned gave rather small sample sizes and so it would be instructive to repeat them with many replicates of each.

NG*w*16. *ccf*-6(*A*) and *ccf*-6(*B*) wild-types gave very similar conversion frequencies in crosses to the same NG*w*16 mutant strain. However, two crosses involving different *ccf*-6(*B*) strains gave rather different conversion frequencies and spectra. This might be due to the action of a hitherto unknown conversion control factor, which cannot be *ccf*-6, or sampling or environmental effects.

These data show that *ccf*-6 is not involved in locus II

conversion control, although this cannot be stated as proven for mutation S*w*26. The lack of influence of *ccf*-6 on NG*w*18 is particularly interesting since its conversion behaviour is investigated in the next Chapter of this thesis.

locus III

B*w*4.2. Small differences in the conversion frequency of this mutation were observed by Lamb and Ghikas (1979) and Helmi (1980), which may have a genetic basis. Similar, but less profound differences were observed in this work. However, it is unlikely that *ccf*-6 is involved, since crosses of different *ccf*-6(*B*) strains to the same B*w*4.2 strain gave slightly different conversion frequencies. However, the cross involving the *ccf*-6(*A*) wild-type strain gave an even higher conversion frequency. Since all three crosses gave conversion frequencies of less than 1% and the sample sizes of two of the crosses were small, there is no direct evidence for any action of *ccf*-6 on B*w*4.2

B*w*3.10. *ccf*-6(*A*) and *ccf*-6(*B*) derived wild-type strains both gave very similar low conversion frequencies when crossed to B*w*3.10 mutant strains, although the strains concerned were different. *ccf*-6 would not seem to be implicated in having any action on B*w*3.10 conversion.

C*w*1.14. Helmi (1980) detected slight variations in the conversion frequency of this mutation in crosses to different derived wild-type strains. These variations were also observed in this experiment and may have a

genetic basis. However, this cannot involve *ccf*-6, since crosses of different *ccf*-6(*B*) derived wild-type strains to the same C*w*1.14 mutant strain show these differences too, while the cross involving *ccf*-6(*A*) showed an even lower conversion frequency.

These data concerning locus III mutations do not point to the involvement of *ccf*-6 modifying their conversion behaviour in any significant way.

locus IV

NG*w*11. All three fertile crosses shown in Table 28 were low converting, with a range of frequencies from 0.25-0.45%. All derived wild-type strains were crossed to the same NG*w*11 mutant strain. There are thus no significant effects of *ccf*-6 on NG*w*11 conversion.

Unfortunately none of the crosses involving mutation NG*w*12 was fertile and no appropriate crosses could be found in past literature.

BB9*w*9

The conversion behaviour of this mutation is under the control of *ccf*-5. In Chapter 2 it was shown that *ccf*-5 has no action on *w*BHj and in this Chapter that *ccfs* 5 and 6 are distinct. The data in Table 28 show that *ccfs* 5 and 6 do not interact in any way to influence BB9*w*9 conversion behaviour. All three crosses involved the same BB9*w*9 strain. Two crosses involved different *ccf*-6(*A*)

carrying derived wild-type strains: one was LCF, the other HCF, due to the *ccf*-5 factors known to be carried by the strains in question. Furthermore, derived wild-type strains carrying *ccf*-6(*A*) or *ccf*-6(*B*) both gave high conversion frequencies of BB9*w*9 in crosses. Thus the *ccf*-6 factors have no influence on BB9*w*9 conversion.

NG*w*14

The data provide no evidence for the action of *ccf*-6 on NG*w*14 conversion.

Summary

This experiment and earlier data provide no evidence for the action of *ccf*-6 on mutations of loci I, II, III and IV and unlinked mutations BB9*w*9 and NG*w*14. As far as the data go it would appear that the action of *ccf*-6 is specific to *w*BHj. It would be extremely valuable to induce mutations closely linked to *w*BHj and of different mutagenic origins and determine the action of *ccf*-6 on them. This specificity of *ccf*-6 to the *w*BHj site is not surprising in view of the possible mode of action of the *ccf*, which will be discussed in Chapter 5.

Origin of the two *ccf*-6 factors

As already noted, all our laboratory strains of *Ascobolus immersus* have been derived from the original two isolates of the Pasadena strains, taken from a dung sample

by Dr. C. Yu-Sun and Professor S. Emerson in 1963. These original wild-type isolates were designated P5- and K5+. Thus any genetic conversion control factors with different forms discovered in the Pasadena collection of strains since 1963 could have originated in 3 ways:

1. They could have been present as a polymorphism in the original P5- and K5+ strains.
2. One form may since have arisen from the other by mutation.
3. One form may since have arisen from the other by various recombinational processes, such as unequal crossing-over, etc.

The obvious way to distinguish between these possibilities for *ccf*-6 was to cross *w*BHj strains of known *ccf*-6 genotype to the original P5- and K5+ wild-type strains to determine whether one strain carried *ccf*-6(*A*) and the other *ccf*-6(*B*). If both apparently carried the same factor, then either a second *ccf* is involved in controlling *w*BHj conversion, or the *ccf*-6 factors arose later in the ancestry of the Pasadena strains. Further crosses would be necessary to distinguish between these possibilities.

As stated in Chapter 2, for many years all sub-cultures of the original P5- and K5+ strains had been completely sterile. Nevertheless, 1 lyophilised culture of each strain remained and these were vegetatively revivable. Many replicates of crosses *w*BHj+ × P5- and *w*BHj- × K5+ were performed. No replicates of the latter cross were fertile, but 5 replicates of the former cross were.

The overall data for this cross are given in Table 29. Cross *w*BHj+ × P5- showed a very low conversion frequency. In fact the frequency was lower than in any other crosses that had been performed involving mutation *w*BHj. Since strain *w*BHj+ carries *ccf*-6(*A*) it would seem that strain P5- carries *ccf*-6(*B*) since the cross is LCF. This is not certain, however, because the conversion frequency is lower than usual for *A* × *B* crosses. This could be due to a second major conversion control factor reducing *w*BHj conversion in *ccf*-6(*A*) × *ccf*-6(*B*) or even *ccf*-6(*A*) homozygous crosses. Alternatively, the low frequency of *w*BHj gene conversion could be due to the action of several minor modifiers of *w*BHj conversion.

Since cross *w*BHj- × K5+ was infertile, the literature was examined for pre-existing data involving crosses of *w*BHj strains to early descendents of K5+ (and P5- too). The data of Lamb and Ghikas (1979) included a cross of *w*BHj+ to KIII-, while cross *w*BHj- × KIV+ was performed in this work. These 'K' derived wild-type strains are second generation derivatives of K5+ and pedigree diagram analysis indicates that each strain has a 0.5 chance of inheriting a particular allele from the original K5+ (or P5-) strain (for ancestry of KIII- and KIV+ see Fig.4, page 175). The data from crosses *w*BHj+ × KIII- and *w*BHj- × KIV+ are given in Table 29.

TABLE 29: Conversion data from crosses *w*BHj+ × P5-, *w*BHj+ × KIII- and *w*BHj- × KIV+

Cross	Octad classes (+:*w*), %					% NRCF	Relative % PMS	% Conv. to +	Total Octads
	4:4	6:2	2:6	5:3	3:5				
*w*BHj+ × P5-	96.08	2.24	0.45	0.87	0.35	3.92	31.12	79.34	4010
*w*BHj+ × KIII-*	94.26	3.82	0.57	-	1.34	5.74	23.34	66.67	1046
*w*BHj- × KIV+	93.05	3.67	0.79	1.29	0.99	6.75	33.78	73.48	1007

* Data of Lamb and Ghikas (1979)

Thus it can be seen that all three crosses are low converting and are therefore most likely to carry *ccf*-6(*B*) since both *w*BHj strains used carry *ccf*-6(*A*). The conversion frequencies for crosses *w*BHj+ × KIII- and *w*BHj- × KIV+ are quite acceptable for *A* × *B* crosses, but as already stated, the value for cross *w*BHj+ × P5- is rather low. Although all 3 crosses exhibit similar conversion spectra in terms of relative frequencies of PMS and conversion to wild-type, the actual spectrum of cross *w*BHj+ × KIII- differs in detail - no 5:3 octads were detected. This might be due to genetic causes or sampling effects. Since no fertile repeats of this cross were obtainable, and the same phenomenon was not seen in any other crosses, the question must remain open.

In conclusion, it would seem that *ccf*-6 factor (*B*) was present in the original P5- strain, probably in conjunction with other minor modifiers of *w*BHj conversion. It was not possible to detect *ccf*-6(*A*) in two second generation derivatives of original strain K5+, but since each of these strains

has only a 0.5 chance of possessing a particular K5+ allele this does not mean that K5+ carried *ccf*-6(*B*) and not *ccf*-6(*A*). Therefore it was not possible to demonstrate that the two forms of *ccf*-6 were present in the two original Pasadena strains as a polymorphism, but this remains likely since this is true of the two forms of *ccf*-1 and the *P* and *K* forms of *ccf*-2 (Emerson and Yu-Sun, 1967) and very probably true of the two forms of *ccf*-5 (Chapter 2 of this thesis).

Discussion

The above experiments have demonstrated that the conversion behaviour of mutation *w*BHj is subject to genetic control, but with small variations in conversion frequency being partly attributable to imperfectly controlled environmental factors. The factor primarily responsible for *w*BHj conversion control in this study is novel, having been shown to be distinct from the hitherto known *ccfs* 1, 2, 3, 4 and 5 and was designated *ccf*-6. *ccf*-6 has been shown to be fairly closely linked to *w*BHj (R.F. = 7.04%), while so far two alternative forms of *ccf*-6 - *A* and *B* - have been discovered in the Pasadena strains. Wild-type and white mutant strains carrying either *ccf*-6(*A*) or *ccf*-6(*B*) were isolated by analysing 4+:4*w*, 6+:2*w* or 2+:6*w* octads from + × *w* crosses heterozygous for the two forms of *ccf*-6. The isolation of such strains made possible a whole cycle of + × *w* crosses with all combinations of the *ccf*-6 factors:

+, *ccf*-6(*A*) × *w*, *ccf*-6(*A*)

+, *ccf*-6(*A*) × *w*, *ccf*-6(*B*)

+, *ccf*-6(*B*) × *w*, *ccf*-6(*A*)

+, *ccf*-6(*B*) × *w*, *ccf*-6(*B*)

In these crosses, the overall conversion frequency, conversion frequency to wild-type and to mutant and the relative frequency of PMS were studied. The overall results for each of the above types of cross are summarized in Table 30. The results for each type of cross were based on pooling data from many crosses of that type. These crosses were either replicates of the same cross or different individual crosses but identical with respect to *w*BHj and *ccf*-6 genotypes. Except in the case of 2 *B* × *B* crosses, no crosses which gave a total sample of less than 1000 dehisced octads were included. The variability among crosses of the same type was tested by the $\Sigma\chi^2$ homogeneity test, applying this to narrower ratio conversion frequency data. The data from all 4 types of cross were heterogeneous: for the 24 *A* × *A* crosses, $\Sigma\chi^2 = 199.96$, $p < 0.01$, 23 df; for the 43 *A* × *B* crosses, $\Sigma\chi^2 = 97.37$, $p < 0.01$, 42 df; for the 6 *B* × *A* crosses, $\Sigma\chi^2 = 22.95$, $p < 0.01$, 5 df; and for the 5 *B* × *B* crosses, $\Sigma\chi^2 = 9.72$, $p = 0.02$-0.05, 4 df. However, it had been shown that replicates of the same cross also showed similar heterogeneity in conversion frequency, at least in the case of LCF cross *w*BHj- × 25-5R+ ($\Sigma\chi^2 = 24.58$, $p < 0.01$, 6 df). This suggested that imperfectly controlled environmental factors were at least partially responsible for the heterogeneity among data from crosses of the same type. This, of course, does not rule out any effects of genetic background on conversion frequencies at the *w*BHj site, but

TABLE 30: Conversion data from + x *w*BHj crosses with different combinations of *ccf*-6 factors

ccf-6 factor in + *w*BHj		Octad classes (+:*w*) Frequency as % of total octads					Total octads	%NRCF ± S.E.	Octad classes (+:*w*) Relative frequency,%				Relative frequency of PMS, %	%Conv. to +	*Some conversion parameters					
															Direction of conversion ratio				Efficiency of correction ratio	
		4:4	6:2	2:6	5:3	3:5			6:2	2:6	5:3	3:5			a	b	c	d	e	f
A	A	86.33	7.68	2.51	1.73	1.47	97878	13.38±0.11	57.38	18.77	12.90	10.95	23.85	70.28	3.06	1.18	2.63	2.59	7.39	3.19
A	B	92.13	4.28	1.52	1.03	0.85	125102	7.68±0.08	55.72	19.78	13.40	11.11	24.51	69.12	2.82	1.21	2.46	2.34	7.16	3.08
B	A	92.52	3.91	1.45	0.99	0.95	36648	7.29±0.14	53.59	19.84	13.55	13.02	26.57	67.14	2.70	1.04	2.29	2.60	6.52	2.76
B	B	87.52	7.84	1.98	1.65	0.81	6556	12.28±0.41	63.85	16.15	13.42	6.58	20.00	77.27	3.95	2.04	3.63	1.94	9.00	4.00

* a-d: direction of conversion ratio (conversion to +/conversion to *w*)

a = value for detected conversion classes with meiotic segregation = 6:2/2:6

b = " " " " " " postmeiotic " = 5:3/3:5

c = overall value for dual hybrid chromatid models = 2(6:2)+5:3/2(2:6)+3:5

d = " " " single " " " = (6:2)(3:5)/(2:6)(5:3)

e-f : efficiency of correction ratio (detected correction events/detected non-corrections)

e = dual hybrid chromatid models = 2(6:2+2:6)+5:3+3:5/5:3+3:5

f = single " " " = 6:2+2:6/5:3+3:5

makes them more difficult to quantify.

Considering the data in Table 30, it can be seen that the conversion frequency of *w*BHj is high in crosses homozygous for either form of the linked *ccf*-6 and low in crosses heterozygous for these factors. There is a slight difference in conversion frequency between the two types of HCF cross, which is nonetheless statistically significant ($\Sigma\chi^2 = 6.62$, p = 0.01-0.02, 1df). The mean conversion frequencies were 13.38% for the 24 *A* × *A* crosses and 12.28% for the 5 *B* × *B* crosses. This difference in conversion frequency may have a genetic basis, most likely involving *ccf*-6 but perhaps interactions between the *ccf*-6 factors and other minor modifiers of gene conversion could be involved. Overall, it can be said that the conversion frequency of *w*BHj is reduced by a little less than one-half in crosses heterozygous for the two forms of *ccf*-6 compared with crosses homozygous for either form. This is a much less drastic reduction in conversion frequency than that reported for mutation BB9*w*9 in crosses heterozygous for the two forms of *ccf*-5 reported in Chapter 2 of this thesis. Furthermore, unlike BB9*w*9 and *ccf*-5, *w*BHj showed a broadly similar conversion spectrum in crosses with all four combinations of *ccf*-6 factors. PMS was relatively frequent, ranging from 20.00% in *B* × *B* crosses to 26.57% in *B* × *A* crosses, while conversion was predominantly to wild-type, ranging from 67.14% in *B* × *A* crosses to 77.27% in *B* × *B* crosses. Thus the conversion spectrum of *w*BHj is of type C (according to the classification of Leblon, 1972 a,b) in all four categories of cross. It was not possible to conclusively determine the

molecular nature of mutation *w*BHj by reversion studies (see Chapter 3, PART TWO).

Considering conversion spectra in more detail, the relative frequencies of each of the narrower ratio conversion classes show relatively good agreement for all four categories of cross. Only the *B* × *B* crosses show any slight differences with respect to the other three types of cross: in *B* × *B* crosses, 6:2 octads were slightly more frequent and 3:5 octads more infrequent than in the other crosses. The relative frequency of PMS was also lowest in this category of cross. These very slight differences in conversion spectrum might be a true attribute of the *ccf*-6 system, but since the overall *B* × *B* data are only based on the pooled data from 5 crosses with a total sample size of less than 7000 octads, it is probably wrong to attach much importance to any slight differences in conversion spectrum. In summary, the *ccf*-6 factors do not seem to have any influence on the conversion spectrum of mutation *w*BHj.

The conversion parameters a, b, c, d, e and f shown in Table 30 enable further information to be gained from the conversion data of *w*BHj in each type of cross. These estimates were defined by Wickramaratne and Lamb (1978) and the defining equations are given in Table 30. From this Table it can be seen that the estimates obtained for each of the six parameters are broadly the same in all four categories of cross. Only the *B* × *B* crosses show any pronounced differences from the other three types of cross. For example, the direction of conversion ratio in PMS octads is much more strongly to wild-type in *B* × *B* crosses.

For all the other five parameters, the estimate for the *B* × *B* crosses are always at one extreme end of the range seen in all four types of cross, but the departures in value are not so great as for parameter (b) described above. As already noted, these slight differences in conversion spectrum of *B* × *B* vis à vis the *A* × *A*, *A* × *B* and *B* × *A* crosses may have more to do with the small sample size scored for the few *B* × *B* crosses than the action of the *ccf*-6 factors themselves. Overall, it can be said that the direction of conversion ratio in all types of cross is more strongly to wild-type among MS than among PMS octads. Furthermore, the overall direction of conversion ratio differs little for single or dual hybrid DNA models, except for *B* × *B* crosses where the conversion to wild-type ratio is greater for dual hybrid chromatid models. The efficiency of correction ratio is greater for dual hybrid chromatid models for all four categories of cross. A consideration of these parameter estimates is interesting, but since the frequency of ab4:4 octads at the *w*BHj site is not known, it was not possible to deduce whether the *w*BHj conversion data fit the symmetrical H-DNA Holliday-type model using the equations of Lamb and Helmi (unpublished).

The data obtained in these experiments and summarized in Table 30 show that the two forms of *ccf*-6 do not show any appreciable *cis/trans* position effects on *w*BHj conversion, either in terms of conversion frequency, spectrum or both. This is similar to the situation reported for BB9*w*9 and *ccf*-5 in Chapter 2 of this thesis, but unlike

the three forms of *ccf*-2 which show clear *cis/trans* position effects on the conversion behaviour of closely linked locus I mutations (Lamb and Helmi, 1978). In fact, since *w*BHj and *ccf*-6 showed a recombination frequency of 7.04% in this work, *cis/trans* position effects of the *ccf*-6 factors on *w*BHj conversion behaviour are much less likely to occur than for the *ccf*-2 factors and the more closely linked locus I mutations. Following on from this, co-conversion of *w*BHj and *ccf*-6 would be much less likely to occur than for the very closely linked BB9*w*9 and *ccf*-5. Indeed, in the relevant experiments there was no evidence of co-conversion of *w*BHj and *ccf*-6 in this work.

The overall data on *w*BHj conversion behaviour clearly point to the existence of a closely linked conversion control factor - *ccf*-6 - which reduced the conversion frequency of mutation *w*BHj in crosses heterozygous for the two forms of the factor. This reduction in conversion frequency was generally about 40%. Most crosses were clearly classifiable as LCF or HCF, but in some few instances there was some ambiguity as the conversion frequency distributions of the two classes can sometimes overlap slightly, as in the case of the data in Table 23. In cases such as this it was necessary to cross such isolates with intermediate conversion frequencies to tester strains carrying *ccf*-6(*A*) and *ccf*-6(*B*) before the *ccf*-6 genotype of the isolate under test could be determined. Such tests always resulted in a clearcut result with no ambiguities. However, the fact that such confirmatory tests of *ccf*-6 genotype were sometimes necessary showed that either the

ccf-6 conversion control system, not producing very large differences in conversion frequencies of *w*BHj, is particularly sensitive to imperfectly controlled environmental conditions or that in the crosses under study, minor modifiers of *w*BHj conversion behaviour were segregating. In all probability both of these possibilities are involved. In this context it is interesting to note that when mutant strain *w*BHj+ was crossed to original wild-type strain P5-, the lowest conversion frequency of *w*BHj (3.92%) was reported in the whole work. This might have been due to the action of an unknown *ccf* of major effect interacting with *ccf*-6 to reduce the conversion frequency of *w*BHj. Alternatively, in cross *w*BHj+ × P5- the genotypes of many minor modifiers of gene conversion could have been such as to quantitatively reduce gene conversion. Unfortunately, the cross of mutant strain *w*BHj- to the other original wild-type strain K5+ was completely infertile. It might have yielded very informative results, particularly if the *w*BHj conversion frequency was higher than usual for a HCF cross. It remains quite likely that future experiments involving mutation *w*BHj may reveal the existence of further conversion control factors modifying *w*BHj conversion behaviour. They may be of major, but more probably minor effect. However, the identification of the latter type of factor would be much more difficult. The existence of such further factors is pointed to not only by the relatively low frequency of *w*BHj conversion in cross *w*BHj+ × P5- but also by the fact that the *w*BHj conversion frequency in

normal LCF crosses still averages from 7 to 8%. This is still fairly high in terms of the LCF crosses for most other mutations whose conversion control systems have been investigated, both in the Pasadena and European strains of *Ascobolus immersus*.

Experiments were undertaken which determined that *ccf*-6 has no effect on the gene conversion behaviour of mutations in loci II, III and IV and unlinked mutations BB9*w*9 and NG*w*14. Other preliminary data obtained earlier in this Chapter also indicated that *ccf*-6 could have no effect on locus I mutations. It is probable that even if further mutations were included in a subsequent experiment, *ccf*-6 would have no effect on their conversion properties either. Its action is probably limited to its own vicinity, although the induction and isolation of other mutations in that vicinity is of great interest for further work. This specificity of *ccf*-6 to a closely linked site and its action in reducing gene conversion frequencies of such a site in heterozygous crosses are strongly indicative of the nature and mode of action of *ccf*-6, topics which are discussed fully in Chapter 5.

What of the possible origins of the two known forms of *ccf*-6? It has been shown that *ccf*-6(*B*) was most probably present in the original P5- wild-type strain isolated in 1963, but this is not absolutely certain as *w*BHj showed a conversion frequency lower than usual for such a $A \times B$ cross. Due to infertility of the other original wild-type strain K5+, it was not possible to demonstrate whether this strain carried *ccf*-6(*A*). Data were available

from crosses of *ω*BHj mutant strains to derived wild-type strains KIII- and KIV+, both second generation derivatives of P5- and K5+. Both derived wild-types apparently carried *ccf*-6(*B*), like P5-. This does not rule out the possibility that K5+ could have carried *ccf*-6(*A*). An interesting point was the unusual conversion spectrum shown by *ω*BHj in cross *ω*BHj+ × KIII-. This spectrum was not seen in any other cross in this work and could thus have been due to sampling effects. Alternatively it could indicate the existence of a third form of *ccf*-6 or the action of other modifiers of *ω*BHj conversion behaviour. It should be noted that not all conversion control factors are limited to having two forms. In the Pasadena strains of *Ascobolus immersus*, *ccf*-2 has three forms (Lamb and Helmi, 1978), while the *ss* recombination modulator gene in *Neurospora crassa* (which like *ccf*-6 reduces intragenic recombination at a closely linked site in heterozygous crosses) has three polymorphic variants (Catcheside, 1981). The data obtained in this work do not, therefore, clearly show that the two known forms of *ccf*-6 existed as a polymorphism in the original two wild-type Pasadena strains, but this still remains most likely. It also remains possible that *ccf*-6(*A*) arose from *ccf*-6(*B*) by mutation or some form of recombinational event in our laboratory stocks. If a possible third variant of *ccf*-6 does exist in our laboratory strains, it might be re-detected in further studies.

Chapter 4. The genetic control of conversion of the NG*w*18 white ascospore pigmentation mutation.

Preliminary experiments

The mutation NG*w*18 was NG-induced and isolated by Ghikas (1978). It is a locus II mutation, along with the spontaneous mutation S*w*26, with which it has not been observed to recombine. Mutation NG*w*16 (also NG-induced) lies at a distance of 2.5 recombination units from NG*w*18 and was not regarded by Ghikas (1978) as being part of locus II. However, since mutations *w*10 and *w*78 show a similar recombination frequency in certain crosses and are regarded as part of locus I, in this work mutation NG*w*16 is regarded as part of locus II. Ghikas (1978) was unable to determine the recombination frequency between S*w*26 and NG*w*16, both of the latter two mutations having been isolated by her. In this Chapter, the bulk of the data is concerned with the conversion behaviour of mutation NG*w*18, but some experiments were also carried out involving mutations NG*w*16 and S*w*26.

Initially it was important to verify the results obtained by Lamb and Ghikas (1979) concerning the conversion properties of mutation NG*w*18 shown in Table 2, which showed that the same mutant strains could show radically different conversion frequencies when crossed to different derived wild-type strains (4.22, 3.31 and 0.18% NRCFs). However, despite repeated attempts, the original 3 crosses performed by Lamb and Ghikas (1979) were no longer fertile. These crosses involved mutant strain NG*w*18+ and derived

wild-types 92-, 42- and KIII-. As a result of this inability to repeat the original crosses, the NGω18+ mutant strain was crossed to a range of derived wild-type strains in our stock collection. At this stage only one NGω18 strain was available, of (+) mating type. 5 fertile crosses were achieved and the data from these crosses are displayed in Table 31, along with data from the 3 original crosses performed by Lamb and Ghikas (1979). From this Table it can be seen that the conversion frequencies of the 8 crosses fall clearly into two categories. The first 4 crosses show very low conversion frequencies of 0.17 to 0.39%, while the remaining 4 crosses show conversion frequencies 10- to 20-fold higher, with values of 3.08 to 4.22%. The variability in conversion frequency among crosses of the same type was assessed by the $\Sigma\chi^2$ homogeneity test. Both the LCF ($\Sigma\chi^2$ = 11.09, p = 0.01-0.02, 3df) and the HCF ($\Sigma\chi^2$ = 10.54, p = 0.01-0.02, 3df) groups of crosses were heterogeneous with respect to narrower ratio conversion frequencies. This conversion frequency heterogeneity may be due to imperfectly controlled environmental effects, but the role of genetic background cannot be ruled out. Later in this Chapter, other possible sources of conversion heterogeneity among LCF crosses will be discussed.

The conversion frequency results obtained in this experiment are broadly in agreement with those of Lamb and Ghikas (1979), with the crosses performed being assignable to LCF and HCF groups. Furthermore, *ccf*-2 is not implicated in these differences in conversion frequency since

TABLE 31: Conversion data from + x NG*w*18 crosses

Mutant strain	Crossed to	Octad classes (+:*w*),%					%NRCF	Relative % PMS	%Conv. to +	Total octads
		4:4	6:2	2:6	5:3	3:5				
NG*w*18+	KIII-*	99.82	0.12	-	0.04	0.02	0.18	11.11	88.89	10260
"	7R7-	99.71	-	0.05	0.24	-	0.29	83.33	83.33	2067
"	16P-	99.82	0.04	0.01	0.12	-	0.17	66.98	91.51	60691
"	P5-	99.61	0.05	-	0.34	-	0.39	88.24	100.00	4371
"	92-*	95.74	2.36	0.71	0.76	0.38	4.22	27.01	74.07	5733
"	42-*	96.67	2.11	0.35	0.59	0.26	3.31	25.68	81.56	8541
"	RS8-1-	96.85	1.53	0.56	0.83	0.23	3.15	33.82	75.00	2162
"	RS17-1-	96.92	1.12	0.70	0.98	0.28	3.08	40.91	68.18	715

* Data of Lamb and Ghikas (1979)

derived wild-type strains carrying the same form of *ccf*-2-*ccf*-2(*P*) can give LCF (strains 7R7- and 16P-) and HCF (strain 92-) crosses.

Considering conversion spectrum, the HCF crosses show relatively high frequencies of PMS, ranging from 25.68 to 40.91% and conversion predominantly to wild-type, ranging from 68.18 to 81.56%. The situation in the LCF crosses is not so clearcut, with the relative frequency of PMS ranging from 11.11% (in the original cross performed by Lamb and Ghikas, 1979) to 88.24%. In all 4 LCF crosses, conversion was again overwhelmingly to wild-type, with values ranging from 83.33 to 100.00%. Thus, in agreement with the original work of Lamb and Ghikas (1979), in both LCF and HCF crosses, mutation NG*w*18 shows a conversion spectrum of type C, according to the criteria of Leblon (1972 a,b). This conversion spectrum type has been deemed typical of base substitution mutations by the latter author. However, the relative frequency of PMS was highly variable in the LCF crosses. A large part of this variability is probably due to the small numbers of conversion octads being scored in the LCF crosses. Small numbers are always subject to greater random variation between samples. Later in this Chapter it will be seen that the phenocopy frequency is very high in LCF crosses and probably variable also, in response to imperfectly controlled environmental conditions. Such variations in phenocopy frequency would affect the apparent conversion spectrum of mutation NG*w*18 in LCF crosses.

In summary, these preliminary crosses broadly confirmed the existing data on the conversion behaviour of NG*w*18, providing LCF and HCF crosses for further analysis. The difference in conversion frequency between the two sets of crosses is of great magnitude (10- to 20-fold differences) and strongly suggests that the conversion behaviour of NG*w*18 is under genetic control. The system involved cannot be *ccf*-2. Neither can it be *ccfs* 5 or 6, since it was shown in Chapters 2 and 3 of this thesis respectively, that these factors have no effect on NG*w*18 conversion behaviour. At the outset of this investigation, a simple easily testable model of conversion control was assumed (a closely linked, monogenic system) and this formed the basis upon which the following experimental programmes of crosses were devised. However, at this early stage in the work, only one NG*w*18 strain of (+) mating-type was available. It was therefore necessary to obtain NG*w*18 strains of (-) mating type to act as tester strains in subsequent experiments. It was realized that such (-) mating type strains might not necessarily carry the same form of the putative *ccf* influencing NG*w*18 conversion behaviour as strain NG*w*18+. Steps were taken to minimize this risk and, in any case, any differences in conversion behaviour between NG*w*18(+) and (-) testers would later become apparent.

Derivation of NG*w*18 mutant strains of (-) mating type

20 white mutant spores were randomly isolated from 4+:4*w* octads from the LCF cross NG*w*18+ × 16P-. 11 of

these spores germinated and 5 gave fertile crosses with derived wild-type strain 25-5R+. The conversion data from these crosses are shown in Table 32.

From Table 32 it can be seen that all 5 random white mutant isolates gave HCF crosses with 25-5R+, with conversion frequencies ranging from 2.82 to 5.56%. Since all

TABLE 32: Conversion data of random white spore isolates from 4+:4*w* octads from cross NG*w*18+ x 16P- when crossed to derived wild-type strain 25-5R+

Isolate No.	Octad classes (+:*w*), %					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
2	95.69	1.35	1.62	0.54	0.81	4.31	1371
3	94.44	2.46	1.21	1.18	0.71	5.56	2807
4	96.85	1.53	0.56	0.83	0.23	3.15	2651
9	96.17	1.86	1.28	0.39	0.29	3.83	1019
15	97.18	2.11	-	0.70	-	2.82	284

isolates gave HCF crosses and none gave low, it is probable that they all carried the same form of the *ccf* acting on NG*w*18 conversion behaviour. If this factor is closely linked to NG*w*18, then it is also very probable that these NG*w*18 isolates of (-) mating type carried the same form of the *ccf* as the original NG*w*18+ strain. Isolate number 3 was chosen as an NG*w*18 tester strain of (-) mating type (since it gave the most fertile and most clearly HCF cross to derived wild-type strain 25-5R+) and was designated strain 3NG*w*18-.

Monogenic conversion control of NG*w*18

The data in Table 31 show that when strain NG*w*18+ was crossed to derived wild-type strains 16P- and RS8-1-, very different conversion frequencies resulted. Since the NG*w*18 strain was the same in each cross, and if the conversion control of this mutation is monogenic, then strains 16P- and RS8-1- must carry different forms of the conversion control factor. Therefore, either LCF cross NG*w*18+ × 16P- or HCF cross NG*w*18+ × RS8-1- must be heterozygous for different forms of the conversion control factor. Due to the fact that gene conversion of the *w*62, BB9*w*9 and *w*BHj mutations in the Pasadena strains of *Ascobolus immersus* is reduced when their respective closely linked conversion control factors are heterozygous in a cross (Emerson and Yu-Sun, 1967; present work) and the same situation applies to the four *cv* modifiers in the European strains (Girard and Rossignol, 1974), it was felt more likely that LCF cross NG*w*18+ × 16P- might be heterozygous for a closely linked conversion control factor, but both LCF and HCF crosses were finally analysed. The theoretical basis for this analysis was explained in Chapter 2 and illustrated in Figure 1 (page123), but a brief resumé is given here: analysis of the 6 wild-type spores from 6+:2*w* convertant octads (whether they arise by symmetrical or asymmetrical H-DNA formation) from a cross heterozygous for two forms of a closely linked *ccf*, should reveal that 4 spores carry the same form of the *ccf* as the wild-type parent, while 2 spores will have inherited the *ccf* form originally present in the white mutant parent. The latter

2 spores should show altered conversion behaviour in back-crosses to the white mutant parent, unless certain forms of dominance are involved or the white mutation and its conversion control factor frequently co-convert. It should also be noted that if the white mutation and its *ccf* are more loosely linked or unlinked, in some octads 4 of the 6 wild-type spores could show altered conversion properties.

Although it was stated above that the LCF cross NG*w*18+ × 16P- was thought more likely to be heterozygous for a closely linked *ccf*, the conversion behaviour of wild-type spores from 6+:2*w* octads from HCF cross NG*w*18+ × RS8-1- was analysed first. This was for 2 reasons: i) 6+:2*w* octads are very rare in LCF crosses and it took quite a time to isolate enough spores for analysis; ii) early results from analysis of 6+:2*w* octads from the LCF cross indicated that such octads were frequently phenocopies and thus not genuine convertant octads.

Conversion behaviour of wild-type isolates from 6+:2*w* octads from HCF cross NG*w*18+ × RS8-1-

Following the programme outlined above, wild-type spores were isolated from 38 6+:2*w* convertant octads, from HCF cross NG*w*18+ × RS8-1-. Germination was poor - in only 1 octad did all 6 wild-type spores germinate - but in 7 octads at least 4 wild-type spores germinated. These isolates were crossed to white mutant strains NG*w*18+ and 3NG*w*18-. All of the isolates gave fertile crosses and the conversion data resulting from these crosses are shown in Table 33.

TABLE 33: Conversion data of wild-type spore isolates from 6+:2*w* octads from cross NG*w*18+ x RS8-1- when crossed to NG*w*18+ or 3NG*w*18-

Isolate No. (octad.spore)	Octad classes (+:*w*),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
3.2-	97.27	1.46	0.39	0.83	0.05	2.73	32.14	83.93	2054
3.4-	95.39	3.05	0.48	0.82	0.21	4.56	22.67	84.88	3774
3.5-	97.85	1.07	0.58	0.50	-	2.15	23.08	73.08	1212
3.6+	97.01	2.15	0.17	0.62	0.06	2.99	22.64	92.45	1771
6.1-	97.81	1.33	0.13	0.60	0.13	2.19	33.33	87.88	1509
6.2+	96.42	1.87	0.34	1.19	0.17	3.58	38.10	85.71	587
6.3-	97.15	2.09	0.22	0.55	-	2.85	19.23	92.31	911
6.4+	97.28	2.16	0.16	0.24	0.16	2.72	14.71	88.24	1252
8.1-	95.50	3.16	0.45	0.77	0.12	4.50	19.82	87.39	2465
8.3+	96.46	2.40	0.38	0.76	-	3.54	21.43	89.29	791
8.4+	96.34	2.73	0.21	0.43	0.09	3.46	15.04	91.15	3264
8.5-	96.41	2.10	0.90	0.30	0.30	3.59	16.67	66.67	334
8.6-	96.69	2.38	0.28	0.45	0.20	3.31	19.64	85.50	4210
10.2-	97.64	1.51	0.31	0.39	0.12	2.32	21.67	81.67	2586
10.4+	96.80	2.13	0.49	0.41	0.16	3.20	17.95	79.49	1219
10.5+	96.80	2.41	0.22	0.57	-	3.20	17.98	93.26	2784
10.6-	97.23	1.51	0.63	0.38	0.25	2.77	22.73	68.18	794
13.1+	96.28	2.82	-	0.80	0.09	3.72	24.05	97.47	2124
13.2+	96.75	2.42	0.33	0.42	0.08	3.25	15.38	87.18	1200
13.3+	96.25	2.71	0.42	0.57	0.05	3.75	16.67	87.50	1919
13.4-	98.21	1.34	0.04	0.40	-	1.79	22.50	97.50	2231
13.5-	98.16	1.25	0.13	0.42	0.04	1.84	25.00	90.91	2391

TABLE 33 (continued)

Isolate No. (octad.spore)	Octad classes (+:w),%					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
24.3+	97.17	2.16	0.25	0.42	-	2.83	14.93	91.04	2364
24.4+	97.51	1.94	0.09	0.28	0.18	2.49	18.52	88.89	1085
24.5+	97.23	2.11	0.15	0.44	0.07	2.77	18.67	92.00	2705
24.6+	97.14	2.14	0.28	0.37	0.06	2.86	15.22	88.04	3218
34.1+	97.27	1.99	0.06	0.68	-	2.73	25.00	97.92	1760
34.2+	97.74	1.76	0.13	0.34	0.04	2.26	16.67	92.59	2385
34.3+	97.82	1.25	0.13	0.73	0.07	2.11	37.50	93.75	1516
34.4-	96.66	2.61	0.21	0.51	-	3.34	15.38	93.59	2333
34.5+	96.28	2.98	0.21	0.35	0.07	3.62	11.76	92.16	2820
34.6-	94.52	4.04	0.28	0.94	0.17	5.43	20.41	91.84	1805

All crosses show 'high' conversion frequency (1.79 - 5.43% NRCF)

From this Table it can be seen that all the wild-type isolates gave HCF crosses with both NGω18 tester strains. The range of conversion frequencies was from 1.79 to 5.43%. This is quite a large range and indeed the conversion frequency data <u>are</u> heterogeneous ($\Sigma\chi^2$ = 141.44, $p < 0.01$, 31 df), but all crosses are clearly HCF. Particular attention should be paid to octad 34 where all 6 wild-type spores gave fertile crosses, with a range of conversion frequencies of 2.11 to 5.43%. Although the latter conversion frequency is a rather high value, there is no sister spore in the octad showing a similarly high conversion frequency, and this high conversion frequency value is probably due to imperfectly controlled environmental conditions. Furthermore, in octads 8 and 13, 5 wild-type spores gave fertile crosses. In each case all were HCF with a conversion frequency range of 3.31 to 4.50% in octad 8, and a range of 1.79 to 3.75% in octad 13. No segregants with altered conversion frequencies were detected.

Therefore, from this experiment it would appear that HCF cross NGω18+ × RS8-1- is homozygous for one form or another of the *ccf* acting on NGω18 - no segregants with altered conversion frequencies were detected. Such a result was expected. However, some notes of caution should be sounded. The HCF cross analysed could still be heterozygous for the *ccf* under investigation if this factor usually co-converts with NGω18. The cross could also be heterozygous if the action of the *ccf* involves dominance of

one form of the *ccf* over the other. Thus with NGω18 tester strains of certain *ccf* genotypes, the altered *ccf* genotype of some of the segregants could not be detected. The results from this experiment need to be considered in conjunction with the next.

The data in Table 33 also provide confirmatory evidence of the conversion spectrum of mutation NGω18 in HCF crosses. Among all 32 crosses the relative frequency of PMS ranged from 11.76 to 38.10% and the frequency of conversion to wild-type from 66.67 to 97.92%. Thus, considering the data as a whole, the C-type conversion spectrum of NGω18 in HCF crosses was confirmed. There was moderate variability in conversion spectrum among the crosses. In this context in should be mentioned that in PART TWO of this thesis some evidence is presented which shows that in NGω18 HCF crosses, the 5:3/6:2 ratio is not always constant throughout the period of octad dehiscence from a cross, tending to decrease with time. Although all octads dehisced from any cross were always scored, if a cross ceased to be fertile relatively early (due, for example, to the medium drying out), such a cross might show a higher relative frequency of PMS. This phenomenon might provide a source of heterogeneity in the conversion spectrum data.

The data from this experiment further show that both NGω18 tester strains showed similar conversion properties and therefore probably both carry the same form of the *ccf* under investigation.

Conversion behaviour of wild-type isolates from 6+:2*w* octads from LCF cross NG*w*18+ × 16P-

As already stated, it was thought that LCF cross NG*w*18+ × 16P- was more likely to be heterozygous for two forms of a closely linked *ccf* than was HCF cross NG*w*18+ × RS8-1-, from a consideration of hitherto known systems. Following a similar experimental procedure to that of the last experiment, wild-type spores were isolated from 27 apparently 6+:2*w* convertant octads. Such octads were infrequent, the 27 octads coming from a total octad population of almost 61000 (frequency of 6:2s = 0.04%). Ascospore germination was poor and in only 4 octads did more than 3 spores germinate (in 2 octads all 6 spores germinated and in another 2 octads 5 spores germinated). However, all the resulting wild-type isolates gave fertile crosses with NG*w*18+ or 3NG*w*18- tester strains. The conversion data from these crosses are presented in Table 34.

Immediately it can be seen that none of the wild-type isolates showed altered conversion frequencies compared with their wild-type parent in crosses to NG*w*18 tester strains (conversion frequency for parental cross NG*w*18+ × 16P- was 0.17%). The conversion frequencies were all low, ranging from 0.0 to 0.68%, while the latter frequency was an extreme, high value. More importantly, the data reveal that many of the supposed wild-type isolates were, in fact, phenocopies, being genotypically NG*w*18 mutant spores. When such isolates were crossed to NG*w*18 tester strains they revealed their true identity by producing only 0+:8*w* octads. There are data from 12 octads in Table 34; phenocopy spores were discovered in 7 of these octads. In

TABLE 34: Conversion data of phenotypically wild-type spore isolates from 6+:2*w* octads from cross NG*w*18+ x 16P- when crossed to NG*w*18+ or 3NG*w*18-

Isolate No. (octad.spore)	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1.2+	100.00	-	-	-	-	0.0	998
4.2+	99.88	0.06	-	0.06	-	0.12	1668
5.1-	All 0:8						
5.2+	99.85	-	-	0.15	-	0.15	1304
5.3+	All 0:8						
5.4-	99.64	-	-	0.36	-	0.36	834
5.5+	100.00	-	-	-	-	0.0	690
6.1-	All 0:8						
6.2-	All 0:8						
6.3+	99.94	-	-	0.06	-	0.06	1555
6.4+	100.00	-	-	-	-	0.0	412
6.5+	100.00	-	-	-	-	0.0	681
6.6+	99.76	-	-	0.12	0.06	0.18	1687
15.5+	All 0:8						
15.6+	99.94	-	-	0.06	-	0.06	1617
18.1+	99.87	-	-	0.13	-	0.13	1586
18.2+	99.97	-	-	-	0.03	0.03	2964
18.3+	99.89	-	0.04	0.07	-	0.11	2832
18.5+	99.18	-	-	0.68	-	0.68	1735
18.6-	All 0:8						
20.1+	All 0:8						
20.5+	All 0:8						
20.6-	99.93	-	-	0.07	-	0.07	1425
21.6-	All 0:8						
22.1-	All 0:8						

TABLE 34: (continued)

Isolate No. (octad.spore)	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
22.2+	100.00	-	-	-	-	0.0	1684
22.3+	100.00	-	-	-	-	0.0	334
22.4+	99.56	0.11	-	0.33	-	0.44	910
22.5+	100.00	-	-	-	-	0.0	457
22.6-	All 0:8						
23.1+	99.88	-	-	0.12	-	0.12	1724
23.3+	99.95	0.05	-	-	-	0.05	2087
24.2+	100.00	-	-	-	-	0.0	1883
24.3+	99.95	-	-	0.05	-	0.05	2007
24.4-	99.91	-	-	0.09	-	0.09	2279
25.4+	99.96	-	-	0.04	-	0.04	2557

fact, in each of 4 octads (numbers 5, 6, 20 and 22) 2 apparently wild-type spores were revealed as genotypically NG*w*18 white mutant spores. Such octads must have truly been 4+:4*w* non-convertant octads. If the data are considered as arising from a random sample of apparently wild-type spores from a population of apparent 6+:2*w* octads, if all the 6+:2*w* octads analysed were phenocopies then the true genotypic ratio of wild-type:white mutant, amongst phenotypically wild-type spores, would be 2:1. The observed ratio of 25:11 closely fits this expected 2:1 ratio if all the octads sampled were phenocopies (χ^2 = 0.12, p = 0.70-0.80, 1df). Thus it is highly likely that most, if not all, of the 6+:2*w* octads analysed were phenocopies, genotypically being 4+:4*w* non-convertants.

Have these findings been biased by the different optimum germination conditions for genuine wild-type spores (dung germination medium, 2 hours heat shock at 50°, then overnight at 37°C) and NG*w*18 white mutant spores (same conditions as above, but 4 hours heatshock at 50°C)? Since all phenotypically wild-type spores were subjected to optimum wild-type germination conditions, it would be expected that relatively fewer genotypically NG*w*18 mutant spores would have germinated. This would not affect the conclusion that most of the 6+:2*w* octads analysed were phenocopies. This optimum germination factor is balanced by the fact that NG*w*18 mutant spores in this work showed higher optimum germination frequencies than did wild-type spores. Therefore at optimum wild-type germination conditions the two germination frequencies would be about equal. Furthermore, it was not known whether phenocopy wild-type spores responded

best to wild-type or NGω18 optimum germination conditions.

How do such phenocopies arise? It is possible for the red pigment or one of its precursors to pass from a wild-type red spore into a white mutant spore through a protoplasmic bridge if the two spores are partly connected (Stadler, Towe and Rossignol, 1970; Ghikas, 1978). Such connections need only be very tenuous because none was observable in any of the octads studied in this experiment. Furthermore, all the apparently wild-type spores in these octads were easily separable in the normal way. No physically attached pairs of spores were seen.

From this experiment it can be concluded that in LCF crosses involving mutation NGω18, the frequency of 6+:2ω phenocopy octads is extremely high compared to genuine 6+:2ω octads. In fact, the phenocopy absolute frequency is probably no higher than in HCF crosses for the same or maybe even other mutations. In HCF crosses the number of genuine 6+:2ω octads would far outnumber the phenocopies (if they remained at the same absolute frequency) and the latter would be far less likely to be detected. In LCF crosses, the frequency of genuine 6+:2ω convertant octads must be far lower than the already low apparent frequency of 0.04%, this frequency probably being reduced by a factor of at least ten (the data are consistent with all 12 6+:2ω octads analysed in this experiment being phenocopies). Following on from this, it is quite conceivable that many of the other apparently convertant octads are also phenocopies. If this were so, the true conversion frequency of NGω18 in a

LCF cross could be as low as 0.01% or even lower. The apparent conversion frequency in such crosses is itself often less than 0.1%. Thus LCF crosses could show a 400-fold reduction in NGω18 conversion frequency compared with HCF crosses. This magnitude of conversion reduction in *Ascobolus immersus* is not exceeded even by the actions of *cv2* in the European strains. It would be the first time that such low frequencies of gene conversion for any site were demonstrated to be under genetic control in the Pasadena strains of *Ascobolus*.

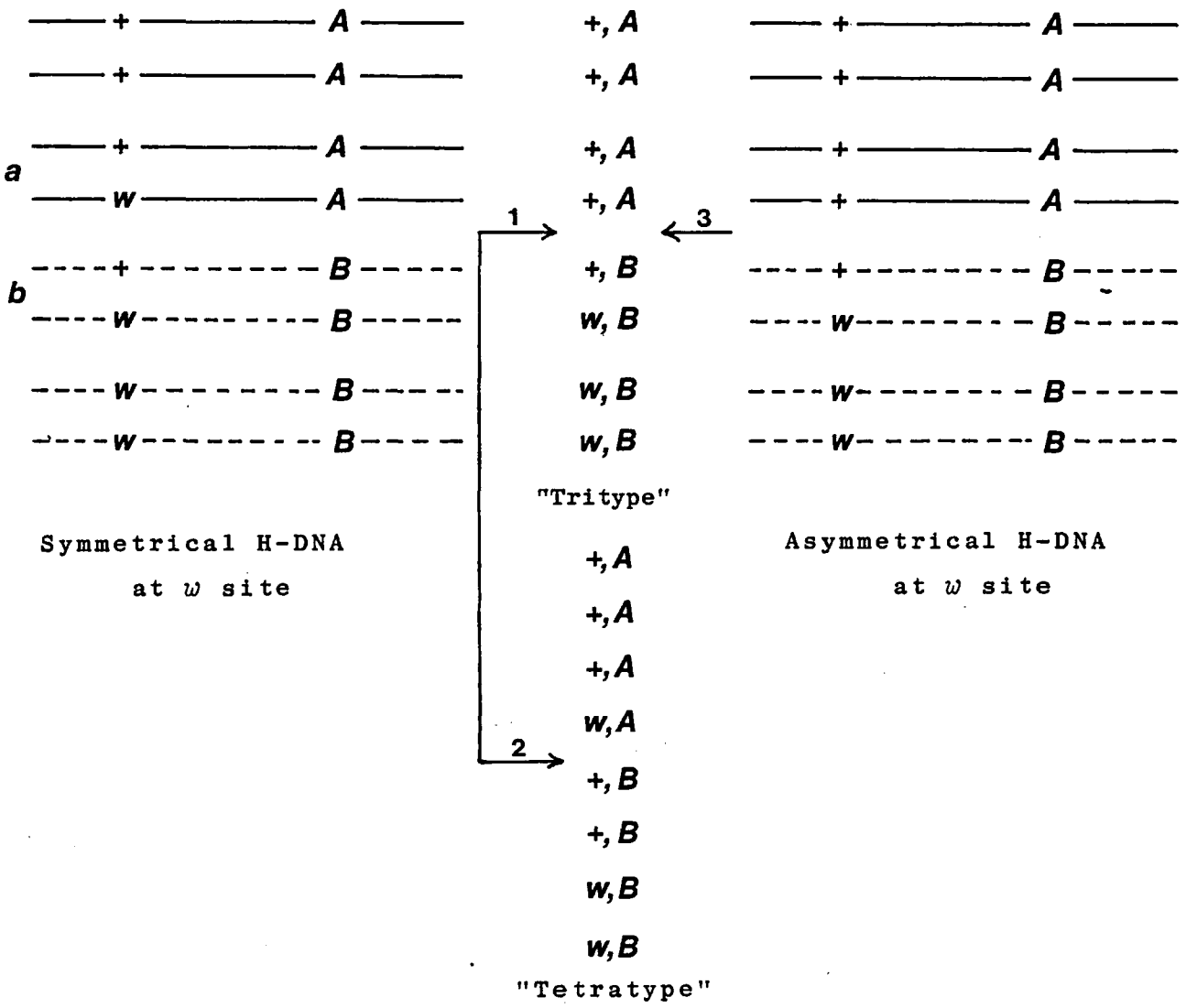
In summary, it was not possible to conclude from this experiment that the LCF cross NGω18+ × 16P- was heterozygous for a closely linked conversion control factor, due to the inability to detect genuine 6+:2ω octads. The detection and analysis of true 6+:2ω convertant octads will be a very laborious process, but should be revealing not only in elucidating the genetic controls of gene conversion at the NGω18 site, but also in providing an estimate of the true frequency of 6+:2ω convertant octads.

Conversion behaviour of wild-type isolates from 5+:3ω octads from LCF cross NGω18+ × 16P-

Since it was not possible to obtain genuine 6+:2ω convertant octads for analysis from the LCF cross NGω18+ × 16P-, it was decided to attempt to analyse the conversion behaviour of wild-type spores from 5+:3ω convertant octads from the same LCF cross. This was done for two reasons. Firstly, in the LCF cross in question apparent 5+:3ω octads were three times as frequent as apparent 6+:2ω octads and were already available for analysis.

(N.B. this was not so in the original NG*w*18+ × KIII-LCF cross performed by Lamb and Ghikas, 1979). Secondly, since the 5+:3*w* octads were more frequent than 6+:2*w* octads, it was hoped that the relative frequency of phenocopies might be less. (However, it should be remembered that to produce a 5+:3*w* phenocopy octad, only 1 phenocopy spore is needed, unlike 2 such spores for phenocopy 6+:2*w*s.) This experiment would also provide a means of estimating phenocopy frequency for this aberrant octad class.

If the LCF cross NG*w*18+ × 16P- were heterozygous for two forms of a closely linked conversion control factor, among the 5 wild-type spores of a 5+:3*w* octad the numbers of spores expected to show altered conversion characteristics with respect to the wild-type parent depends on whether H-DNA formation at the NG*w*18 site is predominantly symmetrical or asymmetrical. The theoretical basis of this analysis is as follows and is illustrated in Figure 6, where *A* and *B* stand for the two states of the conversion control factor: if H-DNA formation at the *w* site is asymmetrical, then using the terminology of Whitehouse (1974) a tritype octad results. Of the 5 wild-type spores, 4 will carry the same form of the *ccf* as the wild-type parent (*A* in this example) while 1 will carry the form of the *ccf* originally present in the white mutant parent. The latter spore isolate should show altered conversion behaviour in backcrosses to the white mutant parent. If H-DNA formation at the *w* site is symmetrical each mispair will have a different fate. There are 2 options: mispair *a* may be corrected to wild-type while mispair *b* remains uncorrected.



1 → mispair *a* corrected to + and mispair *b* uncorrected
Produces Tritype octad (Whitehouse, 1974): 4+, *A* 1+, *B* 3*ω*, *B*

2 → mispair *a* uncorrected and mispair *b* corrected to +
Produces Tetratype octad (Whitehouse, 1974): 3+,*A* 2+,*B*
1*ω*,*A* 2*ω*,*B*

3 → mispair uncorrected. Produces Tritype octad: 4+,*A* 1+,*B* 3*ω*,*B*

Fig. 6: Origin of wild-type segregants with altered conversion properties in 5+:3*ω* octads from + × *ω* crosses, where the conversion control factor is heterozygous, closely linked, but not co-converting with the *ω* site.

Again a tritype octad will result, with 1 of the 5 wild-type segregants showing altered conversion properties in backcrosses to the white mutant parent; alternatively, mispair *a* may remain uncorrected while mispair *b* is corrected to wild-type. In this case a tetratype octad (Whitehouse, 1974) will result. Of the 5 wild-type spores, 3 will carry the same form of the *ccf* as the wild-type parent, while 2 will inherit the form of the *ccf* originally present in the white mutant parent. The latter 2 isolates should show altered conversion behaviour in backcrosses to the white mutant parent.

It can be concluded that in an analysis of the conversion behaviour of the 5 wild-type spores from 5+:3*w* octads resulting from a cross heterozygous for two forms of a closely linked *ccf*, 1 or 2 spores per octad may show altered conversion behaviour in backcrosses to the white mutant parent. This assumes that the white mutation and its conversion control factor do not usually co-convert and that certain forms of dominance of the control factor forms are not involved. If the *w* site and its *ccf* were not so closely linked or were unlinked, up to 4 wild-type isolates showing altered conversion behaviour could arise in some 5+:3*w* octads.

Following this programme of analysis, wild-type spores were isolated from 58 5+:3*w* octads from LCF cross NG*w*18+ × 16P-. Altogether in a total sample size of almost 61000 octads scored from the latter cross, 70 5+:3*w* octads were observed (0.12%). However, in 12 of these octads, pairs of apparently wild-type spores appeared to be very difficult to separate, the spores being fused at their points of

contact. Such octads were almost certainly phenocopies and so the remaining 3 wild-type spores in each were not isolated. From the 58 octads, 72 wild-type spores germinated and 67 of these gave fertile crosses with either NGω18+ or 3NGω18- tester strains. The conversion data obtained from these crosses are displayed in Table 35.

An initial consideration of this Table reveals that none of the wild-type isolates showed altered conversion frequencies compared with their wild-type parent in crosses to NGω18 tester strains (% NRCF for parental cross was 0.17%). The conversion frequencies were all low, ranging from 0.0 to 0.37%. More importantly, the data again reveal that many of the supposed wild-type isolates were in fact phenocopies, being genotypically NGω18 mutant spores. There are data from 38 octads in Table 35; phenocopy spores were discovered in 8 octads. Such octads must in fact have been 4+:4ω non-convertant octads. Thus at first sight it would appear that the relative frequency of 5+:3ω phenocopies (among apparent 5+:3ωs) is lower than the relative frequency of 6+:2ω phenocopies. Unfortunately in this experiment there were no octads where all 5 apparently wild-type spores germinated and gave fertile crosses. In only 2 octads did as many as 4 isolates give fertile crosses. In these octads (numbers 51 and 53) all 4 isolates turned out to be genuine wild-types, all giving LCF crosses to NGω18 tester strains. Thus in these octads there could not have arisen 2 wild-type isolates showing altered conversion properties, as is possible if H-DNA formation at the NGω18 site is symmetrical. In as many as 21 octads only 1 apparently

TABLE 35: Conversion data of phenotypically wild-type spore isolates from 5+:3*w* octads from cross NG*w*18+ x 16P- when crossed to NG*w*18+ or 3NG*w*18-

Isolate No. (octad.spore)	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1.2+	99.63	-	-	0.37	-	0.37	2180
1.4+	All 0:8						
1.5+	99.78	0.04	-	0.17	-	0.22	2289
2.1+	100.00	-	-	-	-	0.0	1335
2.2+	100.00	-	-	-	-	0.0	473
2.3+	All 0:8						
3.2-	All 0:8						
3.3+	99.96	-	-	0.04	-	0.04	2263
3.4+	99.70	0.04	-	0.22	0.04	0.30	2671
4.1-	99.89	0.04	-	0.08	-	0.11	2622
5.1-	99.91	0.03	-	0.06	-	0.09	3297
6.1+	99.89	-	-	0.11	-	0.11	2684
7.1-	99.97	0.03	-	-	-	0.03	3152
8.1-	99.89	-	-	0.11	-	0.11	3627
9.1+	100.00	-	-	-	-	0.0	2440
10.1-	100.00	-	-	-	-	0.0	3068
11.1-	99.96	-	-	0.04	-	0.04	2784
13.2+	100.00	-	-	-	-	0.0	1143
13.4+	100.00	-	-	-	-	0.0	203
14.1+	100.00	-	-	-	-	0.0	901
15.1+	99.65	-	0.17	0.17	-	0.35	1153
16.2+	99.80	-	-	0.10	0.10	0.20	1024
17.2+	All 0:8						
18.1+	99.71	0.04	-	0.22	0.04	0.29	2737
18.2-	99.93	-	-	-	0.07	0.07	2997
19.1-	99.66	0.08	0.04	0.21	-	0.34	2372

TABLE 35: (continued)

Isolate No. (octad.spore)	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
19.3-	99.73	0.04	0.08	0.16	-	0.27	2578
20.1-	99.74	0.08	-	0.18	-	0.26	3817
20.3+	99.61	-	-	0.27	0.08	0.35	2547
20.5+	100.00	-	-	-	-	0.0	887
22.1-	99.96	-	-	0.04	-	0.04	2339
24.2+	99.88	-	-	0.12	-	0.12	2441
24.3+	99.89	0.05	0.05	-	-	0.11	1849
25.1-	99.87	0.07	-	0.07	-	0.13	1519
26.1+	All 0:8						
30.2+	100.00	-	-	-	-	0.0	580
30.3+	99.71	-	-	0.29	-	0.29	691
33.1+	100.00	-	-	-	-	0.0	844
34.1+	All 0:8						
34.4+	99.79	-	-	0.21	-	0.21	1412
34.5+	99.90	-	-	0.10	-	0.10	1959
35.1+	100.00	-	-	-	-	0.0	774
36.5+	99.92	-	0.08	-	-	0.08	1231
38.5-	100.00	-	-	-	-	0.0	2130
39.2-	99.93	0.04	-	0.04	-	0.07	2699
39.4+	99.92	-	0.0 8	-	-	0.08	1218
39.5-	99.64	0.07	0.07	0.14	0.07	0.36	1405
40.1+	99.88	0.06	-	0.06	-	0.12	1648
40.2-	99.91	-	-	0.04	0.04	0.09	2310
47.1-	99.83	0.08	-	0.08	-	0.17	1187
47.2-	99.78	-	-	0.17	0.06	0.22	1781
47.3-	99.95	-	-	-	0.05	0.05	2216

TABLE 35: (continued)

Isolate No. (octad.spore)	Octad classes (+:ω),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
48.1-	100.00	-	-	-	-	0.0	2047
48.4-	99.90	-	-	0.10	-	0.10	2005
50.3-	100.00	-	-	-	-	0.0	2017
50.4+	All 0:8						
50.5-	99.91	-	-	0.09	-	0.09	1142
51.1+	99.77	0.06	-	0.06	0.11	0.23	1762
51.2+	99.76	-	-	0.24	-	0.24	2522
51.3+	99.94	-	-	0.03	0.03	0.06	3292
51.4-	99.92	0.04	-	0.04	-	0.08	2372
52.4+	All 0:8						
53.1+	99.96	0.04	-	-	-	0.04	2607
53.2+	99.79	-	-	0.21	-	0.21	2433
53.3+	99.84	0.03	0.06	0.06	-	0.16	3187
53.5+	99.96	-	-	0.04	-	0.04	2457
56.1+	99.97	-	-	0.03	-	0.03	3103

wild-type spore germinated to give fertile crosses with the NG*w*18 tester strains. 18 of these isolates turned out to be genuine wild-types, while 3 were phenocopies. If all the data are considered as arising from a random sample of apparently wild-type spores from a population of apparent 5+:3*w* octads, if all the 5+:3*w* octads analysed were in fact phenocopies, then the true genotypic ratio of wild-type: white mutant would be 4:1 amongst the phenotypically wild-type spores. The observed ratio of 59:8 just fits this expected 4:1 ratio (χ^2 = 2.72, p = 0.05-0.10, 1df). Thus it is quite probable that most if not all of the 5+:3*w* octads analysed were phenocopies, genotypically being 4+:4*w* non-convertants. This conclusion is quite remarkable since a careful screening procedure was applied before isolating any spores from such 5+:3*w* octads. Any octads in which 1 of the wild-type spores was markedly less pigmented than the other 4 spores were disregarded. So also were those octads in which pairs of wild-type spores occurred which were not easily separable. 12 out of 70 apparently 5+:3*w* octads fell into the latter category.

Overall, the data are consistent with all 38 5+:3*w* octads analysed in this experiment being phenocopies. If this is so, then the frequency of genuine 5+:3*w* octads could be 40-fold lower than the frequency of 0.12% observed in the parental cross. The actual reduction in apparent conversion frequency could be even greater than this, due to the screening of apparent 5+:3*w* octads for phenocopies before any were analysed. Thus, in agreement with the results from the previous experiment, it would seem that the true conversion frequency of NG*w*18 in LCF crosses is

probably less than 0.02% (assuming that all apparent 2+:6*w* and 3+:5*w* octads are not phenocopies; if some are, the true conversion frequency would be even lower). Such a value would indicate a reduction of NG*w*18 conversion frequency in LCF crosses in the region of 200-fold compared with HCF crosses. Evidence of phenocopy frequency from 2 octad classes (6+:2*w* and 5+:3*w*) indicates that very low frequencies of gene conversion can be under genetic control in the Pasadena strains of *Ascobolus immersus*.

As an aside, it should be remembered that only 1 phenocopy spore is needed to turn a 4+:4*w* octad into an apparent 5+:3*w*, while 2 such spores are needed if an apparent 6+:2*w* is to arise. Are the two "crossfeeding" events necessary to create a 6+:2*w* phenocopy octad from a genuine 4+:4*w* octad independent events? An analysis suggested by Dr.Lamb provides a means of testing this:

Parental cross NG*w*18+ × 16P; frequency of 5:3s = 0.12%
frequency of 6:2s = 0.04%

Let the chance of a wild-type spore crossfeeding (with pigment) a *w* spore be x

So the total chance of a phenocopy 5:3 arising is $4x$ per 4:4 octad

If all 5:3s analysed were phenocopies, $4x = 0.12\%$
$x = 0.03\% = 0.0003$

The chance of two wild-type spores independently cross-feeding two *w* spores is $\simeq 4x \times 3x \simeq 12x^2 = 0.00011\%$

(where $4x$ is the total chance of any of the 4 wild-type spores doing it and $3x$ the total chance of any of the 3 remaining wild-type spores doing it).

This estimate is subject to a small reduction as both wild-type spores may crossfeed the same white spore or a small increase as one wild-type spore may crossfeed two whites. Since the observed 6:2 frequency of 0.04% (all apparently phenocopy octads) is much greater than the expected frequency of 0.00011% it would appear that the crossfeeding of two spores is <u>not</u> due to two independent events. One wild-type spore may be able to crossfeed two white spores to produce a 6+:2*w* phenocopy octad.

A further consideration of the data in Table 35 (and also Table 31) shows much variation in the apparent frequency of certain octad classes, particularly the 5:3s. Here the variation in frequency is from 0.0 to 0.37%. The variation in frequency of other classes is much less pronounced. Generally higher % NRCFs seem to be correlated with higher frequencies of 5:3s. Some of this variability is probably due to the random variations produced by sampling small numbers of apparently convertant octads. Minor modifiers of gene conversion may be involved, but since replicates of the same cross can also show variations in 5:3 frequency this is unlikely. Thus variations in phenocopy frequency are likely to account for some of this variation in 5:3 frequency. From another viewpoint, this is highly likely anyway since all or most of the 5:3 octads in LCF crosses <u>are</u> phenocopies. It is not clear what agency might be responsible for producing variations in phenocopy frequency. Wild-type phenocopies arise when red pigment or one of its precursors passes from a wild-type to

a white mutant spore when they are in contact at certain times in their development (Stadler, Towe and Rossignol, 1970; Ghikas, 1978). This process of crossfeeding might be affected by the degree of crowding in an ascus at certain crucial times, and this degree of crowding might well be variable. If phenocopy frequencies are highly variable, the effects will be apparent in LCF crosses involving particular mutations. Thus such mutations might apparently show different conversion spectra in crosses to different derived wild-type strains, while the true agency responsible would be changes in phenocopy frequency. This almost certainly explains why the frequency of 5:3 octads was much lower in the original LCF cross involving NG*w*18 performed by Lamb and Ghikas (1979) than in the original crosses performed in this work. These possible variations in phenocopy frequency should also be borne in mind when considering the conversion spectrum data of Lamb and Ghikas (1979) concerning mutations showing LCFs.

No determinations of phenocopy frequencies were undertaken for 2:6 and 3:5 octads. In the case of these octad classes, phenocopies could not arise by the crossfeeding method described above. However, phenocopies could arise, particularly for 2:6s, by spontaneous mutation to white at any site in the genome. Various workers have obtained estimates of the frequency of spontaneous white ascospore colour mutation which range from 0.01 to 0.8% in the Pasadena strains of *Ascobolus immersus* (Stadler, Towe and Rossignol, 1970; Wickramaratne, 1974; Ghikas, 1978; Helmi, 1980; present work). Thus relatively few to all of the apparent 2:6 octads could be phenocopies.

In summary, it was not possible to conclude from this experiment that the LCF cross NGω18+ × 16P- was heterozygous for a closely linked *ccf*, due to the inability to detect genuine 5+:3ω octads. In the Discussion at the end of this Chapter, suggestions for further work will be presented which should enable the mode of action of the putative *ccf* acting on NGω18 to be elucidated, involving analysis of 2+:6ω octads which are rare but maybe less likely to be phenocopies. Also the necessary derived wild-type tester strains are available. However, at this juncture it was necessary to obtain clear evidence for a genetic system controlling the conversion behaviour of mutation NGω18 and to determine whether this system was monogenic or otherwise.

Conversion behaviour of random wild-type spores isolated from cross 25-5R+ × 7R7- when crossed to NGω18+ or 3NGω18-

A cross was performed between derived wild-type strains believed to carry different forms of the *ccf*(*s*) responsible for the conversion behaviour of NGω18: from Table 31 it can be seen that cross NGω18+ × 7R7- is LCF, while from Table 32 cross 3NGω18- (isolate 3) × 25-5R+ is HCF. Since it seems that mutant strains NGω18+ and 3NGω18- carry the same form of the *ccf*(s) responsible for NGω18 conversion control, then strains 25-5R+ and 7R7- must carry different forms of the factor (s).

Accordingly, cross 25-5R+ × 7R7- was performed and 212 wild-type spores were randomly isolated* from the progeny (all octads observed were 8+:0ω). 42 spores germinated and

*These spores were isolated and germinated by Shirin Mafi from our laboratory

these isolates were crossed to white mutant strains NGω18+ and 3NGω18-. Altogether 27 fertile crosses resulted. The data obtained are shown in Table 36 and Figure 7.

From this Table and Figure it can be seen that of the 27 random isolates which gave fertile crosses with NGω18+ or 3NGω18-, 14 isolates gave LCF crosses (with conversion frequencies of 0.03 to 0.69%, although the latter frequency was an extreme high value), behaving like the 7R7- parent, while 13 isolates gave HCF crosses (with conversion frequencies of 2.01 to 4.55%), behaving like the 25-5R+ parent. This ratio of 14:13 very closely fits the expected 1:1 ratio of LCF:HCF crosses arising from this type of experiment if the conversion frequency differences under study are subject to monogenic control (χ^2 = 0.04, p = 0.80-090, 1df). It should be noted that there is some variation in conversion frequency among both the LCF and HCF crosses. In the case of the LCF crosses this variation in conversion frequency probably arises largely from fluctuations in phenocopy frequency, as described in the last experiment: for the 6:2 and 5:3 octad classes, phenocopies far outnumber true convertant octads. In the case of the HCF crosses, where the range of conversion frequencies was from 2.01 to 4.55%, several factors could be responsible. Minor modifiers of NGω18 conversion frequency could be segregating in the parental cross, acting to "spread out" the range of conversion frequencies attributable to the major monogenic conversion control system shown to exist by this experiment. Imperfectly controlled environmental conditions might also lead to some variation in conversion frequency. The genetic background of the white mutant tester strains does

TABLE 36: Conversion data of random wild-type spores isolated from cross 25-5R+ × 7R7- when crossed to NGw18+ or 3NGw18-

Isolate No.	Octad classes (+:w), %					% NRCF	Relative % PMS	% Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
8-	97.72	1.35	0.29	0.43	0.18	2.24	26.98	79.37	2807
12+	99.94	-	0.06	-	-	0.06	0.0	0.0	1668
14-	97.15	2.07	0.35	0.43	-	2.85	15.15	87.88	1157
19-	99.80	-	0.15	0.05	-	0.20	25.00	25.00	2013
23-	99.85	-	0.15	-	-	0.15	0.0	0.0	682
28+	99.86	-	0.06	0.06	-	0.11	50.0	50.00	3491
39-	99.65	-	-	0.35	-	0.35	100.00	100.00	852
47+	96.28	2.61	0.59	0.35	0.16	3.72	13.77	79.71	3714
61-	99.70	0.15	0.05	0.10	-	0.30	33.33	83.33	1977
80-	99.64	-	0.15	0.22	-	0.36	60.00	60.00	1375
89-	97.86	1.37	0.19	0.54	0.03	2.14	26.87	89.56	3136
90-	99.31	0.19	0.12	0.37	-	0.69	54.55	81.82	1601
95-	99.63	0.11	0.11	0.11	0.05	0.37	42.86	57.14	1882
100+	97.15	2.16	0.21	0.37	0.11	2.85	16.67	88.89	1898
115-	96.05	2.41	0.74	0.63	0.17	3.95	20.29	76.81	1746
117-	95.45	3.31	1.04	-	0.21	4.55	4.55	72.73	483
128+	99.97	0.03	-	-	-	0.03	0.0	100.00	3159
132+	96.03	2.57	0.47	0.78	0.16	3.97	23.53	84.31	1285
138+	99.86	0.06	-	0.06	0.03	0.14	60.00	80.00	3486
154+	96.55	2.67	0.56	0.22	-	3.45	6.45	83.87	898
158+	97.24	1.74	0.55	0.42	0.04	2.76	16.92	78.46	2358
175-	99.72	0.08	0.04	0.14	0.02	0.28	57.14	78.57	5065
194+	97.99	1.47	0.21	0.24	0.09	2.01	16.42	85.07	3339
204+	99.84	-	-	-	0.16	0.16	100.00	0.0	1228
210+	96.54	2.54	0.42	0.38	0.12	3.46	14.44	84.44	2602
211-	95.94	2.59	0.39	0.95	0.13	4.06	26.61	87.10	3051
212+	99.94	-	-	0.06	-	0.06	100.00	100.00	3621

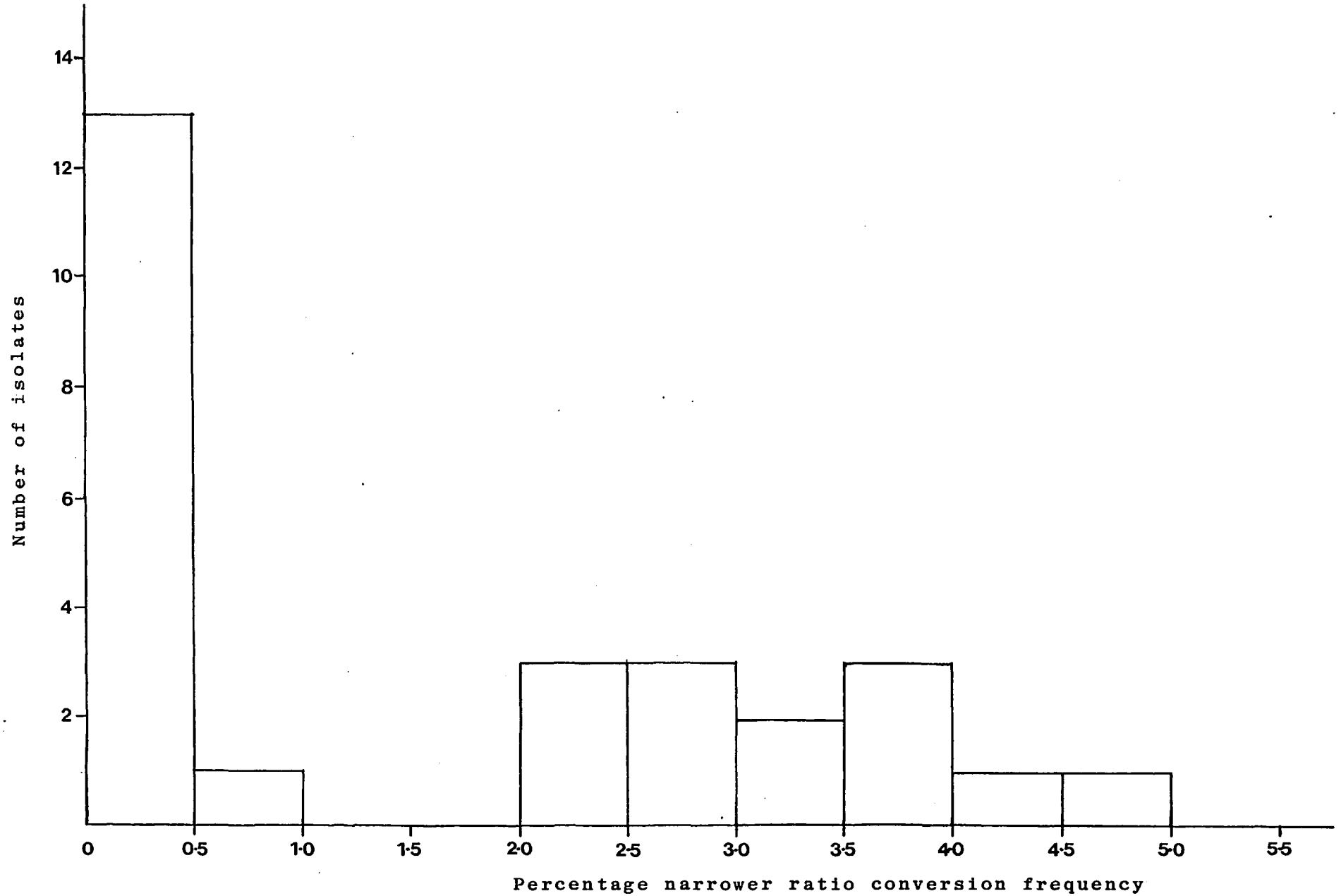


Fig. 7: Narrower ratio conversion frequencies of random wild-type isolates from cross 25-5R+ × 7R7- when crossed to NGω18+ or 3NGω18-

not seem to be implicated: wild-type isolates (giving HCF crosses) gave a range of conversion frequencies of 2.14 to 4.55% when crossed to NG*w*18+ and 2.01 to 3.97% when crossed to 3NG*w*18-. Following on from this, it can be seen from Table 36 that both white mutant tester strains were capable of giving both LCF and HCF crosses to different random wild-type isolates. This is further evidence that both strains carry the same form of the *ccf* controlling NG*w*18 conversion behaviour.

Table 36 also contains data on the conversion spectrum of NG*w*18 in HCF and LCF crosses. In HCF crosses the relative frequency of PMS ranges from 13.77 to 26.98% and the frequency of conversion to wild-type from 76.81 to 89.56% (excluding 2 crosses with a total sample size of less than 1000) i.e., all crosses were broadly of conversion spectrum type C. As might have been expected, the conversion spectrum was highly variable among LCF crosses, with the relative frequency of PMS ranging from 0.0 to 100.0% and the relative frequency of conversion to wild-type from 0.0 to 100.0%! This again shows that the conversion spectrum of mutations in crosses showing very low conversion frequencies cannot be determined with much certainty. Variability arises from variations in phenocopy frequency and also since small numbers of apparently convertant octads are being scored, these small numbers are subject to much random sampling variation.

In summary, these results are inconsistent with oligo- or poly-genic control of NG*w*18 conversion behaviour and instead point to the existence of a single conversion control factor acting on mutation NG*w*18, although its mode of

action remains unknown. This factor can be designated conversion control factor 7 or *ccf*-7, since it was the seventh such factor to be discovered in the Pasadena strains of *Ascobolus immersus*. Its action would appear to be profound since there is usually a 20-40-fold difference in apparent conversion frequencies between LCF and HCF crosses, while the difference between true conversion frequencies is probably much greater.

Linkage relations of NG*w*18 and *ccf*-7

Initially the hypothesis was put forward that the conversion control system acting on NG*w*18 was monogenic and closely linked to NG*w*18 since this was the most sraightforward assumption to test. The results from the experiments already outlined are in agreement with one major genetic conversion control factor (*ccf*-7) acting on NG*w*18 although it was not possible to determine the mode of action of this factor. Furthermore, it was not possible to determine whether HCF or LCF crosses involving NG*w*18 were heterozygous for the supposed two forms of *ccf*-7. However, it was thought more likely that the LCF cross would be heterozygous for *ccf*-7: analysis of 6:2 octads from an HCF cross had not revealed any wild-type segregants with altered conversion properties; gene conversion of mutations *w*62, BB9*w*9 and *w*BHj is reduced in crosses heterozygous for their respective closely linked *ccfs* in the Pasadena strains of *Ascobolus immersus* (Emerson and Yu-Sun, 1967; present work) while the *cv* conversion modifiers in the European strains function in the same way (Girard and Rossignol, 1974). Thus

it was hoped to obtain some information on the linkage relationship of NG*w*18 and *ccf*-7 by analysis of the LCF cross NG*w*18+ × 16P-. If this cross was heterozygous for the two forms of *ccf*-7 and the latter site is closely linked to NG*w*18, most if not all wild-type spores randomly isolated from 4+:4*w* non-convertant octads should carry the same form of *ccf*-7 as the wild-type parent and show similar conversion behaviour. Any isolates showing altered conversion behaviour in backcrosses to the white mutant parent would be recombinants for the two sites in question.

Conversion behaviour of wild-type spores randomly isolated from 4+:4*w* octads from cross NG*w*18+ × 16P-

40 wild-type spores were randomly isolated from 4+:4*w* octads from cross NG*w*18+ × 16P-. 24 spores germinated and 18 of these gave fertile crosses with NG*w*18+ and 3NG*w*18- as tester strains. The results from these crosses are shown in Table 37.

From this Table it can be seen that all 18 crosses were LCF, with a range of conversion frequences of 0.0 to 0.21%, with one extreme value of 0.62% resulting from a cross with a very small sample size of dehisced octads. Therefore these 18 isolates, when crossed to NG*w*18 tester strains showed conversion properties like their wild-type parent. If the parental cross had been heterozygous for the two known forms of *ccf*-7, and no dominance is involved, then all 18 isolates in Table 37 would be non-recombinant for the NG*w*18 and *ccf*-7 sites, indicating close linkage of these sites. Further data concerning the linkage relationship

TABLE 37: Conversion data of random wild-type isolates from 4+:4*w* octads from cross NG*w*18+ × 16P- when crossed to NG*w*18+ or 3NG*w*18-

Isolate No.	Octad classes (+ *w*), %					% NRCF	Total Octads
	4:4	6:2	2:6	5:3	3:5		
1+	99.91	-	-	0.05	0.05	0.09	2185
2-	99.82	-	-	0.12	-	0.12	1659
3+	99.89	-	-	0.05	-	0.05	1854
4+	99.97	-	-	0.03	-	0.03	3579
7+	99.79	0.05	-	0.16	-	0.21	1899
13+	99.81	0.05	-	0.14	-	0.19	2157
16+	99.97	-	-	0.03	-	0.03	3155
17+	99.84	-	-	0.16	-	0.16	2532
20-	99.38	-	-	0.62	-	0.62	360
21-	100.00	-	-	-	-	0.0	573
22+	99.82	-	-	0.18	-	0.18	545
24+	99.96	0.04	-	-	-	0.04	2844
25-	99.94	-	-	0.06	-	0.06	1615
27+	99.96	0.04	-	-	-	0.04	2347
29+	99.88	0.03	-	0.09	-	0.12	3365
31+	99.97	-	-	0.03	-	0.03	3282
32+	100.00	-	-	-	-	0.0	370
33+	100.00	-	-	-	-	0.0	2866

of NG*w*18 and *ccf*-7 can be obtained from Tables 34 and 35. The former Table contains conversion data from wild-type isolates from apparent 6+:2*w* octads while the latter contains similar data from 5+:3*w* octads. However, analysis of the data showed that they were consistent with all the octads analysed being phenocopies, the octads being genotypic 4+:4*w* non-convertant octads. There are data from 12 octads in Table 34 and from 38 octads in Table 35. No wild-type spores showing altered behaviour in backcrosses to NG*w*18 strains were detected. Therefore, if the LCF parental cross had been heterozygous for the two forms of *ccf*-7 and no dominance is involved, the overall data suggest very close linkage of NG*w*18 and *ccf*-7 - no recombinants were observed among 102 wild-type spores isolated from 4+:4*w* octads. This cannot be a definite conclusion since the mode of action of *ccf*-7 is not known.

Originality of *ccf*-7

ccf-7 is probably a truly original conversion control factor and not in any way associated with the six hitherto known *ccfs* in the Pasadena strains of *Ascobolus immersus*: *ccf*-1 determines the conversion behaviour of closely linked white ascospore pigmentation mutation *w*62. Prior to this work the linkage relations of *w*62 and NG*w*18 were not known. They were accordingly mapped and found to be unlinked (R.F. = 49.10%). If *ccf*-7 is closely linked to NG*w*18 (and this has not been shown categorically) then *ccf*-1 and *ccf*-7 would be quite separate, distinct factors. To confirm this, crosses of several derived wild-type strains carrying differ-

ent forms of *ccf*-7 to the same *w*62 strain should be performed. *ccf*-2 is closely linked to locus I (Wickramaratne and Lamb, 1978; Lamb and Helmi, 1978) and may even lie within the locus (Helmi, 1980). The data presented in Table 31 indicate that the same NG*w*18 strain can show LCF or HCF when crossed to derived wild-type strains carrying the same form of *ccf*-2 - *ccf*-2(*P*). Thus *ccf*-2 and *ccf*-7 must be quite separate factors. Helmi (1980) reported that *ccf*-3 is linked to the mating type locus, while in this work NG*w*18 showed no signs of linkage to the mating type. If *ccf*-7 is closely linked to NG*w*18 then *ccf*-3 and *ccf*-7 would be quite distinct factors. *ccf*-4 is reported as being loosely linked to locus I, while Ghikas (1978) showed that NG*w*18 is unlinked to this locus. Again, if *ccf*-7 is closely linked to NG*w*18, *ccf*-4 and *ccf*-7 would be quite separate factors. *ccfs* 3 and 4 comprise the "Super" system of locus I conversion control (see Chapter 1, Introduction). From Table 31 it can be seen that "non-Super" strains (all the wild-type strains in the Table) can give both LCF and HCF crosses involving mutation NG*w*18. This is not conclusive proof that *ccfs* 3 and 4 are distinct from *ccf*-7 due to the interactions of the former two *ccfs* and their mode of action. Confirmatory crosses of different "Super" strains (all of genotype *ccf*-3(*E*), *ccf*-4(*r*)) to the same NG*w*18 tester strain should be carried out. If some crosses are LCF and others HCF, then almost certainly the *ccf*-3, -4 and -7 factors are quite distinct. *ccf*-5 and *ccf*-7 must be completely distinct factors since it was shown in Chapter 2 of this work that mutation NG*w*18 gave LCF crosses to derived wild-type strains carrying either form of

ccf-5, while crosses involving other derived wild-types carrying one form of *ccf*-5 were HCF. Similarly, *ccf*-6 and *ccf*-7 must be separate factors since derived wild type strains carrying *ccf*-6(*A*) gave LCF and HCF crosses to the same NG*w*18 strain. Furthermore, derived wild-type strains carrying *ccf*-6(*A*) or *ccf*-6(*B*) both gave LCF crosses to the same NG*w*18 strain (see Chapter 3 of this thesis).

Thus, in summary, it can be concluded that *ccf*-7 is certainly distinct from *ccfs* 2, 5 and 6 and probably also distinct from *ccfs*1, 3 and 4, although this has not been absolutely proven. Confirmatory experiments remain to be performed. In conclusion, *ccf*-7 is probably a novel conversion control factor in the Pasadena strains of *Ascobolus immersus*. This is supported by the fact that none of the hitherto known conversion control systems is able to act to produce such low conversion frequencies as *ccf*-7 can for NG*w*18.

The action of *ccf*-7 on other locus II mutations (S*w*26 and NG*w*16)

The original NG*w*18 and NG*w*16 mutant strains were NG-induced and isolated by Ghikas (1978) from the same parental cross involving derived wild-type strains 61- and 9r+ (both carrying *ccf*-2(*P*)) while the original S*w*26 mutant strain arose spontaneously in the progeny of cross BHR2- × 9r+ (both derived wild-types carry *ccf*-2(*P*)) and was isolated by the same author. In mapping experiments, she did not observe any recombinants for S*w*26 and NG*w*18 among 5000 octads scored (i.e., the mutations are either very closely

linked or one is a point mutation and the other (probably S*w*26) an overlapping deletion), while mutations NG*w*18 and NG*w*16 showed a recombination frequency of 2.5%. In this thesis all 3 mutations are regarded as part of locus II.

Lamb and Ghikas (1979) crossed all 3 mutants to the same 3 derived wild-type strains. The conversion data obtained are shown in Table 38.

TABLE 38: Conversion data of S*w*26, NG*w*16 and NG*w*18 mutant strains when crossed to 3 different derived wild-type strains.

Mutant strain	Crossed to	Octad classes (+:*w*),% 4:4	6:2	2:6	5:3	3:5	% NRCF	Relative %PMS	% Conv. to +	Total Octads
S*w*26+	92-	99.60	0.20	0.07	0.12	0.02	0.40	33.33	79.17	6000
	42-	99.60	0.01	0.04	0.20	0.15	0.40	87.50	53.13	8060
NG*w*16+	92-	95.98	0.67	0.20	2.41	0.75	4.02	78.43	76.47	2535
	42-	94.25	1.50	0.30	3.15	0.78	5.73	68.54	81.13	5270
	KIII-	95.35	1.35	0.18	2.65	0.25	4.43	65.54	90.40	3999
NG*w*18+	92-	95.74	2.36	0.71	0.76	0.38	4.22	27.01	74.07	5733
	42-	96.67	2.11	0.35	0.59	0.26	3.31	25.68	81.56	8541
	KIII-	99.82	0.12	-	0.04	0.02	0.18	11.11	88.89	10260

All data of Lamb and Ghikas (1979)

From this Table it can be seen that the 3 mutations do not show the same conversion behaviour. Mutation S*w*26 shows LCF in crosses to derived wild-type strains 92- and 42-. Both of the latter wild-type strains gave HCF crosses with NG*w*16 and NG*w*18 strains. This could be because the S*w*26 strain used carries the other form of *ecf*-7 to that carried

by NGω18. Unfortunately cross Sω26+ × KIII- was not fertile and could not be repeated in this work. This cross might reveal whether Sω26+ and NGω18+ carry different forms of *ccf*-7. The two crosses involving Sω26+ also show different conversion spectra which may be due to the action of conversion control factors, cryptic heterozygosity in one of the crosses or imperfectly controlled environmental effects. Mutant strains NGω16+ and NGω18+ both gave HCF crosses to derived wild-type strains 92- and 42-, with conversion frequencies of broadly similar values (4.02 and 5.73% for NGω16 and 4.22 and 3.31% for NGω18). However, the conversion spectra of both mutations differed, with much more frequent PMS in crosses involving NGω16. NGω16+ gave a similar HCF cross to derived wild-type strain KIII - while NGω18+ gave a LCF cross. The experiments described in this Chapter suggest that derived wild-types 92- and 42- on one hand and KIII- on the other, carry different forms of a *ccf*(*ccf*-7) affecting the conversion behaviour of NGω18. However, crosses involving NGω16 gave HCF with all 3 derived wild-type strains. These results cannot be explained if NGω16 is responding to the same conversion control system as NGω18 and this system acts by reducing gene conversion in crosses heterozygous for both forms of *ccf*-7, which would almost certainly be closely linked in such a case. Therefore either NGω16 does not respond to *ccf*-7, or *ccf*-7 does not act by reducing gene conversion of (closely linked) mutations when heterozygous.

It was decided to investigate the response of mutations Sω26 and NGω16 in crosses to many derived wild-type strains known to carry the different forms of *ccf*-7. Such derived

wild-type strains had been isolated from cross 25-5R+ × 7R7- and their conversion behaviour in crosses to NG*w*18 mutant strains already determined (see Table 36). In fact, it was this experiment which demonstrated the monogenic control of NG*w*18 conversion.

Conversion behaviour of random wild-type spores isolated from cross 25-5R+ × 7R7- when crossed to S*w*26- and NG*w*16-

42 derived wild-type isolates were available from parental cross 25-5R+ × 7R7-. 27 of these isolates had given fertile crosses with either NG*w*18+ or 3NG*w*18- tester strains, revealing that 14 of the derived wild-type strains carried one form of *ccf*-7 while 13 strains carried another form. At this stage the only S*w*26 and NG*w*16 strains showing fertility were both of (-) mating type. Thus all the derived wild-type isolates from cross 25-5R+ × 7R7- known to be of (+) mating type were crossed to S*w*26- and NG*w*16- mutant strains. Virtually all of these crosses were fertile and the conversion data obtained from them, plus data from crosses involving the same derived wild-type strains and 3NG*w*18-, are displayed in Table 39.

From this Table it can be seen that the conversion frequency of S*w*26 varied from 0.0 to 1.91%, although only 3 out of 14 crosses gave conversion frequencies of more than 0.83%. However, in all previous crosses involving mutation S*w*26 performed by Lamb and Ghikas (1979), Helmi (1980) and in earlier experiments in this work, the mutation had never shown a conversion frequency greater than 0.40%. Prior to this experiment it had been thought

TABLE 39: Conversion data of random wild-type spores isolated from cross 25-5R+ x 7R7- when crossed to Sw26-, NGw16- and 3NGw18- mutant strains

Isolate No.	Crossed to	Octad classes (+:w),%					% NRCF	Total Octads
		4:4	6:2	2:6	5:3	3:5		
	Sw26-	99.66	-	-	0.34	-	0.34	292
12	NGw16-	98.26	0.76	0.04	0.85	0.09	1.74	2240
	3NGw18-	99.94	-	0.06	-	-	0.06	1668
	Sw26-	98.67	0.11	-	1.11	0.11	1.33	902
28	NGw16-	97.62	0.47	0.05	1.81	0.05	2.38	1932
	3NGw18-	99.86	-	0.06	0.06	-	0.11	3491
	Sw26-	98.09	0.09	-	1.82	-	1.91	1101
47	NGw16-	99.33	-	0.27	0.41	-	0.67	745
	3NGw18-	96.28	2.61	0.59	0.35	0.16	3.72	3714
	Sw26-	99.17	-	-	0.83	-	0.83	480
100	NGw16-	98.74	0.33	0.07	0.73	0.13	1.26	1505
	3NGw18-	97.15	2.16	0.21	0.37	0.11	2.85	1898
128	Sw26-	99.81	-	-	0.19	-	0.19	534
	3NGw18-	99.97	0.03	-	-	-	0.03	3159
	Sw26-	99.66	0.34	-	-	-	0.34	294
132	NGw16-	98.55	0.38	0.13	0.82	0.13	1.45	1583
	3NGw18-	96.03	2.57	0.47	0.78	0.16	3.97	1285
138	Sw26-	99.76	-	-	0.24	-	0.24	820
	3NGw18-	99.86	0.06	-	0.06	0.03	0.14	3486

TABLE 39: (continued)

Isolate No.	Crossed to	Octad classes (+:w),%					% NRCF	Total octads
		4:4	6:2	2:6	5:3	3:5		
	Sw26-	99.81	-	-	0.19	-	0.19	1033
154	NGw16-	98.52	0.29	0.06	1.01	0.12	1.48	3457
	3NGw18-	96.55	2.67	0.56	0.22	-	3.45	898
158	Sw26-	100.00	-	-	-	-	0.0	811
	3NGw18-	97.24	1.74	0.55	0.42	0.04	2.76	2358
	Sw26-	99.89	-	-	0.11	-	0.11	1852
194	NGw16-	98.54	0.49	0.16	0.76	0.05	1.46	3706
	3NGw18-	97.99	1.47	0.21	0.24	0.09	2.01	3339
	Sw26-	99.51	0.10	0.10	0.29	-	0.49	1023
204	NGw16-	97.28	0.65	0.12	1.86	0.09	2.72	3384
	3NGw18-	99.84	-	-	-	0.16	0.16	1228
205	Sw26-	99.96	-	-	0.04	-	0.04	2465
	NGw16-	98.41	0.58	0.14	0.72	0.14	1.59	694
	Sw26-	100.00	-	-	-	-	0.0	326
210	NGw16-	94.68	1.06	1.42	2.13	0.71	5.32	282
	3NGw18-	96.54	2.54	0.42	0.38	0.12	3.46	2602
	Sw26-	98.85	0.10	-	0.96	-	1.15	1040
212	NGw16-	97.92	0.56	0.04	1.24	0.20	2.04	2501
	3NGw18-	99.94	-	-	0.06	-	0.06	3621

possible that the S*w*26+ strain used in crosses by Lamb and Ghikas (1979) might have carried the alternative form of *ccf*-7 not carried by NG*w*18+ and 3NG*w*18- and S*w*26 might respond to *ccf*-7 in the same way as NG*w*18. Although a different S*w*26 strain was used in this experiment, the results refute that possibility, since there is no correlation between the conversion behaviour of strains S*w*26- and 3NG*w*18-. S*w*26 shows high conversion frequencies in crosses involving wild-type isolates numbers 28, 47 and 212. Isolates 28 and 212 gave HCF crosses to 3NG*w*18- while isolate 47 gave a LCF cross. In the remaining 11 crosses, S*w*26 showed a conversion frequency variation of 0.0 to 0.83%. This variation cannot be correlated with the conversion behaviour of NG*w*18 nor with the sample sizes of the crosses. Some variation is certainly due to random fluctuations in the small numbers of convertant octads and imperfectly controlled environmental conditions. Fluctuations in phenocopy frequencies may also be involved. However, 3 isolates certainly showed higher conversion frequencies (of 1.15, 1.33 and 1.91%) than ever reported for S*w*26 before. It is interesting that in these 3 crosses a greatly increased 5:3 frequency contributed largely to the high % NRCFs. If these elevated conversion frequencies do have a genetic origin, *ccf*-7 cannot be the factor responsible, unless its behaviour is being greatly modified by a second unknown factor. The 3 "HCF" crosses should be repeated to determine whether the observed elevated conversion frequencies were merely the result of chance random variation or imperfectly controlled environmental conditions.

Table 39 also shows that in the 11 crosses involving mutation NG*w*16, its conversion frequency varied from 0.67 to 5.32%. These values were extremes, resulting from small sample sizes. Excluding these 2 crosses, the range in conversion frequency was from 1.26 to 2.72%. These values were much lower than in the 3 original crosses performed by Lamb and Ghikas (1979) and shown in Table 38 (4.02, 4.43 and 5.73%). However, the conversion spectra were broadly similar: excluding the 2 crosses with extreme conversion frequencies, in the remaining 9 crosses 5:3s were the most frequent aberrant octad class, followed by 6:2s. There is no correlation between the conversion behaviour of NG*w*16 and NG*w*18 in crosses of mutant strains to the same derived wild-type strains. Therefore either NG*w*16 does not respond to *ccf*-7 or the mode of action of *ccf*-7 is such that its response could not be detected in this experiment, i.e. one form of *ccf*-7 is dominant over the other. Say *A* is a dominant enhancer of gene conversion and NG*w*16- carries *A*, while some of the derived wild-type isolates carry *A* and some *a* (the recessive form). In such a situation all crosses involving NG*w*16(*A*) would be HCF. Furthermore, if 3NG*w*18- carried *a*, then half of the crosses involving this mutation would be LCF and half HCF, which is the situation observed in Table 39. Why are the NG*w*16 conversion frequencies observed in this experiment lower than those observed in the original crosses of Lamb and Ghikas (1979)? Firstly, a different NG*w*16 strain was used in this work. However, the NG*w*16- strain used in this experiment was also used in the crosses shown in Table 28 (Chapter 3). Three crosses were performed. One of these gave a NG*w*16 conver-

sion frequency (of 1.70%) similar to those seen in this experiment (but the conversion spectrum differed, with 6:2s as the most frequent aberrant class), while the remaining two crosses gave conversion frequencies (of 4.01 and 4.81%) similar to those observed in the crosses of Lamb and Ghikas (1979). Thus the NG*w*16- strain used in this experiment is capable of giving higher conversion frequencies of 4-5%, similar to those observed in the original crosses. These NG*w*16 conversion frequencies of 1.26-2.72% and 4-5% could represent two classes of LCF and HCF crosses, brought about by the action of *ccf*-7. However, it is impossible to explain all the conversion data on this basis even supposing that *ccf*-7 acts in one of the following ways: as a heterozygous reducer of gene conversion; a heterozygous enhancer; a dominant reducer or a dominant enhancer. Alternatively, NG*w*16 may not respond to *ccf*-7, or its response may not be apparent due to the effect of other unknown *ccfs* segregating in the crosses. The lower conversion frequencies of NG*w*16 seen in this experiment may not involve the *ccf*-7 factors - other factors in the genetic background of the NG*w*16- strain may be responsible.

In conclusion, it was not possible to determine in this experiment whether mutations S*w*26 and NG*w*16 respond to *ccf*-7. Hitherto unknown, elevated conversion frequencies of S*w*26 were observed, but this was not a simple response to *ccf*-7. All the crosses involving NG*w*16 showed basically the same conversion frequency.

Discussion

The above experiments have demonstrated that the conversion behaviour of mutation NG*w*18 is subject to genetic control and the control system seems to be monogenic. The *ccf* involved has been termed *ccf*-7 and is almost certainly novel, having been shown to be distinct from *ccfs* 2, 5 and 6 and probably also distinct from *ccfs* 1, 3 and 4 although this was not proven. If *ccf*-7 acts in a similar way to *ccfs* 1, 5 and 6 and the *cv* modifiers, then it would be very closely linked to NG*w*18 (no recombinants were observed among 102 wild-type spores isolated from 4+:4*w* octads from LCF cross NG*w*18+ × 18P-). It was not possible to determine the mode of action of *ccf*-7, but two forms of it were apparently segregating in these experiments. In all the crosses performed, NG*w*18 conversion frequency fell clearly into 2 classes - LCF and HCF. The overall results for these 2 classes of cross are summarized in Table 40. The results for each type of cross were based on pooling data from many crosses of that type. These crosses were either replicates of the same cross or different individual crosses but apparently identical with respect to NG*w*18 and *ccf*-7 genotypes. No crosses which gave a total sample size of less than 1000 dehisced octads were included.

The variability among crosses of the same type was tested by the $\Sigma\chi^2$ homogeneity test, applying this to narrower ratio conversion frequency data. The data from both types of crosses were highly heterogeneous: for the 96

TABLE 40: Overall conversion data from LCF and HCF crosses involving mutation NGω18.

Cross type	Octad classes (+:ω),%					%NRCF ±S.E.	Rel. %PMS	%Conv. to +	Total octads
	4:4	6:2	2:6	5:3	3:5				
"LCF"	99.86	0.03	0.01	0.09	0.01	0.14± 0.01	69.98	85.23	288 339
"HCF"	96.74	2.16	0.38	0.56	0.15	3.26± 0.05	22.04	83.70	111 343

LCF crosses $\Sigma\chi^2 = 214.84$, $p < 0.01$, 95 df; and for the 45 HCF crosses $\Sigma\chi^2 = 263.82$, $p < 0.01$, 44 df. Imperfectly controlled environmental conditions may be partially responsible for the heterogeneity among crosses of the same type. In the case of LCF crosses, variations in phenocopy frequency for at least the 6:2 and 5:3 octad classes could also produce variability in %NRCFs. A spread of conversion frequencies was observed in the HCF crosses which could be due to other modifiers of NGω18 conversion frequency segregating in some HCF crosses.

Considering the data in Table 40 it can be seen that the apparent mean conversion frequency of NGω18 in LCF crosses was 0.14% and 3.26% in HCF crosses. This represents a 23-fold difference in conversion frequency between the two types of cross. If this difference in conversion behaviour is due to the action of a single conversion control factor - and this certainly seems so - then it has a more profound effect on the conversion frequency of the

target mutation than any of the other 6 *ccfs* known in the Pasadena strains of *Ascobolus immersus*: when heterozygous in a cross, *ccf*-1 can reduce the conversion frequency of closely linked mutation *w*62 by a maximum of about 5-fold, compared to crosses homozygous for one form of *ccf*-1; the *ccf*-2 factors can bring about up to 13-fold differences in the conversion frequency of closely linked locus I mutation *w*78 in appropriate crosses; the *ccf*-3 and *ccf*-4 factors which comprise the "Super" conversion control system can approximately double whatever gene conversion frequency at locus I is determined by the *ccf*-2 factors; when heterozygous in a cross, *ccf*-5 can reduce the conversion frequency of closely linked mutation BB9*w*9 by about 5-fold compared to crosses homozygous for either form of *ccf*-5; *ccf*-6 acts similarly on a less closely linked mutation *w*BHj, but the conversion frequency of this mutation in crosses homozygous for either form of *ccf*-6 is less than double that shown in crosses heterozygous for *ccf*-6.

In actual fact, the true difference in conversion frequency between the LCF and HCF crosses is almost certainly much greater than 23-fold. In an analysis of the conversion behaviour of wild-type spores from 6:2 and 5:3 octads from LCF cross NG*w*18+ × 16P-, data are available from 12 6:2 and 38 5:3 octads. A large number of phenotypically wild-type spores turned out to be NG*w*18 white mutant spores. The overall data are consistent with all the 6:2 and 5:3 octads under study in the LCF cross

having been phenocopies and genotypically being 4:4 non-convertant octads. Thus the true NG*w*18 conversion frequency must be far lower than 0.14% in LCF crosses. Even if all the 2:6 and 3:5 octads were genuine convertants (and they may very well not be), the true NG*w*18 conversion frequency could be reduced to less than 0.02% and could even be much lower than this. Conversely, in an analysis of the conversion behaviour of wild-type spores from 6:2 octads from an HCF cross, no phenocopy spores were detected in 7 octads. It is worth noting that Girard and Rossignol (1974) analysed a sample of aberrant octads from various + × *w* crosses homozygous (HCF) or heterozygous (LCF) for the corresponding *cv* modifier to determine whether such octads were genuine convertants or of spurious origin. Most of the aberrant octads obtained from LCF crosses did not result from conversion, while most of those obtained from HCF crosses did. In most cases the apparently convertant octads resulted from false clusters of ascospores rather than phenocopies as in this work.

In summary, the observed NG*w*18 conversion frequency of 3.26% is probably a very close estimate of the true conversion frequency of NG*w*18 in HCF crosses. This is expected if the absolute phenocopy frequency is roughly the same in LCF and HCF crosses. It can be concluded that the difference in true NG*w*18 conversion frequency between LCF and HCF crosses is probably of the order of several hundred-fold. Only the *cv2* conversion modifier in the European strains of *Ascobolus immersus* is known to have a similarly great effect on the conversion behaviour of some of its

target mutations (Girard and Rossignol, 1974). However, in this case also the true difference in conversion frequency between HCF and LCF crosses may be greater than the apparent differences in conversion frequency.

Considering the conversion spectrum of NG*w*18 in both LCF and HCF crosses, it can be seen from Table 40 that the mutation shows a C-type spectrum (frequent PMS, conversion overwhelmingly to wild-type) in both types of cross. Apparently, PMS is much less frequent in HCF crosses. However, this may not be so. The conversion spectra of all HCF crosses were broadly similar, while the conversion spectra of LCF crosses fluctuated wildly. In each LCF cross only small numbers (usually 1 or 2 only) of apparently convertant octads arose, and small numbers are particularly susceptible to random, sampling variations. By pooling the data from a large number of crosses, this might be overcome (the data in Table 40 come from 96 LCF crosses, each with sample sizes of over 1000). However, a more important factor is involved. It was shown that most 5:3 and 6:2 octads in LCF crosses were in fact phenocopies. The phenocopy frequency among 3:5 and 2:6 octads was not detected, but for reasons already outlined may be much lower. Thus the true conversion spectrum of NG*w*18 in LCF crosses remains quite unknown.

It was not possible to determine whether closely linked mutations NG*w*16 and S*w*26 responded to *ccf*-7 in the same way as NG*w*18. In all the experimental crosses, NG*w*16 showed broadly the same conversion frequency, while NG*w*18 gave LCF and HCF crosses. This behaviour could be

explained if *ccf*-7 has two forms, one of which is a dominant enhancer of gene conversion. If the NG*w*16 strain used in these experiments carried *A*, the dominant enhancing form and the NG*w*18 strains used carried the recessive form *a*, then these results are explicable. The conversion data involving mutation S*w*26 are not so easily explicable. This mutation does not seem to respond to *ccf*-7 in any simple way, if it does at all. This might be due to the molecular nature of the mutation or the interaction of *ccf*-7 with other unknown *ccfs* segregating in the appropriate crosses.

Here it is appropriate to consider various ways in which the *ccf*-7 factors might control NG*w*18 conversion behaviour and then describe experiments which could distinguish between these possibilities.

Models of *ccf*-7 action

1. A heterozygous reducer of gene conversion.

Many systems of this type are known, including *ccfs* 1, 5 and 6 in the Pasadena strains (Emerson and Yu-Sun, 1967; present work) and *cvs* 1, 2, 4 and 6 in the European strains of *Ascobolus immersus* (Girard and Rossignol, 1974); the *ss* recombination modulator in *Neurospora crassa* (Catcheside, 1981); and the system whereby recombination is reduced in the *adh1* locus of *Zea mays* (Freeling, 1978). If this were the mode of action of *ccf*-7 on NG*w*18 conversion, then HCF crosses would be homozygous for one form of *ccf*-7. Thus no wild-type isolates showing altered conversion behaviour in backcrosses to the white mutant parent would be recoverable in such a cross. LCF crosses would be heterozygous

for the *ccf*-7 factors and thus if genuine 6:2 convertant octads could be found, 2 wild-type spores with altered conversion properties should be detectable in each octad if NG*ω*18 and *ccf*-7 are closely linked but do not usually co-convert. If the sites were less closely linked, 4 wild-type spores showing altered conversion behaviour could be detected in some octads. If *ccf*-7 acted in this way, the conversion data involving mutation NG*ω*16 are difficult to explain, unless another *ccf* is capable of overriding *ccf*-7. Alternatively, NG*ω*16 may not respond to *ccf*-7.

2. A dominant enhancer of gene conversion.

Many systems of this type are already known in fungi: The *cog* genes in *Neurospora crassa*, of which only 1 has been positively identified (Catcheside, 1977); the YS17 mutation in the *buff* gene of *Sordaria brevicollis* (MacDonald and Whitehouse, 1979); the *rec* genes in *Coprinus cinereus* (Katy and Moore, 1980); and *ccf*-3 in the Pasadena strains of *Ascobolus immersus* (Helmi and Lamb, 1981). The *cog* and YS17 systems are linked to the target mutations whose conversion they enhance while the *rec* genes and *ccf*-3 are unlinked to their target mutations. If this were the mode of action of *ccf*-7 on NG*ω*18 conversion, then LCF crosses would be homozygous for the recessive form (*a*), while HCF crosses involving the same NG*ω*18 strain would be of the +, *A* × *w*, *a* type (where *A* is the dominant enhancing form) i.e. *A*/*a* heterozygous. Thus in an analysis of the conversion behaviour of wild-type spores isolated from 6:2 octads from an HCF cross, 4 would carry *A* while 2 would carry *a* and show

altered, low conversion frequencies in backcrosses to the NGω18 parent. In this work such altered conversion segregants were not observed. This could be due to co-conversion of NGω18 and *ccf*-7. Certainly on this system *ccf*-7 could not be unlinked to NGω18 because some octads would reveal 4 wild-type spores with altered conversion segregants. This mode of *ccf*-7 action could explain the conversion behaviour of NGω16 strains if those used in this work carried *A*.

3. A dominant reducer of gene conversion.

Such systems have also been reported, including the *rec* genes in *Neurospora crassa* (Catcheside, 1977), the recombination control of the mating type loci in *Schizophyllum commune* (work of Stamberg and collaborators, summarized by Fincham, Day and Radford, 1979), the *recR* gene in *Coprinus cinereus* (which can also act as a recombination enhancer in other parts of the genome (Katy and Moore, 1980)) and *ccf*-4 in the Pasadena strains of *Ascobolus immersus* (Helmi and Lamb, 1981). These systems may be linked or unlinked to the affected mutations. If *ccf*-7 were to operate in such a way, then HCF crosses would be homozygous for the recessive form of the factor and no isolates showing altered conversion characteristics in crosses to the appropriate parental strain could be isolated from such crosses. However, LCF crosses would be heterozygous for the two forms of *ccf*-7. The dominant, reducing form would be in the wild-type strain in the LCF cross analysed in this work, since the same NGω18 strain was capable of giving HCF crosses to other derived wild-type strains. An analysis of the conversion behaviour

of wild-type isolates from 6:2 octads from such a LCF cross should reveal that 2 of the 6 isolates show altered, high, conversion frequencies in backcrosses to the white mutant parent. In this work no genuine 6:2 convertant octads were detected and so this mode of action for *ccf*-7 cannot be ruled out, but if *ccf*-7 does act in this way, it cannot be unlinked to NG*w*18 since if it were, segregants showing altered, elevated conversion frequencies would be detectable in 4:4 non-convertant octads. This mode of *ccf*-7 action cannot explain the conversion behaviour of mutation NG*w*16, if it responds to *ccf*-7.

4. A heterozygous enhancer of gene conversion.

There seems to be only one known example of recombination enhancement in crosses heterozygous for a controlling genetic factor and this concerns crosses between *ad-3A* and *ad-3B* in *Neurospora crassa* (De Serres, 1971). If *ccf-7* were to act by enhancing NG*w*18 conversion frequencies in crosses heterozygous for its two forms, then in 6:2 octads isolated from HCF crosses 2 wild-type isolates should show altered, reduced gene conversion frequencies in backcrosses to the white mutant parent. None was observed in this experiment, but this could be due to co-conversion of NG*w*18 and *ccf*-7, if tightly linked. On this model of *ccf*-7 action NG*w*18 and *ccf*-7 could not be unlinked or loosely linked since then 4 wild-type isolates showing altered conversion properties could be detected in 6:2 octads from a HCF cross. This model of *ccf*-7 action is unable to explain the conversion

behaviour of mutation NGω16 if this mutation responds to *ccf*-7 in the same way as NGω18 does.

5. An incompletely dominant enhancer/reducer of gene conversion.

The 3 forms of *ccf*-2 show incomplete dominance in their control of locus I conversion frequencies in the Pasadena strains of *Ascobolus immersus* (Lamb and Helmi, 1978). The M26 mutation in the *ade6* gene of *Schizosaccharomyces pombe* increases gene conversion frequencies in this gene in an additive rather than a dominant fashion (Gutz, 1971b; Goldman and Smalletts, 1979). This is not true incomplete dominance, but the situation is similar. Suppose the two forms of *ccf*-7 were to act in such a way. An analysis of the conversion behaviour of wild-type spores isolated from 6:2 octads (from both LCF and HCF crosses) in backcrosses to the white mutant parent would not be very revealing, giving conversion data that would be in agreement with either *ccf*-7 acting as a heterozygous reducer or enhancer of NGω18 gene conversion. NGω18 strains carrying either form of *ccf*-7 would be needed as tester strains. The LCF and HCF crosses already known represent heterozygous and homozygous for one form of *ccf*-7 crosses, although which corresponds to which is not known. Crosses homozygous for the other form of *ccf*-7 would need to be performed. Either NGω18 strains carrying either form of *ccf*-7 should be obtained and crossed to derived wild-types carrying either form of *ccf*-7 (the latter strains are already available) or the programme of analysing the conversion behaviour of white

mutant isolates from 2:6 octads from LCF and HCF crosses outlined below should be carried out. Such a programme would reveal whether *ccf*-7 acts as an incompletely dominant enhancer/reducer of NG*w*18 conversion and, if so, the conversion frequency of the latter mutation in crosses homozygous for the form of *ccf*-7 for which, as yet, data are not available.

Suggested experimental programme to characterize *ccf*-7

One of the problems in this work was that all the available NG*w*18 tester strains seemed to carry the same form of *ccf*-7. Without knowing the mode of action of this factor it would be difficult to isolate NG*w*18 strains carrying the other form of *ccf*-7. Were such strains of both mating types available, they should be crossed to the wild-type isolates from 6:2 octads from HCF cross NG*w*18+ × RS8-1-. The conversion results obtained would distinguish between some of the possibilities of *ccf*-7 action outlined above. A second, major, problem was the inability to isolate genuine 6:2 convertant octads from a LCF cross, due to the high incidence of phenocopies. Both of these problems might be overcome by determining the conversion behaviour of white mutant isolates from 2:6 octads from both LCF and HCF crosses: although such octads were very infrequent in LCF crosses (generally less than 0.01%), it was felt that they were less likely to be phenocopies than 6:2s by virtue of the way in which phenocopy spores usually arise (by passage of red pigment from a wild-type to a white mutant spore by a proto-

plasmic connection). Furthermore, wild-type tester strains of both mating types and carrying either form of *ccf*-7 are already available - strains 16P- and 7R7- carry one form of *ccf*-7, while 92- carries the other (Table 31) as does 25-5R+. Wild-type isolates of (+) mating type carrying the same form of *ccf*-7 as 16P- and 7R7- can be selected from those giving LCF crosses in Table 36.

White mutant spores should be isolated from 2:6 octads from both LCF and HCF crosses involving mutation NG*w*18, using the optimum germination conditions established in PART TWO (Chapter 1) of this thesis (dung germination medium; 4 hours heat shock at 50°C then leave overnight in dark at 37°C). The true genotypic nature of each phenotypic white mutant isolate should be determined by crossing to NGw18 mutant strains of both mating types. This is because some 2:6 octads could be phenocopies, with some of the white spores having arisen by spontaneous mutation at other sites in the genome. Each genuine NG*w*18 isolate from genuine 2:6 octads should be crossed to pairs of derived wild-type strains carrying both forms of *ccf*-7. The pattern of conversion results so obtained should clearly distinguish between the 5 possible modes of *ccf*-7 action outlined above.

Finally, what of the possible origins of the two forms of *ccf*-7 detected in the experiments described? From Table 31 it can be seen that original wild-type strain P5- gave a LCF cross with NG*w*18+ and thus apparently carried one form of *ccf*-7. However, crosses involving the other original wild-type strain K5+ were not fertile. It can also be seen from Table 31 that the cross NG*w*18+ × KIII- was LCF. Thus

strain KIII- — a second generation derived wild-type — probably also carries the same form of *ccf*-7 as P5-. It remains highly likely that original wild-type strain K5+ carries the alternative form of *ccf*-7. If this is so, the two known forms of *ccf*-7 would exist as a polymorphism in the original wild-type isolates of the Pasadena strains of *Ascobolus immersus*. Alternatively, one form could have later arisen from the other by mutation or some recombinational event.

Chapter 5: Discussion - comparison of the nature and actions of *ccfs* 5, 6 and 7 on their target mutations, comparisons with other systems and possible mechanisms of action.

Introduction

The experiments described in the previous Chapters of this thesis have demonstrated that the conversion behaviour of the 3 unlinked mutations BB9*w*9, *w*BHj and NG*w*18 is under the control of 3 separate conversion control systems, each of which is apparently monogenic. These systems are *ccf*-5, acting upon BB9*w*9; *ccf*-6 acting upon *w*BHj; and *ccf*-7, acting upon NG*w*18. The actions of the *ccf*-5 and -6 factors on their target mutations show very great similarities, as well as some differences. It remains possible that *ccf*-7 acts upon NG*w*18 in a similar way, although this was not proven. In all 3 systems only small variations in the conversion frequency of the target mutation were attributable to imperfectly controlled environmental conditions or minor genetic modifiers of conversion behaviour, although the latter 2 factors might have played a relatively greater rôle in the case of *w*BHj conversion. In this Chapter the nature and actions of *ccfs* 5, 6 and 7 on their target mutations will be compared. These systems will also be compared with other apparently similar systems in the same and other organisms and finally possible mechanisms of action will be discussed.

The nature and action of *ccfs* 5, 6 and 7 on their target mutations

The nature and action of each *ccf* will be discussed in terms of its linkage to the mutations whose conversion it

controls; the number of alternative forms of each *ccf*; the resulting conversion frequencies and spectra of the target mutations in the range of crosses performed; possible *cis/trans* position effects of the *ccfs* on their target mutations - both in terms of conversion frequency, spectrum or both; possible co-conversion of each *ccf* and its target mutation; the specificity of each *ccf* and finally, their possible origins.

The *ccf*-5 factor responsible for BB9*w*9 conversion control is novel, having been shown to be distinct from the other known *ccfs* in the Pasadena strains of *Ascobolus immersus*. The same applies to *ccf*-6, responsible for *w*BHj conversion control, while *ccf*-7, acting on NG*w*18, is certainly distinct from *ccfs* 5 and 6 and is likely to be novel too, although this was not proven in this work.

ccf-5 is closely linked to BB9*w*9 (no recombinants among 53 progeny analysed). *ccf*-6 is also fairly closely linked to *w*BHj, but no so close as *ccf*-5 and BB9*w*9. In a suitable doubly heterozygous cross, *ccf*-6 and *w*BHj showed a recombination frequency of *c*. 7%. For the various reasons outlined in Chapter 3 this might be an over-estimate of the true recombination frequency, but it can certainly be concluded that *ccf*-6 and *w*BHj are <u>not</u> so closely linked as *ccf*-5 and BB9*w*9. This difference in linkage relations might account for important differences in the degree of response of BB9*w*9 and *w*BHj to their respective *ccfs*. Uncertainty surrounds the linkage relations of *ccf*-7 and NG*w*18 since it was not possible to conclusively determine the mode of action of *ccf*-7 in this work. If *ccf*-7 acts in a similar way to *ccfs* 5 and 6, then it would be very closely linked to NG*w*18 - no

recombinants were observed among 102 progeny. On this basis, *ccf*-7 and NG*w*18 could be the most closely linked, followed by *ccf*-5 and BB9*w*9, known to be closely linked, with *ccf*-6 and *w*BHj the most loosely linked *ccf* and target mutation.

In the experiments carried out in this work, 2 forms each of *ccfs* 5, 6 and 7 were segregating in the crosses performed. This does not preclude the later discovery of further forms of any of these factors in the existing Pasadena strains, which could arise from any of the existing forms by mutation or recombination. In fact, 3 forms of *ccf*-2 are known (Lamb and Helmi, 1978). It is also quite conceivable that further polymorphic variants of any of these *ccfs* could exist among wild "populations" of the Pasadena strains of *Ascobolus immersus*.

In the case of mutations BB9*w*9 and *w*BHj + × *w* crosses were performed with all 4 possible combinations of the 2 *ccf*-5 and the 2 *ccf*-6 factors respectively. This was not possible for *ccf*-7 and NG*w*18 since not all the desired wild-type and white mutant strains could be isolated. These crosses demonstrated that the conversion frequencies of mutations BB9*w*9 and *w*BHj were high in crosses homozygous for either form of their closely linked *ccf* and low in crosses heterozygous for these factors.

Considering the data involving mutation BB9*w*9 first, it was seen that there was a slight, but statistically significant difference in BB9*w*9 conversion frequency in the 2 *ccf*-5 homozygous crosses - the mean conversion frequencies were 11.08% and 9.24%. These differences might be due to the

nature of the *ccf*-5 factors themselves, or minor modifiers of gene conversion could be responsible. Overall, BB9*w*9 conversion frequency was reduced about 5-fold in crosses heterozygous for the 2 forms of *ccf*-5.

In the data involving mutation *w*BHj there was also seen a slight, but statistically significant difference in *w*BHj conversion frequency in the 2 types of *ccf*-6 homozygous cross - the mean conversion frequencies were 13.38% and 12.28% (rather higher than the BB9*w*9 HCFs). Again this difference in conversion frequency could have a genetic basis, involving either the *ccf*-6 factors or minor modifiers of gene conversion. Overall, the *w*BHj conversion frequency was reduced by a little less than one-half in crosses heterozygous for the 2 forms of *ccf*-6 compared with crosses homozygous for either form. This is a much less drastic reduction in conversion frequency than was observed in the parallel situation of *ccf*-5 and BB9*w*9 - a possible consequence of the less close linkage of *ccf*-6 and *w*BHj. This point will be considered more fully later.

As already pointed out, it was not possible to perform crosses involving NG*w*18 with all possible combinations of the *ccf*-7 factors. However, the crosses that were performed could easily be classified into HCF and LCF categories, although it was not possible to determine which category of cross was homozygous for 1 form of the *ccf*-7 and which was heterozygous. There was an apparent 23-fold difference in conversion frequency between the mean LCF (0.14%) and HCF

(3.26%) values for NG*w*18. The magnitude of this effect of *ccf*-7 on NG*w*18 conversion frequency is greater than the effect not only of *ccfs* 5 and 6 but of any of the known *ccfs* on their target mutations in the Pasadena strains of *Ascobolus immersus*. However, it was shown that the difference between true NG*w*18 conversion frequencies is certainly much greater than 23-fold between LCF and HCF crosses, since in LCF crosses the overwhelming majority of apparent 6:2 and 5:3 octads were shown to be phenocopies. All 6:2 octads analysed from HCF crosses were apparently genuine. Although this was not tested, it remains possible that many apparent 2:6 and 3:5 octads in LCF crosses may also be phenocopies. Even if this were not so, the difference in true conversion frequencies between LCF and HCF crosses brought about by the action of *ccf*-7 could be of the order of several hundred-fold. In *Ascobolus immersus* only the *cv2* modifier in the European strains is known to have such a profound effect on some of its closely linked target mutations (Girard and Rossignol, 1974). In summary, *ccf*-7 has a far more profound effect in modifying NG*w*18 conversion frequency than *ccfs* 5 and 6 have on their target mutations. Unfortunately, it was not possible to demonstrate whether *ccf*-7 acted on NG*w*18 in the same way that *ccfs* 5 and 6 act upon their target mutations, i.e. by reducing conversion frequencies of these mutations in crosses heterozygous for the respective *ccf*.

What of the effect of these *ccfs* on the conversion spectrum of their target mutations? BB9*w*9 showed a conversion spectrum of type C in *ccf*-5(*A*) homozygous crosses and a broadly A-type spectrum in the other 3 types of cross, according to the criteria of Leblon (1972 a,b). In fact,

the relative frequency of PMS was twice as high in *ccf*-5(*A*) homozygous crosses as it was in the other 3 types of cross. Thus there was no consistent correlation between reduction in conversion frequency and reduction in PMS. Accordingly, the mechanism by which gene conversion of BB9*w*9 is reduced by *ccf*-5 cannot intrinsically be involved with these changes in PMS. It would further seem that the involvement of cryptic heterozygosity close to BB9*w*9 is unable to explain these relative changes in PMS. Some attribute of the *ccf*-5 system or interaction of the *ccf*-5 factors with minor modifiers of conversion behaviour could be involved.

Unlike *ccf*-5 and BB9*w*9, *w*BHj showed a broadly similar C-type conversion spectrum in crosses with all 4 combinations of the *ccf*-6 factors. PMS was relatively frequent, ranging from 20.00% (in *ccf*-6(*B*) homozygous crosses) to 26.57% (in +, *ccf*-6(*B*) × *w*, *ccf*-6(*A*) crosses). Only very slight differences in conversion spectrum were discernible between the 4 types of cross. Certainly PMS was not reduced in crosses heterozygous for the 2 forms of *ccf*-6.

NG*w*18 showed a C-type conversion spectrum in both LCF and HCF crosses, but apparent PMS was more frequent in LCF crosses. It should be remembered that the true conversion spectrum of NG*w*18 could not be accurately ascertained in LCF crosses since most 6:2 and 5:3 octads were shown to be phenocopies. Thus in the 2 cross types analysed, it cannot be said whether *ccf*-7 has any effect on the conversion spectrum of NG*w*18.

Possible *cis*/*trans* position effects of the *ccf*-5 and *ccf*-6 factors on the conversion frequency, spectrum, or

both, of their closely linked target mutations were looked for. None was found in either system. It had been thought that such *cis/trans* position effects were more likely for the *ccf*-5 factors on BB9*w*9 conversion for 2 reasons: BB9*w*9 is much more closely linked to *ccf*-5 than *w*BHj is to *ccf*-6. It is difficult to conceive how more distant *ccfs* might show such *cis/trans* position effects, although this might still be possible at the separation of *w*BHj and *ccf*-6 (7 recombination units). Furthermore, *cis/trans* position effects on *w*BHj conversion spectrum would be most unlikely as this spectrum was closely similar in all the crosses analysed prior to the *cis/trans* test. This latter factor did not apply in the case of mutation BB9*w*9 where clear differences in conversion spectrum as well as frequency had been observed. Since the mode of action of *ccf*-7 on NG*w*18 could not be determined, it was not possible to construct the desired strains to perform the appropriate crosses to look for *cis/trans* position effects of the *ccf*-7 factors on NG*w*18 conversion behaviour.

Circumstantial evidence was accumulated for some co-conversion of *ccf*-5 and BB9*w*9 although it was not possible to quantify this. Indeed, co-conversion of *ccf*-5 and BB9*w*9 is quite likely to occur, owing to their close linkage. However, not all *ccfs* and very closely linked target mutations need necessarily show co-conversion. Of paramount importance is the mechanism of action of the *ccf* in question. Co-conversion of *ccf*-6 and *w*BHj would be much less likely to occur owing to their distance of 7 recombination units apart. No known sites this widely separated have shown co-conversion in any studies performed to date. Since studies on the *ccf*-7 system

did not yield enough data to characterise this system, it was not possible to determine whether *ccf*-7 and NG*w*18 can co-convert. If *ccf*-7 acts in the same manner as *ccfs* 5 and 6 (or indeed if it acts in some other ways too) then *ccf*-7 and NG*w*18 would be very closely linked (no recombinants among 102 progeny analysed) and some co-conversion would be a distinct possibility. This point must remain open until further experiments are completed by other workers.

The action of *ccf*-5 was shown to be specific to BB9*w*9, having no effect on mutations of loci I, II, III and IV and unlinked mutations *w*BHj and NG*w*14. Similarly *ccf*-6 acts specifically on mutation *w*BHj, having no effect on the conversion behaviour of mutations of loci I, II, III and IV and unlinked mutations BB9*w*9 and NG*w*14. Thus both *ccfs* 5 and 6 act specifically on closely linked mutations. These findings are most revealing and will be of great help in considering the possible mechanisms by which these *ccfs* act. It is almost certain that it is the close location of the affected mutations that is of primary importance, rather than their molecular nature. It had been hoped to investigate this further by inducing and isolating further white ascospore colour mutations of different molecular natures close to both BB9*w*9 and *w*BHj. However, this was not achieved (see Chapter 3, PART TWO). If it had been, much further useful data might have been obtained relating to the molecular actions of both *ccf*-5 and *ccf*-6. It was not possible to determine the specificity of *ccf*-7 in advance of the mode of action of this *ccf* being elucidated. Furthermore, it was not possible to determine whether 2 other locus II mutations (other than NG*w*18) responded to *ccf*-7. If this *ccf* acts like *ccfs* 5 and

6 it would seem that these 2 mutations do not respond, or their response is not apparent due to the action of a further, unknown *ccf*. This might be evidence that *ccf*-7 acts in a different manner from *ccfs* 5 and 6.

At this juncture it is worth pointing out that it is shown in Chapter 6 of PART ONE of this thesis that mutations BB9*w*9, *w*BHj and possibly NG*w*18 do not respond to the "Super" conversion control system. This system consists of 2 unlinked factors, *ccf*-3 and *ccf*-4, certain combinations of which (*ccf*-3(*E*), *ccf*-4(*r*)) can enhance conversion frequencies of locus I mutations in crosses in the absence of other combinations (i.e. *ccf*-4(*R*)). The "Super" system is discussed in Chapter 6. Suffice it to say here that Helmi and Lamb (1981) postulated that *ccf*-3(*E*) codes for a diffusible product which reaches the unlinked locus I where it increases H-DNA initiation frequency, probably through interaction with the *ccf*-2 factors adjacent to or within locus I. According to this theory, *ccf*-4(*R*) could be an alternative binding site for the *ccf*-3(*E*) product or could code for a diffusible product combining with that from *ccf*-3(*E*) so deterring it from enhancing conversion at locus I. This is mentioned here because the data discussed in Chapter 6 show that *ccfs* 5 and 6 cannot be alternative binding sites for the *ccf*-3(*E*) diffusible product. This is further evidence that *ccfs* 5 and 6 do not act in the same way as *ccf*-2, although the data already discussed make this most unlikely anyway - unlike *ccfs* 5 and 6 the *ccf*-2 factors modify the conversion frequencies of closely linked locus I mutations (except for 1 aberrant case) in an incompletely dominant way and show *cis/trans*

position effects on both the conversion frequencies and spectra of these mutations.

What of the possible origins of the alternative forms of each of *ccfs* 5, 6 and 7 which were segregating in the crosses performed in this work? Both forms of each could have been present in the original 2 wild-type isolates of the Pasadena strains as a polymorphism. Alternatively, one form could have later arisen from the other by mutation or a recombination process such as unequal crossing-over. In this work it was shown that 1 form of each *ccf* was present in one of the 2 original wild-type strains (P5-). Since the other original wild-type strain (K5+) was no longer fertile it was not possible to show that this strain carried the alternative form of each factor. However, this seems highly likely. In fact, in the case of *ccf*-5 a second generation wild-type derivative of the original 2 strains was shown to carry the alternative form of *ccf*-5, making it extremely likely that both forms of this *ccf* were present in the 2 original Pasadena strains as a polymorphism. Furthermore, Emerson and Yu-Sun (1967) showed that the 2 forms of *ccf*-1 and the *P* and *K* forms of *ccf*-2 were present as a polymorphism in the 2 original Pasadena strains. Helmi (1980) also attempted to trace the ancestry of the 2 forms each of *ccfs* 3 and 4 to the same 2 original wild-type isolates. Thus, in conclusion, it would seem that the Pasadena strains of *Ascobolus immersus* are rich in natural variants of elements which act to control gene conversion (and probably crossing-over) frequencies at a variety of sites and in a variety of

ways. It would seem almost certain that the conversion control factors studied in this work were naturally occurring and polymorphic, fulfilling a function in nature, rather than being artificially induced variants in the laboratory.

Comparison of *ccfs* 5, 6 and 7 with other known conversion control systems

Comparisons of *ccfs* 5 and 6 with other known conversion control systems will primarily be considered here, since the mode of action of *ccf*-7 is not known. 5 basic types of conversion control system have been reported in the literature:

1. Heterozygous reducers of gene conversion. Many systems of this type are known, including *ccf*-1 in the Pasadena strains of *Ascobolus immersus* (Emerson and Yu-Sun, 1967); *cvs 1, 2, 4* and *6* in the European strains of the same organism (Girard and Rossignol, 1974); cryptic mutation G234 in the *b2* spore locus also in the European strains of *Ascobolus* (Hamza et al. 1981); the *ss* recombination modulator in *Neurospora crassa* (Catcheside, 1981); the system which controls gene conversion in the *rosy* locus of *Drosophila melanogaster* (Chovnick et al. 1971); and the system whereby intragenic recombination is reduced in the *adh1* locus of *Zea mays* (Freeling, 1978). Since *ccfs* 5 and 6 both act to reduce the recombination frequency of closely linked mutations when heterozygous in a cross, it is with these systems that they will be compared. The other 4 classes of conversion control systems reported in the literature will only be briefly mentioned here.

2. Dominant enhancers of gene conversion. Many systems of this type are also known in fungi: the *cog* genes in *Neurospora crassa* of which only 1 has been positively identified (Catcheside, 1977); the YS17 mutation in the *buff* gene of *Sordaria brevicollis* (MacDonald and Whitehouse, 1979); the *rec* genes in *Coprinus cinereus* (Katy and Moore, 1980); and *ccf*-3 in the Pasadena strains of *Ascobolus immersus* (Helmi and Lamb, 1981). Since *ccfs* 5 and 6 do not resemble any of these systems they will not be considered further.

3. Dominant reducers of gene conversion. Systems of this type have been reported, including the *rec* genes in *Neurospora crassa* (Catcheside, 1977); the recombination control of the mating type loci in *Schizophyllum commune* (work of Stamberg and collaborators, summarized by Fincham, Day and Radford, 1979); the *recR* gene in *Coprinus cinereus* (which can also act as a recombination enhancer in other parts of the genome (Katy and Moore, 1980)); and *ccf*-4 in the Pasadena strains of *Ascobolus immersus* (Helmi and Lamb, 1981). *ccfs* 5 and 6 do not act in the same way as any of these systems so they will not be discussed further.

4. Heterozygous enhancers of gene conversion. There is only 1 known example of recombination enhancement in crosses heterozygous for a controlling genetic factor and this involves crosses between *ad-3A* and *ad-3B* in *Neurospora crassa* (De Serres, 1971). *ccfs* 5 and 6 modify the conversion behaviour of closely linked mutations in exactly the opposite way to this, so this system will not be considered any further.

5. Incompletely dominant enhancers or reducers of gene conversion. The 3 forms of *ccf*-2 show incomplete dominance in their control of locus I conversion frequencies in the Pasadena strains of *Ascobolus immersus* (Lamb and Helmi, 1978); the M26 mutation in the *ade6* gene of *Schizosaccharomyces pombe* increases gene conversion frequencies in this gene in an additive rather than a dominant fashion (Gutz, 1971b; Goldman and Smallets, 1979). The latter example is not true incomplete dominance, but the situation is similar. These 2 systems bear no relation to *ccfs* 5 and 6 in their mode of action and so will not be discussed further.

From the above it can be seen that among a whole range of different types of conversion control systems reported in the literature, there are several examples of systems which control the conversion behaviour of target mutations in the same way as *ccfs* 5 and 6, i.e. by reducing the conversion frequencies of these mutations when heterozygous in a cross. *ccfs* 5 and 6 will be compared with each of these systems in turn, and comparisons with *ccf*-7 will be made where valid.

The *ccf*-1 system in the Pasadena strains of *Ascobolus immersus*

This system was described by Emerson and Yu-Sun (1967) and shows strong resemblances to *ccfs* 5 and 6. Like the latter 2 *ccfs*, *ccf*-1 reduces the conversion frequency of a closely linked white ascospore colour mutation *w*62 when heterozygous in a cross. This close linkage was demonstrated by the fact that no recombinants (for *ccf*-1 and *w*62) were observed

among 31 progeny analysed. This suggests that *ccf*-1 and *w*62 are more closely linked than are *ccf*-6 and *w*BHj, although this cannot be stated with certainty. The data are insufficient to determine whether *ccf*-1 and *w*62 are more or less closely linked than *ccf*-5 and BB9*w*9.

It may be remembered that in crosses homozygous for either form of *ccf*-5 or *ccf*-6 the conversion frequencies of the linked target mutations were slightly, but significantly different (9.24 and 11.08% for BB9*w*9 and 12.28 and 13.38% for *w*BHj). The difference in *w*62 conversion frequency in crosses homozygous for either form of *ccf*-1 was much greater than this - the mean conversion frequencies based on the data of Emerson and Yu-Sun (1967) were *c*.6.3% and *c*. 11%. These differences in *w*62 conversion frequency could be indicative of molecular differences in the 2 forms of *ccf*-1. This will be considered when the possible molecular natures and modes of action of *ccfs* 5 and 6 are discussed.

The conversion frequencies in crosses heterozygous for *ccf*-1 are *c*. 2.0% and *c*. 2.6% depending on which *ccf*-1 factor is in *cis* to *w*62. Thus the difference in *w*62 conversion frequency in *ccf*-1 homozygous and heterozygous crosses ranges from *c*. 2.5 to 5.5-fold, depending upon which particular homozygous or heterozygous crosses are considered. This reduction of gene conversion in heterozygous crosses is of the same order, or rather less than that observed for *ccf*-5 and BB9*w*9, but greater than that observed for *ccf*-6 and *w*BHj. As already mentioned, this might be a consequence of the relative degrees of linkage of the *ccfs* and their target

mutations, or the nature of the *ccfs* themselves might be involved. These points will be considered when the possible mechanisms of *ccf*-5 and *ccf*-6 action are discussed. It is interesting to note that when the data for one type of cross heterozygous for the *ccf*-1 factors are compared with one of the homozygous crosses, the frequency of PMS octads seem preferentially reduced - by approximately 19-fold compared to the overall reduction in conversion frequency by *c.* 5.5-fold. This preferential reduction in PMS frequency applies far less strongly if at all when the other *ccf*-1 homozygous cross is considered. This situation is roughly similar to that appertaining to *ccf*-5 and BB9*w*9, but different from the action of *ccf*-6 and *w*BHj.

Differences occur between *ccf*-1 on one hand and *ccfs* 5 and 6 on the other in that the *ccf*-1 factors seem to show *cis/trans* position effects on *w*62 conversion behaviour, in terms of both conversion frequency and spectrum, although these effects are not great. The *ccf*-5 and *ccf*-6 factors do not show *cis/trans* position effects on the conversion behaviour of their closely linked target mutations. Unfortunately the data of Emerson and Yu-Sun (1967) were insufficient to reveal whether co-conversion of *ccf*-1 and *w*62 can occur. The action of *ccf*-1 is apparently specific to closely linked *w*62.

In summary, the general action of *ccf*-1 on *w*62 conversion behaviour is closely similar to the actions of *ccf*-5 and *ccf*-6 on BB9*w*9 and *w*BHj respectively, but some differences in detail do occur.

The *cv* conversion modifiers in the European strains of *Ascobolus immersus*.

These systems, originally reported by Rizet et al.(1969), were fully described by Girard and Rossignol (1974). Altogether 4 conversion modifiers are known (*cvs 1, 2, 4* and *6*) each closely linked to a spore colour locus (*b1, b2, b4* and *b6* respectively). Like *ccfs* 5 and 6, each *cv* modifier reduces the conversion frequencies of mutations in the respective closely linked locus when the modifier is heterozygous in a cross. *cv1* and *b1* on the one hand and *cv2* and *b2* on the other, would seem to be very closely linked (no recombinants in 38 progeny and no recombinants in 92 progeny respectively) - certainly more closely linked than *ccf*-6 and *w*BHj (R.F. ≃ 7%). It is conceivable that *cv2* and *b2* may be more closely linked than *ccf*-5 and BB9*w*9 but in the absence of much larger sample sizes of progeny being analysed for both systems this must remain speculative. Direct tests for linkage were not performed for *cv4* and *b4* and *cv6* and *b6*. However, close linkage is inferred because the same genetic distance is observed between genes *b4* and *b6* and between *cv4* and *cv6*. These data are of course insufficient to compare the inferred close linkage of *cv4* and *b4* and *cv6* and *b6* with the clearly demonstrated linkage relations of *ccf*-5 and BB9*w*9 and especially *ccf*-6 and *w*BHj.

If data involving the *cv1* modifier are considered first, Girard and Rossignol (1974) crossed 10 *b1* mutants to wild-type strains carrying either form of *cv1*. The reduction in conversion frequency in crosses heterozygous for *cv1* compared to crosses homozygous for 1 form of *cv1* ranged from 2 to 120-

fold, according to the individual mutation. This range of heterozygous reduction of conversion frequencies encompasses the roughly 2-fold reduction in *w*BHj conversion frequency brought about by *ccf*-6, the approximately 5-fold reduction in BB9*w*9 conversion frequency due to heterozygosity of *ccf*-5 in a cross and the apparent 23-fold reduction in NG*w*18 conversion frequency brought about by *ccf*-7 (remembering that the reduction in true conversion frequency is far greater than this). The great variation in these results illustrates the importance of inducing and isolating further white ascospore colour mutations close to both *ccf*-5 and *ccf*-6 before really definite conclusions about the magnitude of the effect of these factors on the gene conversion behaviour of closely linked mutations can be drawn. The molecular nature of the mutation and more especially its distance from the *ccf* could play some rôle.

Girard and Rossignol (1974) also crossed 21 *b2* mutants to wild-type strains carrying either form of *cv2*. The reduction in conversion frequency in crosses heterozygous for *cv2* compared with crosses homozygous for 1 form of *cv2* ranged from around 3 to 64-fold, according to the individual mutation. For 14 of these mutations the difference in conversion frequency was over 15-fold. Indeed, in some crosses, the "suppression" of gene conversion was almost total. Considering the data overall, it seems that heterozygosity of the *cv2* factors has a very strong effect on reducing the conversion frequencies of closely linked mutations. When false cluster frequencies are taken into account, the true conversion reduction for some mutations

may be of the order of several hundred-fold or even more. Thus *cv2* seems to have a much more drastic effect on the conversion behaviour of closely linked mutations than do *ccfs* 5 and 6, although as stated above, it is most desirable to induce and isolate further mutations close to these *ccfs*. It should also be remembered that the effect of *ccf*-7 (whatever its mode of action) on NG*w*18 conversion may be as great as the effect of *cv2* on certain closely linked mutations.

There are far less data relating to *cv4* and *cv6*. It has been conclusively shown that when *cv4* is heterozygous in a cross, the conversion frequency of closely linked mutations is reduced, but much less drastically so compared with the action of *cv2*. Its behaviour is more akin to *ccf*-5 in this respect. Although not conclusively demonstrated, it appears that *cv6* acts in the same way as *cvs 1*, *2*, and *4*.

Unfortunately Girard and Rossignol (1974) did not perform complete cycles of crosses with all 4 possible combinations of any of the *cv* modifiers for any single mutation. At best, all that exists for any one mutation are 2 crosses - one homozygous for 1 form of a particular *cv* and the other heterozygous for the *cv*. Thus, from their data it is impossible to determine if crosses homozygous for either form of the *cv* would give roughly similar or radically different conversion frequencies. It will be remembered that the former situation applied to *ccfs* 5 and 6, while for *ccf*-1 the 2 conversion frequencies are markedly different. Furthermore, it is not possible to determine whether any of

the *cv* modifiers show *cis/trans* position effects on the conversion behaviour of closely linked mutations. Similarly, this lack of complete data makes it more difficult to determine whether the *cv* modifiers have any effect on the conversion spectrum of a mutation as well as its frequency. In any case this is difficult to determine since the actions of *cv1* and *cv2* at least, on many mutations with widely different conversion spectra were investigated. Also, in many LCF crosses, many apparent conversion octads were shown to be false clusters. All that can be said is that for some *b2* mutations at least, PMS octads were absent when *cv2* was heterozygous, although quite common in crosses homozygous for 1 form of *cv2*. It is also not possible to determine whether any of the *cv* modifiers shows any co-conversion with any closely linked mutations from the data available. However, good data are available which show that each *cv* modifier acts specifically on its closely linked spore colour locus. Also, when heterozygous, *cv2* reduces intragenic crossing-over, while *cv4* seems to decrease the recombination frequency between *b4* and *b6* when *cv4* is heterozygous in a cross.

These *cv* modifiers have been discussed in considerable detail, because as far as comparable data are available, they are very similar to the *ccf*-5 and *ccf*-6 factors described in this work and the *ccf*-1 factor described by Emerson and Yu-Sun (1967). The main differences between these systems seem primarily the degree of conversion reduction of closely linked mutations in crosses heterozygous for the controlling factor in question. It is highly probable that all of these systems

act in the same way but before discussing this most important point, other known systems which also reduce conversion or crossing-over frequencies in heterozygous crosses must be considered and compared with *ccfs* 5 and 6.

The *ss* recombination modulator in *Neurospora crassa*

This system was described by Catcheside (1981). Unlike the previous systems discussed the *ss* recombination modulator has 3 alleles, ss^E, ss^S and ss^C, each associated with a different wild-type strain. When heterozygous for any of these alleles, *ss* reduces intragenic recombination 2 to 20-fold within the closely linked *nit-2* locus. This recombination reduction depends upon the *nit-2* mutations in question and the *ss* alleles which are heterozygous. These data are therefore concerned with recombination frequencies rather than conversion frequencies, but since most intragenic recombination probably occurs by gene conversion it is quite appropriate to compare this system with *ccfs* 5 and 6. Of course, for many aspects of the systems, directly comparable data will not be available. Nevertheless, the degree of conversion reduction of mutation BB9*w*9 by *ccf*-5 is encompassed within the range of values of intragenic recombination frequency reduction by *ss*. The action of *ccf*-6 heterozygosity on *w*BHj conversion would be at the extreme lower end of the recombination reduction range exhibited by the *ss* modulator. However, it should be remembered that the recombination frequency between 2 alleles of the same gene is a compound of the conversion frequencies of both alleles

plus any reciprocal recombination that may be occurring (in this work only the frequency of prototrophs arising from crosses between different auxotrophic mutants were scored).

It is extremely interesting to note that crosses homozygous for different *ss* alleles did not give the same recombination frequencies between any pair of *nit-2* alleles. This situation is analogous to the slight, but significant differences in BB9*w*9 and *w*BHj conversion frequencies in crosses homozygous for either form of *ccf*-5 and *ccf*-6 respectively. Similar, but more pronounced, differences in *w*62 conversion frequency were observed in crosses homozygous for either form of *ccf*-1 (Emerson and Yu-Sun, 1967). These striking similarities are strongly indicative of a common mechanism of action for all of these conversion/recombination modifiers. On this basis it would seem highly likely that if complete data existed, the same phenomenon might be observed for the *cv* conversion modifiers in *Ascobolus immersus* (Girard and Rossignol, 1974).

As already noted, the *ss* modulator is closely linked to the *nit-2* locus. In fact, according to the data of Catcheside (1981), *ss* may even lie within *nit*-2. Since the data on which these conclusions were based are not directly comparable to the data on linkage relations of *ccf*-5 and BB9*w*9 and *ccf*-6 and *w*BHj, it is not possible to deduce the relative degrees of linkage of these sites.

It was not possible to determine the effects of *ccf*-5 and *ccf*-6 on crossing-over in intervals adjacent to their target mutations because no suitable flanking mutations could be isolated. It would be extremely interesting to

determine this in future studies, since unlike *ss* the otherwise highly similar *cv4* in the European strains of *Ascobolus immersus* seems to affect intergenic recombination in the *b4-b6* interval (Girard and Rossignol, 1974). However, the flanking intervals considered for both systems are very different. Furthermore, *ss* interacts multiplicatively with *rec-1*: together these genes modulate recombination within the *nit-2* locus over a range exceeding 100-fold. As yet, no such interactions of either *ccf*-5 or *ccf*-6 with any other factors have been observed, but these cannot be ruled out. Perhaps all strains used in experiments involving *ccfs* 5 and 6 have been isogenic for such interacting factors. Without the segregation of different alleles for such factors they could not be detected. No interactions with the *ccf*-3 and *ccf*-4 "Super" factors were observed in the experiments described in Chapter 6 of this thesis.

In summary, despite differences in the nature of the data it seems clear that the mode of action of the *ss* recombination modulator is very similar to the actions not only of *ccfs* 5 and 6 but also *ccf*-1 in the Pasadena strains and the *cv* modifiers in the European strains of *Ascobolus immersus*. It is highly probable that all of these systems act in the same way.

The control of intragenic recombination in the *adh1* locus of *Zea mays*

This system was described by Freeling (1978). Again these data are concerned with intragenic recombination frequencies rather than conversion frequencies but strong parallels of this system with *ccfs* 5 and 6 may still be drawn. Freeling

(1978) examined the recombinational behaviour of 22 *adh1* mutants induced within 5 naturally occurring *alcohol dehydrogenase-1* alleles. In this discussion only the results involving *adh1-F* and *adh1-S*-derived mutants are of interest. These 16 mutants were derived in such a way that all had equivalently randomized genetic backgrounds, with the exception of that portion of the genome linked to *adh1*. Several of these mutants were put together in the heterologous combination *adh1-S mutant/adh1-F mutant* with the result of complete recombination restriction. Furthermore, even *adh1-F*-derived mutants which were capable of recombining among themselves failed to generate adh^+ gametophytes when put with *adh1-S* mutants. These results indicate drastic reduction or even total elimination of intragenic recombination in crosses heterozygous for regions closely linked to *adh1*. Thus the factor(s) responsible for this reduction of *adh1* intragenic recombination frequencies when heterozygous would appear broadly similar to *ccfs* 5 and 6 and indeed to other systems already described.

Freeling (1978) also observed that the *adh1-S* and *adh1-F* cistrons displayed significantly different mean frequencies of intragenic recombination. This could well be due to homozygosity for either form of the apparently closely linked recombination modifier producing different levels of intragenic recombination. If such an interpretation is correct, this would parallel the situation already described for *ss* and *nit-2* intragenic recombination in *Neurospora crassa* (Catcheside, 1981) and be analogous to the conversion

frequency differences of mutations *w*62, BB9*w*9 and *w*BHj in crosses homozygous for either form of *ccfs* 1, 5 and 6 respectively.

In summary, the *adh1* intragenic recombination control system seems very similar to the *ccf*-5 and *ccf*-6 systems and the other systems compared with them. Again, it would seem most probable that all these systems operate by a common mechanism. The reporting of such a system in a higher eukaryote (*Zea mays*) is of great interest since it is a further indication that much evidence relating to recombination obtained from fungi may also be applicable to higher eukaryotes in general.

The control of gene conversion and crossing-over in the *rosy* gene of *Drosophila melanogaster*

Gene conversion and crossing-over in the *rosy* gene of *Drosophila melanogaster* were investigated by Chovnick et al. (1971), utilizing half-tetrad analysis. Strong genetic and biochemical evidence indicates that this gene is the structural gene for the enzyme xanthine dehydrogenase. The gene conversion and crossing-over data obtained by Chovnick et al. (1971) relate to a large group of mutants which are enzymatically inactive and exhibit a brownish mutant eye colour phenotype resulting from a reduction in the red (drosopterin) pigments. The progeny of *rosy* mutant heterozygous females were grown on a medium which selected for survival only those offspring receiving a ry^+ meiotic product. Analysis of these ry^+ survivors allowed their classification as arising from either classical crossovers or conversion events.

The data of relevance to this discussion are those which show that the effect of a heterozygous short deletion in a region adjacent to the *rosy* gene (in the Karmoisin gene) is to reduce both crossovers and conversions within that gene. These data will not be discussed in detail as they are not directly comparable to the data concerning *ccfs* 5 and 6 - the deletion is lethal when homozygous and so it is only possible to compare conversion and crossover data from meioses where this deletion is absent or heterozygous. When heterozygous, this deletion seems to reduce conversion frequencies in a polarized manner - the closer the sites to the deletion, the more conversion is reduced. This is unlike the behaviour of the *cv* modifiers in *Ascobolus* (Girard and Rossignol, 1974) but no comparable data exist for *ccfs* 5 and 6. However, the degree of conversion reduction when the deletion is heterozygous is of the same order as that due to heterozygosity of *ccfs* 5 or 6 in a cross, being much less than that due to the action of at least *cvs 1* and *2*.

In summary, this system is similar to *ccfs* 5 and 6 in that heterozygosity of a region close to the *rosy* cistron acts to reduce gene conversion (and crossing-over) when heterozygous. Again, the reporting of such a system - apparently similar to those seen in fungi - in a higher eukaryote lends further support to the hypothesis that much evidence relating to recombination obtained from fungi may also be applicable to higher eukaryotes in general.

Cryptic mutation G234 in the *b2* locus of the European strains of *Ascobolus immersus*

This system was described by Hamza et al.(1981) and seems to differ from *ccfs* 5 and 6 and other systems described above in several important aspects. G234 is a cryptic mutation located within spore colour locus *b2* of the European strains of *Ascobolus immersus*. When heterozygous, G234 decreases the frequency of aberrant segregations of the mutations located to its right, towards the low conversion end of the locus. However, G234 does not change the aberrant segregation frequency of the mutations located to its left, towards the high conversion end of the locus. Most importantly, unlike *ccfs* 5 and 6 (and *ccf*-1 and the *cv* modifiers), this polar reduction in conversion frequencies is almost entirely accomplished by virtually eliminating ab4:4 asci for mutations showing PMS, while it reduces the disparity betwen 6:2 and 2:6 asci with an overall reduction in gene conversion for mutations giving only MS conversion octads. This G234 heterozygous modification of conversion frequency is quite different from the effects of *ccf*-5 and *ccf*-6 on their closely linked target mutations. The frequency of ab4:4 asci was not determined for these mutations, but if these control systems acted primarily by reducing the frequency of such asci, little overall effect on the detected conversion classes would be seen (PMS is fairly frequent for both BB9*ω*9 and *ω*BHj mutations, at least in HCF crosses). Also, *ccfs* 5 and 6 did not have any effect on the 6:2/2:6 disparity among crosses with all possible combinations of the *ccf* factors.

A further difference is that, when homozygous, G234 has little or no effect on the aberrant segregation patterns of

any *b2* mutation, compared to $+^{G234}$ homozygous crosses. In other systems examined, different conversion or intragenic recombination frequencies were recorded for closely linked mutations in crosses homozygous for either form of the respective control factor, although this effect was only slight for *ccfs* 5 and 6 (N.B. data were not available to determine this for the *cv* modifiers or for the *Drosophila rosy* gene system). G234 does show similarity with *ccfs*5 and 6 in that it has no effect on the conversion behaviour of mutations lying elsewhere in the genome.

It is interesting to note that Lamb (1975) predicted that a segregating cryptic mutation lying between a known marker and the end of the polaron from which H-DNA formation starts might reduce or prevent conversion of the known marker. This prediction was based on an explanation of polarity being caused by mispairs interfering with further H-DNA formation. The G234 system is the first known example in eukaryotes of cryptic heterozygosity reducing the conversion frequencies of known mutations in such a manner.

Overall, it would seem that G234 shows little similarity with *ccfs* 5 and 6 and the other systems discussed, apart from the fact that its action in reducing or otherwise modifying conversion frequencies is limited to closely linked mutations (and then only in a polar way) and occurs when heterozygous in a cross. It is likely that the action of G234 occurs via a completely different mechanism to those of *ccfs* 5 and 6 and the other systems described. These possible mechanisms will now be considered.

Possible mechanisms of *ccf*-5 and *ccf*-6 conversion control

First, the possible nature and mechanisms of conversion control by *ccfs* 5 and 6 will be discussed in general terms and then possible explanations of the differences in detail between these systems will be considered. Since it was not possible to fully characterize *ccf*-7 it would be fruitless to speculate upon its molecular nature and mode of action, although some attributes of this system will be considered where appropriate.

A simple explanation of the reduction of BB9*w*9 and *w*BHj conversion frequencies in crosses heterozygous for closely linked *ccfs* could be that the 2 forms of each *ccf* consist of structural rearrangements of this region of the chromosome in question. Such structural differences could arise from translocations or inversions in which at least one breakpoint is close to the spore colour mutation in question. Heterozygosity for such rearrangements might reduce gene conversion (and reciprocal recombination) by interfering with correct chromatid pairing. However, this explanation seems most unlikely since abnormally high frequencies of ascospore abortion are expected in crosses heterozygous for a chromosomal rearrangement. Very few aborted ascospores were observed in crosses heterozygous for either *ccf*-5 or *ccf*-6.

A second possible explanation for the reduced frequency of convertants in crosses heterozygous for a closely linked *ccf* is that these *ccfs* are genes which determine a step in the recombination process occurring in their vicinity. According

to Meselson and Radding (1975) this recombination process proceeds as follows: following synapsis of homologous non-sister chromatids, an endonuclease catalyses a cut in one strand of one DNA duplex at a specific site; single-strand displacement by DNA polymerase occurs, resulting in D-loop formation and the generation of a single-strand bridge, so forming an asymmetric heteroduplex; this structure isomerizes to generate a two-strand bridge (Holliday structure) and a symmetric heteroduplex. Failure to repair mismatched bases (at the white mutant sites in this case) would result in PMS, while repair can either result in "restoration" to the original allele or MS gene conversion. This process is completed by scission of the cross-strand structure.

Since heterozygosity for each *ccf* is associated with a reduction in conversion frequency, the possible rôles that each *ccf* might play in the recombination process are limited. The most straightforward explanation is to propose that *ccfs* 5 and 6 determine the pairing closeness of homologous DNA duplexes in their vicinity. The degree of this pairing closeness could determine the frequency of formation of intimately paired duplexes, the substrate upon which enzymes promoting H-DNA can act. Inclusion in a length of H-DNA is a pre-condition of the "opportunity to convert" for any mutation. According to this explanation, the *ccfs* would consist of DNA sequences rather than individual base pairs. Heterozygosity of these sequences would reduce the pairing closeness of the DNA duplexes in their vicinity and so could interfere with the binding of a DNA

pairing protein (such as DNA synaptase reported in *E.coli* by Potter and Dressler (1980)), so reducing the frequency of formation of intimately paired DNA duplexes and hence H-DNA formation. It would seem that such DNA pairing proteins would act non-specifically preferentially on regions of closely paired DNA duplexes. The existence of region-specific DNA pairing proteins would be most unlikely. Not only would a specific protein be required for each *ccf*, but since it is proposed that intimate pairing is frequent in crosses homozygous for either form of the *ccf*, similar polymorphic variants of the DNA pairing proteins would also be required. Such a situation would create great evolutionary problems. In summary, on this model initial pairing would be poor in crosses heterozygous for the *ccf*, leading to a reduced frequency of intimately paired DNA duplexes and hence H-DNA formation, with the conversion frequencies of closely linked (but not unlinked) mutations concomitantly low. In crosses homozygous for either form of the *ccf* initial pairing would be expected to be more effective leading to more frequent intimate pairing of DNA duplexes and hence H-DNA formation, with the resulting conversion frequencies higher. This model of *ccf*-5 and *ccf*-6 action neatly fits the data presented in this thesis and is basically the explanation favoured by Girard and Rossignol (1974), Catcheside (1981) and Chovnick et al.(1971) to explain the action of the *cv* modifiers, the *ss* recombination modulator and the conversion control of the *rosy* gene respectively - all put forward explanations of the mode of action of these systems based on the respective

factors interfering with close DNA duplex pairing when heterozygous.

Alternatively, the *ccfs* could be directly involved in a step in H-DNA formation itself, such as acting as recognition sites for an endonuclease. However, this seems unlikely, since it is difficult to see how such an endonuclease, initiating the primary single-strand break in one duplex (on the Meselson-Radding (1975) model), and acting preferentially on identical sequences (e.g. *ccf*-5(*A*) - *ccf*-5(*A*)), could be aware of what is on the other chromatid at the corresponding position.

Might it be possible for *ccfs*5 and 6 to influence an event later in the conversion process instead of, or as well as, H-DNA formation? Asymmetry in the paired duplexes due to heterozygosity for the *ccf* factors could interfere with D-loop formation or with migration of the single- or double-strand cross-connections. A need for close symmetry in any of the other steps of recombination is not apparent. Is there any evidence that *ccfs* 5 and 6 might act in one of these alternative ways? There is no positive evidence for this and some contrary evidence can be obtained by considering the data relating to cryptic mutation G234 in the *b2* locus of *Ascobolus immersus* and comparing this system with *ccfs* 5 and 6.

Hamza et al.(1981) do indeed propose that mutation G234 acts to reduce gene conversion frequencies of mutations to its right by specifically blocking symmetric H-DNA propagation without preventing the formation of asymmetric H-DNA

when heterozygous in a cross. This model fits their data well. Savage and Hastings (1981) also explain some marker effects in the *his1* locus of *Saccharomyces cerevisiae* by proposing that heterozygosity of certain sites interferes with the migration of cross-strand exchanges. However, as already pointed out, the *ccf*-5 and *ccf*-6 factors seem to bear little resemblance to G234: when heterozygous in a cross, G234 reduces the conversion frequency of mutations to its right primarily by reducing the frequency of ab4:4 octads for those mutations showing frequent PMS and by reducing the 6:2/2:6 disparity with an overall reduction in conversion frequency for those mutations showing infrequent PMS. This is quite unlike the behaviour of *ccfs* 5 and 6. Furthermore, G234 and $+^{G234}$ homozygous crosses show very similar conversion frequencies for the mutations examined. This is certainly unlike the behaviour of *ccf*-1 and the *ss* modulator. *ccfs* 5 and 6 also show slight, but significantly different conversion frequencies of their target mutations in crosses homozygous for either form of the respective *ccf*, although these differences are much less pronounced than for the *ccf*-1 and *ss* systems. This finding that homozygosity for different *ccf* alleles is not associated with the same conversion frequency is not a prediction of the hypothesis that *ccf* heterozygosity interferes with D-loop formation or single- or double-strand branch migration. Conversely, if *ccfs* 5 and 6 act to determine the efficiency of local pairing, there is no compelling reason why different forms of each *ccf* should

be equally effective in establishing pairing. In fact, for genes whose action is to modulate the recombination process, it would be quite reasonable to expect different conversion and crossover frequencies to be associated with specific alleles. In this context, it is probably significant that where suitable data exist, this phenomenon is observed to some degree for all the heterozygous reducers of nearby conversion and recombination frequencies in the literature - with the exception of G234.

These comparisons would seem to point to a similar molecular nature and mode of action for most of the heterozygous modulators, excepting G234. The latter mutation is believed to correspond to a small deletion or insertion (despite its cryptic nature) according to Hamza et al.(1981). The *ccf*-5 and *ccf*-6 alleles could correspond to non-identical base sequences. Alternatively, one allele could contain a larger deletion of a base sequence present in the other allele. For example, one allele could consist of more repeats of the same sequence than the other allele of the same *ccf*. A third, and perhaps less likely possibility would involve one allele of the *ccf* containing a transposable element while this would be lacking in the other allele. Transposable elements have not been reported in *Ascobolus*, but they are known in yeast (Cameron, Loh and Davis, 1979).

Thus, in summary, it seems most likely that *ccfs* 5 and 6 correspond to DNA sequences non-identical in some way between the two forms of each factor, which determine the pairing closeness of DNA duplexes in their vicinity. This

close pairing is necessary for the initiation of recombination events from as yet undetected specific sites. Non-specific interference with a step in recombination subsequent to synapsis cannot be ruled out, but on the available data seems improbable.

If *ccfs* 5 and 6 do correspond to genes which control the degree of intimate pairing in their vicinity, can the slight differences between both systems be explained on this basis? The main difference between the systems is that when heterozygous in a cross, *ccf*-5 causes a much greater reduction in the conversion frequency of a closely linked mutation than does *ccf*-6. This could be explained in 2 ways - *ccf*-5 and BB9*w*9 are very closely linked (maybe even co-converting to some extent) while *ccf*-6 and *w*BHj lie at a distance of approximately 7 recombination units. It would seem almost certain that, beyond a certain threshold value, the further a site is from a gene determining intimate pairing, the less the effect of this gene will be. Thus, on this theory, *ccf*-6 would have less effect on *w*BHj conversion frequency than would *ccf*-5 on BB9*w*9, giving less difference in conversion frequencies between crosses homozygous and heterozygous for the *ccf*. Here it is worth noting that if *ccf*-7 acts in the same way as *ccfs* 5 and 6, the data point to it being very closely linked to mutation NG*w*18 and *ccf*-7 does indeed have a very drastic effect on NG*w*18 conversion frequencies. Alternatively, it is probable that the greater the DNA sequence difference between the 2 forms of any *ccf* (i.e. the more heterozygosity

there is), the greater will be the associated conversion reduction when heterozygous in a cross. On this theory there would be more sequence differences between the *ccf*-5 alleles than between the *ccf*-6 alleles. This might be a less likely source of the difference between the degree of action of *ccfs* 5 and 6, but the 2 hypotheses are not mutually exclusive. A further source of difference could involve the molecular nature of the mutations in question, rather than the *ccfs* themselves. In the data of Girard and Rossignol (1974) particularly, different mutations in the same locus could show very different conversion frequencies and also very different degrees of conversion reduction when the corresponding *cv* modifier was heterozygous in a cross. It is quite probable that all 3 factors contribute towards the differences in the degree of action, not only of *ccfs* 5 and 6, but also *ccf*-1 in the Pasadena strains of *Ascobolus immersus* and the other heterozygous recombination modulators considered in this discussion (again excepting G234 in the European strains of *Ascobolus*).

Finally, there is considerable evidence from this and other studies that sites controlling effective pairing in their vicinity seem to be very common, being found not only in fungi, but also in higher eukaryotes. Such genes could also play a role in somatic cells since Jack and Judd (1979) point out that in diploid organisms the pairing of genes may be important for gene regulation. Furthermore, naturally occurring polymorphic variants of these sites exist, making

their detection possible. It is remarkable that, to date, 3 such polymorphic sites (*ccfs* 1, 5 and 6) have been discovered in the Pasadena strains of *Ascobolus immersus*, all these strains having been derived from only 2 wild-type isolates. 3 other polymorphic *ccfs* are also known in the same strains of *Ascobolus* (*ccfs* 2, 3 and 4). Thus there is much polymorphism with respect to the local controls of recombination. This variation is either a result of natural selection bringing about a population balance, or is selectively neutral and of little or no account to the organism. Whatever the case, the study of such genes is highly rewarding and illuminating of some of the mechanisms whereby local controls of recombination may operate.

Chapter 6: The effect of the "Super" conversion control system on mutations BB9*w*8, BB9*w*9, BB*w*11, BB*w*21, *w*BHj, NG*w*18 and *w*62

Introduction

The "Super" conversion control system, first reported by Helmi and Lamb (1979) has been shown to consist of two unlinked, interacting components, *ccf*-3 and *ccf*-4, and apparently approximately doubles whatever gene conversion frequency at locus I is determined by the *ccf*-2 factors. *ccf*-3 is unlinked to locus I, while *ccf*-4 is loosely linked to it. Of the two conversion enhancing "alleles" of the "Super" combination — *ccf*-3(*E*) and *ccf*-4(*r*) — *ccf*-3(*E*) is dominant while *ccf*-4(*r*) is recessive, with *ccf*-4(*R*) completely epistatic to *ccf*-3 and *ccf*-3(*e*) recessively epistatic to *ccf*-4 (Helmi and Lamb, 1981). These *ccf* "alleles" were designated according to their effects on conversion frequecies where *E* implies a dominant enhancer; *e* a recessive reducer; *R* a dominant reducer; and *r* a recessive enhancer. From data originating from large numbers of different crosses and from pedigree studies, Helmi and Lamb (1981) postulated that *ccf*-3(*E*) codes for a diffusible product which reaches the unlinked locus I where it increases H-DNA initiation frequency, probably through interaction with the *ccf*-2 factors adjacent to or within locus I. According to this theory *ccf*-4(*R*) could be an alternative binding site for the *ccf*-3(*E*) product or could code for a diffusible product combining with that from *ccf*-3(*E*) so deterring it from enhancing conversion at locus I.

Helmi (1980) carried out experiments hoping to determine the specificity of the "Super" factors: were these factors able to influence the conversion frequencies of mutations unlinked to locus I, or was their action confined to locus I? He crossed "Super" and "non-Super" derived wild-type strains to 6 strains carrying mutations of loci II (S*w*26), III (B*w*4.2 and C*w*1.14) and IV (NG*w*11) and mutations *w*BHj and NG*w*14 (the latter 2 mutations being unlinked to loci I, II, III and IV). The results obtained showed no clear evidence that "Super" is definitely able to enhance the conversion of mutations unlinked to locus I. There was a possible indication that the "Super" factors enhanced conversion of mutation NG*w*11, although some other *ccf* in the "Super" strain might have been responsible for this. Conversely, Helmi's (1980) results could not completely prove that the "Super" system is unable to enhance the conversion frequency of mutations other than those of locus I for two main reasons: i) the enhancing "allele" of *ccf*-3 — *E* — enhances gene conversion only in the absence of the reducing "allele" of *ccf*-4 — *R*. Possibly some of the mutant strains tested carried *ccf*-4(*R*) and hence any enhancing effect of the "Super" system would not be detectable; ii) only 6 different mutations were tested and only one cross was performed for each. The effect of "Super" on a far greater number of mutations would need to be determined before it could be concluded that the system was completely specific to locus I.

In this work the aim was to determine whether the "Super" system had any effect on the gene conversion behaviour of the mutations for which further genetic conversion control syst-

ems had been demonstrated. This would enable a fuller understanding of the genetic controls of gene conversion at these sites to be obtained and also provide more information on the specificity of the "Super" system. The mutations involved in this study were BB9*w*9 (and BB9*w*8, BB*w*11 and BB*w*21, which may be re-isolations of BB9*w*9), *w*BHj and NG*w*18. The effect of "Super" on *w*62 was also sought since the conversion behaviour of this mutation is controlled by a system (*ccf*-1) which acts in a similar manner to the systems shown to control the conversion behaviour of mutations BB9*w*9 (*ccf*-5) and *w*BHj (*ccf*-6). It might be expected that these 3 mutations would respond (or show a lack of response) to "Super" in the same manner.

Preliminary experiments

Initially "Super" derived wild-type strains were crossed to strains mutant for BB9*w*8, BB9*w*9, BB*w*11, BB*w*21, *w*BHj, NG*w*18 and *w*62 to determine whether any of these mutations showed an immediately detectable response to "Super", which they might do if not carrying *ccf*-4(*R*). In the case of mutations BB9*w*9 and *w*BHj, "Super" derived wild-type strains were crossed to pairs of mutant strains carrying either form of the *ccf* controlling the conversion of each mutation. This was unfortunately not possible for the other mutations since such strains were not available. The conversion data obtained from these crosses are shown in Table 41.

TABLE 41: Conversion data from crosses involving BB9*w*8, BB9*w*9, BB*w*11, BB*w*21, *w*BHj, NG*w*18 and *w*62 mutant strains and "Super" derived wild-type strains.

Mutant strain	Crossed to "Super" strain	Octad classes (+:*w*), %					% NRCF	Total Octads
		4:4	6:2	2:6	5:3	3:5		
BB9*w*8+	7114-	97.84	0.96	1.12	-	0.08	2.16	1250
BB9*w*9+	"	98.39	1.15	0.26	0.20	-	1.61	3471
94-1*w*9+	"	94.06	4.44	0.51	0.95	0.04	5.94	5066
BB*w*11+	"	98.12	1.19	0.50	0.20	-	1.88	1010
BB*w*21+	"	97.39	1.22	1.05	0.17	0.17	2.61	574
*w*BHj+	"	89.38	6.59	1.62	1.58	0.61	10.40	4434
14-4*w*BHj+	"	94.09	3.76	0.86	0.96	0.20	5.79	4976
NG*w*18+	"	97.36	1.10	0.20	1.00	0.35	2.64	2007
3NG*w*18-	1114+	95.58	2.96	0.38	0.88	0.15	4.37	7300
207-5-*w*62+	7114-	94.57	3.36	1.15	0.50	0.42	5.43	5751

From this Table it can be seen that mutant strains BB9*w*8+, BB9*w*9+, BB*w*11+ and BB*w*21+ all gave LCF crosses to the same "Super" derived wild-type strain, with conversion frequencies ranging from 1.61 to 2.61%. These are typical LCFs for these mutations, arising from crosses heterozygous for the 2 forms of *ccf*-5, which control the conversion behaviour of these mutations (compare with the conversion frequencies of 1.10 to 3.14% in crosses of the same 4 mutant strains to derived wild-type "non-Super" strain 92-: see Table 13). The fact that all 4 mutant strains show the same apparent lack of response to the "Super" *ccfs* is perhaps a further indication that mutations BB9*w*8, BB*w*11 and BB*w*21 are actually re-isolations of BB9*w*9. It should also be noted that while mutant strain BB9*w*9+ (carrying *ccf*-5(*A*)) gave a LCF cross with "Super" derived wild-type strain 7114-, strain 94-1*w*9+ (also mutant for BB9*w*9, but carrying *ccf*-5(*B*)) gave a HCF cross with the same "Super" strain, although the conversion frequency of 5.94% is rather lower than the normal frequency of c 7.8-11.5% for this mutation in HCF crosses of this type (apparently *ccf*-5(*B*) homozygous - see below). If the "Super" factors are not responsible for this slightly depressed conversion frequency, other hitherto unknown conversion modifiers might be acting in the cross concerned. Taking the data from the above 2 crosses together, it would appear that "Super" strain 7114- carries *ccf*-5(*B*), since *A* × *B* crosses are LCF and *B* × *B* crosses HCF. At this point it is worth recalling the data in Table 12 which showed that the same

BB9*w*9 strain could give LCF and HCF crosses to different derived wild-type "Super" strains. From this preliminary data it would appear that the "Super" system cannot enhance the conversion frequency of BB9*w*9 (or BB9*w*8, BB*w*11 and BB*w*21 if they are not re-isolations of BB9*w*9) in +, *ccf*-5(*B*) × *w*, *ccf*-5(*A*) or +, *ccf*-5(*B*) × *w*, *ccf*-5(*B*) crosses, but this cannot be stated conclusively since the mutant strains under study could carry *ccf*-4(*R*).

*w*BHj mutant strains carrying *ccf*-6(*A*) (*w*BHj+) and *ccf*-6(*B*) (14-4*w*BHj+) were crossed to the same "Super" derived wild-type strain. From Table 41 it can be seen that the cross involving strain *w*BHj+ was HCF(10.40%) while the cross involving strain 14-4*w*BHj+ was LCF(5.79%). The conversion frequencies in each case were a little lower than expected for HCF and LCF crosses involving this mutation, but the data nevertheless indicate that the "Super" strain 7114- must carry *ccf*-6(*A*), since *A* × *A* crosses are HCF and *A* × *B* crosses LCF. Accordingly, there are no apparent enhancing effects of the "Super" factors on *w*BHj conversion frequency in +, *ccf*-6(*A*) × *w*, *ccf*-6(*A*) or +, *ccf*-6(*A*) × *w*, *ccf*-6(*B*) crosses, but again this cannot be stated conclusively at this stage since both *w*BHj mutant strains could carry *ccf*-4(*R*).

Unfortunately NG*w*18 mutant strains known to carry both forms of the *ccf* (*ccf*-7) shown to affects its conversion behaviour were not available. Instead, 2 different NG*w*18 mutant strains were crossed to 2 different derived wild-type "Super" strains. The data in Table 41 show that both crosses gave conversion frequencies (of 2.64 and 4.37%)

typical of HCF crosses for this mutation, with no apparent enhancing effect of the "Super" ccfs.

Various *w*62 mutant strains of both mating types were crossed to derived wild-type "Super" strains 7114- and 1114+. Only 1 cross was fertile, involving strain 207-5-*w*62+, giving a conversion frequency of 5.43%. This value is typical of crosses homozygous for *ccf*-1(*K*) in the work of Emerson and Yu-Sun (1967). Accordingly, there are no apparent enhancing effects of the "Super" factors on *w*62 conversion frequency in *ccf*-1(*K*) homozygous crosses. Again, this cannot be stated conclusively at this stage since mutant strain 207-5-*w*62+ could carry *ccf*-4(*R*).

In summary, these initial experiments suggested that the 2 "Super" *ccfs* are unable to enhance the conversion frequencies of mutations BB9*w*9 (and BB9*w*8, BB*w*11 and BB*w*21 if not re-isolations of BB9*w*9), *w*BHj, NG*w*18 and *w*62 in crosses carrying particular arrangements of the *ccfs* known to control their conversion behaviour. However, no definite conclusions to this effect can be drawn since some or all of the mutant strains used could have carried *ccf*-4(*R*) and in the presence of this "allele" in a cross, the enhancing combination of the "Super" factors (*ccf*-3(*E*), *ccf*-4(*r*)) would be unable to enhance the conversion frequency of the mutation in question.

Accordingly, it was decided to carry out more exhaustive experiments in order to determine conclusively whether the "Super" *ccfs* are able to enhance or otherwise modify the conversion behaviour of the mutations under study. The

aim was to randomly isolate white mutant spores from 4+:4*w* octads arising from all the crosses shown in Table 41 (with the exception of those involving mutations BB9*w*8, BB*w*11 and BB*w*21 which are probably re-isolations of BB9*w*9) and cross colonies from the spores which germinated to derived wild-type "Super" strains of both mating types. In this case, if any particular mutant strain had carried *ccf*-4(*R*), half of the progeny arising from the parental cross to a "Super" wild-type strain (*ccf*-3(*E*), *ccf*-4(*r*)) would carry *ccf*-4(*R*) while half would carry *ccf*-4(*r*). In crosses to "Super" wild-type strains, white mutant progeny of the latter type would be able to show enhanced conversion frequencies if the "Super" *ccfs* are able to enhance the conversion frequency of the mutation under study. The above assumes that none of the mutations concerned is linked to *ccf*-4. If any were, less than half of the white mutant progeny might be expected to show enhanced conversion frequencies in crosses to "Super" derived wild-type strains.

The action of "Super" on mutation BB9*w*9

Conversion behaviour of white mutant spores randomly isolated from 4+:4*w* octads from cross BB9*w*9+ × 7114-

40 white spores were randomly isolated from 4+:4*w* non-convertant octads from the LCF cross BB9*w*9+ × 7114-. 16 of these spores germinated and 12 of these isolates gave fertile crosses with derived wild-type "Super" strains 7114- or 1114+. The data obtained from these crosses are displayed in Table 42.

TABLE 42: Conversion data of random white mutant isolates from 4+:4*w* octads from cross BB9*w*9+ × 7114- when crossed to "Super" wild-types 7114- or 1114+

Isolate No.	Octad classes (+:*w*), %					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1+	99.30	0.42	0.28	-	-	0.70	711
2-	92.54	4.97	0.70	1.63	0.16	7.46	1287
3+	99.32	0.38	0.04	0.15	0.11	0.68	2633
4+	98.97	0.65	0.17	0.21	-	1.03	2908
5+	98.55	1.04	0.30	0.11	-	1.45	2685
9+	97.96	1.19	0.48	0.40	0.04	2.10	2519
10-	89.41	7.31	0.57	2.63	-	10.51	1218
13-	93.58	4.26	0.34	1.61	0.09	6.30	3238
14-	91.95	4.83	0.33	2.61	0.17	7.94	1802
15-	94.15	3.28	0.57	2.00	-	5.85	701
17-	89.27	6.77	0.58	3.17	0.07	10.59	1388
18+	98.40	0.96	0.12	0.52	-	1.60	2496

From this Table it can be seen that all 6 white mutant isolates of (+) mating type gave LCF crosses (with conversion frequencies of 0.68 to 2.10%) to the parental "Super" strain 7114-. No segregants with enhanced conversion frequencies were observed. If the white mutant parental strain had carried *ccf*-4(*R*) some of the white mutant progeny should have been recombinants, carrying *ccf*-4(*r*): *ccf*-4 is loosely linked to locus I while BB9*w*9 is unlinked to locus I. Thus BB9*w*9 and *ccf*-4 may be unlinked or loosely linked, but not tightly linked. These results suggest that the "Super" factors are unable to enhance BB9*w*9 conversion frequencies in LCF +, *ccf*-5(*B*) × *w*, *ccf*-5(*A*) crosses. It can also be seen from Table 42 that all 6 white mutant isolates of (-) mating type gave HCF crosses (with conversion frequencies of 5.85 to 10.59%) to the "Super" derived wild-type strain 1114+. These results are simply explained if the "Super" strain 1114+ carries *ccf*-5(*A*), then all the crosses would be *A* × *A* homozygous and hence high converting for this mutation. The observed conversion frequencies of 5.85 to 10.59% are rather lower than the normal range of c. 9 to 15% seen in *A* × *A* crosses. This might be due to one of the "Super" factors acting in such a way as to slightly reduce BB9*w*9 conversion frequencies or other minor modifiers of conversion behaviour could be responsible. None of the (-) mating type isolates showed conversion frequencies enhanced above the normal *A* × *A* HCF cross levels. This indicates that the "Super" factors can have no enhancing effect on BB9*w*9 conversion in *ccf*-5(*A*) homozygous crosses.

Conversion behaviour of white mutant spores randomly isolated from 4+:4*w* octads from cross 94-1*w*9+ × 7114-

40 white spores were randomly isolated from 4+:4*w* non-convertant octads from the HCF cross 94-1*w*9+ × 7114-. 14 of these spores germinated and 12 of these isolates gave fertile crosses with derived wild-type "Super" strains 7114- or 1114+. The data obtained from these crosses are displayed in Table 43.

From this Table it can be seen that all 6 white mutant isolates of (+) mating type gave HCF crosses to the parental "Super" strain 7114-. No segregants with conversion frequencies enhanced compared to the parental cross (%NRCF = 5.94%) were observed. All conversion frequencies fell in the range 5.41 to 6.98%, except for one extreme value of 9.38% which resulted from a cross giving a very small sample size of dehisced octads. Strain 94-1*w*9+ carries *ccf*-5(*B*) while all results obtained in these experiments are consistent with derived wild-type "Super" strain 7114- carrying *ccf*-5(*B*) also. Thus all white mutant progeny from a cross between these strains would carry *ccf*-5(*B*) and give *ccf*-5(*B*) homozygous crosses to "Super" strain 7114-, which are high converting. The observed conversion frequencies of 5.41 to 6.98% plus 9.38% are rather lower than the normal range of c 7.8 to 11.5% for this mutation seen in *B* × *B* crosses. Again, this might be due to one of the "Super" factors acting in such a way as to slightly reduce BB9*w*9 conversion frequencies or other minor modifiers of conversion behaviour

TABLE 43: Conversion data of random white mutant isolates from 4+:4w octads from cross 94-1w9+ x 7114- when crossed to "Super" wild-types 7114- or 1114+

Isolate No.	Octad classes (+:w),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
6+	93.78	3.95	1.01	1.25	-	6.22	2074
12+	93.02	4.44	0.63	1.90	-	6.98	1473
13-	98.39	1.07	0.31	0.15	0.08	1.61	2610
14-	98.85	0.86	0.29	-	-	1.15	847
15+	94.33	3.36	1.01	1.13	0.16	5.67	2467
17-	99.19	-	-	-	0.81	0.81	124
21+	94.59	3.33	1.25	0.83	-	5.41	721
22+	93.77	3.96	0.75	1.29	0.03	6.03	2952
23-	97.29	0.81	0.81	1.08	-	2.71	369
24-	99.64	-	-	0.36	-	0.36	276
26-	98.81	0.89	0.30	-	-	1.19	336
27+	90.63	7.29	-	2.08	-	9.38	396

could be responsible. None of the (+) mating type isolates showed conversion frequencies enhanced above the normal *B* × *B* HCF cross levels. It can also be seen from Table 43 that all 6 white mutant isolates of (-) mating type gave LCF crosses (with conversion frequencies of 0.36 to 2.71%) to the "Super" derived wild-type strain 1114+. It has already been postulated that the latter strain carries *ccf*-5(*A*) and this fits the data from this experiment well. All the LCF crosses would then be of the +, *ccf*-5(*A*) × *w*, *ccf*-5(*B*) type. No (-) mating type segregants showing conversion frequencies enhanced above the levels expected for such crosses were observed. This indicates that the "Super" factors can have no enhancing effect on BB9*w*9 conversion in +, *ccf*-5(*A*) × *w*, *ccf*-5(*B*) crosses.

Summary

The data from the above 2 experiments clearly demonstrate that the "Super" *ccfs* can have no enhancing effect on BB9*w*9 conversion frequency in crosses with all 4 possible combinations of the closely linked *ccf*-5 factors, which primarily control the conversion behaviour of mutation BB9*w*9. These crosses were:

+, *ccf*-5(*A*) × *w*, *ccf*-5(*A*)	HCF
+, *ccf*-5(*B*) × *w*, *ccf*-5(*A*)	LCF
+, *ccf*-5(*A*) × *w*, *ccf*-5(*B*)	LCF
+, *ccf*-5(*B*) × *w*, *ccf*-5(*B*)	HCF

However, in both types of HCF cross the observed conversion frequencies were somewhat lower than those recorded in Chapter 2 of this thesis. No such effect was easily detec-

table in the LCF crosses, but a reduction in conversion frequency by the same proportion in HCF and LCF crosses would be more difficult to detect in the latter type of cross. If such a reduction in conversion frequency in the HCF crosses has a genetic basis, and this seems probable, this might be explained by minor modifiers of gene conversion being present in both the "Super" derived wild-type strains used in this experiment. Alternatively, one of the "Super" factors could be responsible: Helmi (1980) proposed that *ccf*-3(*E*) acts by coding for a product which enhances H-DNA formation at locus I (probably by interacting with the *ccf*-2 factors), either by increasing the frequency of intimate pairing before H-DNA formation actually starts or by enhancing a step during H-DNA formation. It is possible that there may be an illegitimate binding site for the *ccf*-3(*E*) product close to BB9*w*9 (it may even be part of the *ccf*-5 sequence), which prevents some binding of a similar product which normally acts to enhance H-DNA formation in the BB9*w*9 region. There is no genetic evidence that any such gene exists coding for such a product and it is probably more likely that the slightly reduced BB9*w*9 conversion frequencies in HCF crosses in these experiments were caused by minor modifiers of BB9*w*9 conversion frequency. The involvement of one of the "Super" factors remains a possibility which might be tested by crossing white mutant progeny from the above 2 experiments to other "Super" derived wild-type strains and observing whether the BB9*w*9 conversion frequency was always slightly depressed in HCF crosses. Some relevant data already exist in Table 12. Here 2 *ccf*-5(*A*) homozygous crosses involving strain BB9*w*9+ and 2 derived wild-type

"Super" strains gave conversion frequencies of 8.09 and 10.51% which are much more nearly within the normal range of c9 to 15% for such crosses. Thus it would seem that other unknown conversion modifiers are responsible for the somewhat depressed HCF values observed in these experiments. The "Super" *ccfs* certainly had no enhancing effect on BB9*w*9 conversion frequency in the experiments undertaken.

The action of "Super" on mutation *w*BHj

The experiments were carried out jointly with Dr.Helmi who isolated and germinated the white mutant spores from the parental crosses provided, crossed the white mutant progeny which germinated to the "Super" derived wild-type strains and assisted in the scoring of most of these crosses. Accordingly, the data will be considered in detail elsewhere. Suffice it to say here that the experiments provided no evidence that the "Super" factors are able to enhance the conversion frequency of mutation *w*BHj in *ccf*-6(*A*) homozygous HCF crosses or +, *ccf*-6(*A*) × *w*, *ccf*-6(*B*) LCF crosses. However, the data did provide some evidence that unexpected variations in *w*BHj conversion frequencies might be explicable by the action of hitherto unknown conversion modifiers segregating in both crosses analysed. Further experiments need to be undertaken before this can be definitely concluded and the modifier(s) traced and characterized.

The action of "Super" on mutation *w*62

In the case of mutation *w*62 only 1 parental cross had been fertile, involving strains 207-5-*w*62+ and 7114-. As

can be seen from Table 41, *w*62 showed a conversion frequency of 5.43% in this cross, which is a value typical of crosses homozygous for one form of *ccf*-1 – *K* (*ccf*-1 is closely linked to *w*62 and was shown by Emerson and Yu-Sun (1967) to be primarily responsible for *w*62 conversion control). Thus the preliminary data showed no evidence that the "Super" system was able to enhance *w*62 conversion frequencies - in one type of cross at least.

Conversion behaviour of white mutant spores randomly isolated from 4+:4*w* octads from cross 207-5-*w*62+ × 7114-

40 white spores were randomly isolated from 4+:4*w* non-convertant octads from the HCF cross 207-5-*w*62+ × 7114-. 14 of these spores germinated and 9 of these isolates gave fertile crosses with derived wild-type "Super" strains 7114- or 1114+. The data obtained from these crosses are displayed in Table 44.

From this Table it can be seen that all 3 isolates of (+) mating type gave HCF crosses (with conversion frequencies of 4.77 to 6.19%) to the parental "Super" strain 7114-. The conversion frequencies agreed well with the *w*62 conversion frequency of 5.43% observed in the parental cross, i.e. none of the 3 segregants showed enhanced conversion frequencies. The results again suggest that these HCF crosses are homozygous for *ccf*-1(*K*) and seemingly the "Super" factors are unable to enhance *w*62 conversion frequencies in such crosses. However, results from far more than 3 segregants would be needed before this could

TABLE 44: Conversion data of random white mutant isolates from 4+:4*w* octads from cross 207-5-*w*62+ x 7114- when crossed to "Super" wild-types 7114- or 1114+

Isolate No.	Octad classes (+:*w*),%					% NRCF	Total octads
	4:4	6:2	2:6	5:3	3:5		
1-	97.68	1.74	0.10	0.39	0.10	2.32	1035
3-	97.17	2.04	0.68	0.11	-	2.83	883
4-	98.72	0.91	0.16	0.21	-	1.28	1872
5+	95.15	3.39	0.93	0.26	0.19	4.77	2683
6+	93.72	3.59	1.97	0.45	0.18	6.19	1115
7-	97.78	1.85	0.13	0.25	-	2.22	2383
8-	98.63	0.87	0.41	0.08	-	1.37	2416
10-	98.30	0.81	0.35	0.46	0.08	1.70	2590
12+	94.15	3.97	1.34	0.20	0.34	5.85	1487

be stated with any certainty. It can also be seen from Table 44 that all 6 white mutant isolates of (-) mating type gave LCF crosses to the "Super" derived wild-type strain 1114+, with conversion frequencies of 1.28 to 2.83%. These results are easily explained if the "Super" strain 1114+ carries *ccf*-1(*P*) since all the LCF crosses would then be of the +, *ccf*-1(*P*) × *w*, *ccf*-1(*K*) type. Such crosses typically show a *w*62 conversion frequency of about 2.5%. None of the (-) mating type isolates showed conversion frequencies enhanced above the normal +, *P* × *w*, *K* levels. This indicates that the "Super" factors are unable to enhance *w*62 conversion frequencies in the latter type of cross. This can be stated with more certainty since 6 white mutant isolates all showed broadly the same conversion behaviour. *ccf*-4 is not thought to be linked to *w*62, so even if the parental 207-5-*w*62+ strain had carried *ccf*-4(*R*), some of the 9 white mutant progeny tested in this experiment might have been expected to inherit *ccf*-4(*r*) from the "Super" parent, and thus if "Super" was capable of enhancing *w*62 conversion frequencies, this would have occurred in these cases.

This experiment showed that the "Super" factors almost certainly could not enhance *w*62 conversion frequencies in 2 types of cross

+, *ccf*-1(*K*) × *w*, *ccf*-1(*K*)
+, *ccf*-1(*P*) × *w*, *ccf*-1(*K*)

The effect of "Super" in the following 2 categories of cross was not tested, due to the lack of fertile crosses:

+, *ccf*-1(*K*) × *w*, *ccf*-1(*P*)
+, *ccf*-1(*P*) × *w*, *ccf*-1(*P*)

If "Super" were only able to enhance *w*62 conversion frequencies by interacting with the *ccf*-1 factors, it is extremely unlikely that it could enhance the conversion frequency seen in the latter 2 categories of cross: *P* and *K* factors were available to interact with in the first 2 crosses which were investigated. However, the possibility remains that in some *w*62 (or indeed wild-type) strains in our stock collection, factors close to the *w*62 region do exist with which the postulated *ccf*-3(*E*) diffusible product could interact. Setting aside this possibility, it would seem that the "Super" factors are unable to enhance *w*62 conversion frequencies.

The action of "Super" on mutation NG*w*18

The preliminary data shown in Table 41 suggested that the "Super" conversion control system was unable to enhance NG*w*18 conversion frequencies. The 2 crosses performed (involving different NG*w*18 and "Super" strains) both gave typical HCF crosses for NG*w*18. Although white mutant spores were randomly isolated from 4+:4*w* octads from both crosses and germinated, it was decided not to proceed further with similar analyses to those described above since it had not been possible to elucidate the manner in which *ccf*-7 controls the conversion behaviour of mutation NG*w*18. Without this information the results might not have been interpretable and certainly could not have conclusively shown that the "Super" system was able or unable to enhance NG*w*18 conversion frequencies.

Summary

In the experiments described, the "Super" conversion control system was unable to enhance the conversion of mutations BB9*w*9, *w*BHj and *w*62. A possibility does remain, of course, that in other mutant or derived wild-type strains in our stock collection, factors close to any of these 3 mutant sites may exist, with which the *ccf*-3(*E*) proposed diffusible product might interact, hence enhancing the conversion frequency of the mutation in question. There is no evidence for such factors and they probably do not exist. Thus it seems highly probable that the "Super" factors are genuinely unable to enhance conversion of mutations BB9*w*9, *w*BHj and *w*62. The experiments described above also produced some evidence that further modifiers of conversion behaviour may occur which are able to modify the conversion frequencies of BB9*w*9 and *w*BHj in crosses with at least certain combinations of their closely linked *ccfs*. Overall, it now seems more likely that the action of the "Super" *ccfs* is specific to locus I, but the conversion behaviour of many more non-locus I mutations should be studied in experiments similar to those described above.

P A R T T W O

Miscellaneous Studies

Chapter 1: Germination studies.

Introduction

Obviously, poor spore germination is a great disadvantage in any fungus used in genetical research. Ascospore germination in *Ascobolus immersus* is beset by many problems (Van Brummelen, 1968). Germination frequencies of about 90% were reported for *Ascobolus* ascospores by Yu-Sun (1964a), working with the Pasadena strains. Her bacto-peptone germination medium was used by different workers with varying degrees of success until Ghikas in 1976 found that the germination frequency for all types of ascospores (wild-type as well as ascospore colour mutant) was usually below 20% (Ghikas, personal communication), while in the work of Helmi (1980) the germination frequency almost reached 0% in some experiments. Ghikas (1978) unsuccessfully tried various methods of improving germination, including supplementing the bacto-peptone germination medium with thiamine and biotin or furfural. The latter activates dormant ascospores of *Neurospora crassa* (Emerson, 1954). Ghikas (1978) also tried replacing the bacto-peptone medium with *Sordaria brevicollis* germination medium (Yu-Sun et al. 1977), which again did not yield satisfactory results. She found no relationship between germinability and the age of the spores, their time of dehiscence (i.e. from young or old crosses) or the presence or absence of pronase treatment. Ghikas (1978) was unable to establish any standard optimum germination conditions. However, Helmi (1980) was able to establish such optimum germination conditions for three different

ascospore genotypes: wild-type, *w*78 white mutant and *w*3C1 white mutant. *w*78 ascospores have the "collapsing" phenotype described by Ghikas (1978) - they look normal for about 1 week after dehiscence, then they begin swelling (due to water absorption) becoming larger than wild-type spores. At this stage the spores lose their smooth surfaces, becoming rough and fragile and cannot be germinated. As the spore collection medium dries out, *w*78 ascospores shrink to a size about half that of wild-type spores. *w*3C1 ascospores exhibit the "non-collapsing" phenotype, remaining smooth and turgid on the collecting lid as long as the medium is not allowed to dry out. Such ascospores have been observed to retain their normal appearance for periods up to 4 months. Helmi (1980) showed that the optimum germination conditions for these 3 types of ascospore depended upon their genotype and were: wild-type spores - dung germination medium and 2 hours' heat shock at 50° C; *w*78 and *w*3C1 spores - plain agar germination medium and 0-½ hour heat shock at 50°C. However, *w*3C1 spores were able to germinate on dung medium much better than *w*78 spores. The thicker walls of the former spores seemed better able to withstand the high osmotic pressure exerted by the dung medium and perhaps also longer periods of heat shock. Thus Helmi's (1980) results indicated that optimum ascospore germination conditions could be achieved and seemed to be dependent on ascospore phenotype. Various factors also seemed to indicate that white mutant spore phenotypes probably resulted from mutations affecting the spore wall, rather than steps in a pigment biochemical pathway. Thus it might be expected that optimum germination conditions could be highly variable among ascospores carry-

ing different white mutations.

During parts of this work the problem of poor ascospore germination returned. Wild-type (red) spores always germinated relatively well using the optimum conditions established by Helmi (1980), but on occasion the germination frequency fell below 25%. The real germination difficulties were experienced with BB9*w*9 white mutant ascospores. Initially the germination frequency was almost zero, with only 5 ascospores germinating out of 700. None of the previously established optimum conditions produced satisfactory results. BB9*w*9 ascospores were "non-collapsing". Indeed, under the light microscope there were definite suggestions that these ascospores had very thick walls indeed. This suggested that heat shocks even longer than 2 hours might be successful in producing spore germination. Accordingly, it was decided to establish optimum germination conditions, wherever possible, for a range of white ascospore colour mutants, particularly those whose conversion behaviour was to be investigated.

The establishment of optimum germination conditions for various white ascospore colour mutations

In this experiment the aim was to establish optimum germination conditions for 4 different white ascospore colour mutations. These were BB9*w*9, *w*BHj, NG*w*18 and UV*w*M7. The spores used came from the following crosses:

BB9*w*9+ × 7R7-
*w*BHj+ × 15-1*w*BHj-
NG*w*18+ × 3NG*w*18-
UV*w*M7+ × UV*w*M7-

i.e., from self crosses for all mutations except BB9*w*9, since for the latter mutation no fertile self crosses were obtainable (see Chapter 2, PART ONE). Spore suspensions consisting of white mutant spores only, could be prepared from the *w* × *w* self crosses, but in the case of BB9*w*9 only a mixed suspension of wild-type and white mutant spores could be prepared (too many spores were needed in this experiment to permit manual isolation of white mutant spores from 4+:4*w* octads from the parental + × *w* cross). Two germination media were used: plain agar and horse dung medium. Heat shock treatments at 50°C ranged from 0 to 8 hours. There were 3 replicates for each treatment with about 300 white spores plated per replicate. The methods used for preparing the germination media and spore suspensions were described in GENERAL MATERIALS AND METHODS.

The results obtained from this experiment are shown in Table 45. From this Table it can be seen that there were clear differences in ascospore germination requirements - both in terms of germination medium and length of heat shock treatment - between the 4 ascospore genotypes tested. The differences between optimum and sub-optimum germination conditions were shown to be statistically significant for each ascospore colour mutant by applying the $\Sigma\chi^2$ homogeneity test to the numbers of germinating and non-germinating ascospores arising from the relevant germination conditions. Only *w*BHj and UV*w*M7 mutant ascospores germinated well without any heat shock treatment. However, *w*BHj ascospores showed optimum germination (of 70.4%) with a ½ hour heat shock. (For plain agar, $\Sigma\chi^2$ comparing 0 and ½ hour heat shocks for

TABLE 45: Germination of BB9*w*9, *w*BHj, NG*w*18 and UV*w*M7 ascospores on plain agar and dung media, with different periods of heat shock at 50°C.

Mutant genotype	Heat shock at 50°C (hours)	% Germination Medium	
		Plain	Dung
BB9*w*9	0	0.0	0.0
	1	0.0	0.0
	2	1.0	11.4
	4	6.6	54.0
	6	7.2	4.4
	8	0.0	0.0
*w*BHj	0	63.1	58.7
	½	70.4	66.2
	1	16.3	9.8
	2	1.2	0.0
	4	0.0	0.0
NG*w*18	0	0.0	0.0
	1	0.3	2.5
	2	5.8	41.6
	4	27.4	59.1
	6	4.3	10.9
	8	0.0	0.0
UV*w*M7	0	54.8	26.5
	½	19.0	10.1
	1	9.5	8.9
	2	2.3	1.0
	4	0.0	0.0

homogeneity = 8.38, $p < 0.01$, 1df; for dung, $\Sigma\chi^2$ comparing 0 and ½ hour heat shocks for homogeneity = 8.23, $p < 0.01$, 1df; for plain agar, $\Sigma\chi^2$ comparing ½ and 1 hour heat shocks for homogeneity = 417.78, $p < 0.01$, 1df; for dung, $\Sigma\chi^2$ comparing ½ and 1 hour heat shocks for homogeneity = 470.64, $p < 0.01$, 1df) and germinated similarly well on plain and dung media (for ½ hour heat shock, $\Sigma\chi^2$ comparing plain agar and dung for homogeneity = 2.97, $p = 0.05-0.10$, 1df). These optimum requirements were similar to those established for *w*3C1 ascospores in the work of Helmi (1980) - both genotypes exhibit the "non-collapsing" phenotype. In contrast to this behaviour, the germination frequency of UV*w*M7 ascospores was significantly lower in the ½ hour heat shock treatment as opposed to zero heat shock and these ascospores germinated optimally on plain agar (for plain agar, $\Sigma\chi^2$ comparing 0 and ½ hour heat shocks for homogeneity = 63.34, $p < 0.01$, 1df; for 0 heat shock, $\Sigma\chi^2$ comparing plain agar and dung for homogeneity = 39.95, $p < 0.01$, 1df). UV*w*M7 spores are "collapsing", with thin walls which are probably less protective against the high osmotic pressure and high concentration of salts diffusing from the dung medium and seemingly less resistant to longer periods of heat shock. In this context, it is interesting to compare the germination behaviour of UV*w*M7 spores in this experiment with that of *w*78 spores in the work of Helmi (1980). Both genotypes exhibit the "collapsing" spore characteristic. Indeed, UV*w*M7 spores collapse more readily and do seem less resistant to heat shock than *w*78 spores, although the germination of both types of spore seems similarly inhibited by dung medium.

BB9*w*9 and NG*w*18 ascospores needed much longer heat shock treatments before they germinated well. Both genotypes germinated much better on dung than plain agar media. BB9*w*9 ascospores showed an optimum germination frequency of 54% on dung with 4 hours' heat shock at 50°C (for dung, $\Sigma\chi^2$ comparing 2 and 4 hours' heat shock for homogeneity = 137.83, $p < 0.01$, 1df; for 4 hours' heat shock, $\Sigma\chi^2$ comparing plain agar and dung for homogeneity = 122.19, $p < 0.01$, 1df). This was the first time that such unusual germination conditions had been encountered for any genotypes in *Ascobolus immersus*. As noted above, BB9*w*9 white mutant ascospores appear to be very thick-walled under the light microscope, having far thicker walls than any of the other spore genotypes used in this experiment. This correlates well with their optimum germination requirements. Since they require a heat shock of a duration twice as long as that needed to obtain optimum germination of wild-type (red) spores, it is quite probable that BB9*w*9 ascospores have thicker walls than wild-type spores. It was not possible to confirm this under the light microscope due to the heavy red pigmentation of the wild-type ascospores. NG*w*18 ascospores also showed optimum germination (of 59.1%) on dung medium with 4 hours' heat shock (for dung, $\Sigma\chi^2$ comparing 2 and 4 hours' heat shock for homogeneity = 31.45, $p < 0.01$, 1df; for 4 hours' heatshock, $\Sigma\chi^2$ comparing plain agar and dung for homogeneity = 39.59, $p < 0.01$, 1df). However, unlike BB9*w*9 ascospores, NG*w*18 ascospores showed relatively good germination with only 2 hours' heat shock on dung medium (41.6%) and also germinated relatively well on plain agar with

4 hours' heat shock (27.4%), although the resulting germination frequencies were statistically significantly lower than the optimum frequency (see above $\Sigma\chi^2$ homogeneities). In agreement with this, NG*w*18 ascospores were not so noticeably thick-walled. It should be noted that in all of the above treatments there was relatively good agreement between all 3 replicates for the same treatment.

In the case of BB9*w*9 and NG*w*18 mutant ascospores it would be helpful to refine the optimum germination conditions established in this experiment still further: although 54% of BB9*w*9 ascospores germinated on dung medium with a heat shock treatment of 4 hours, the optimum heat shock period could be shorter or longer than this, despite the fact that significantly far fewer spores germinated with heat shocks of 2 hours (11.4%) and 6 hours (4.4%) (for dung, $\Sigma\chi^2$ comparing 2 and 4 hours' heat shock for homogeneity = 137.83, $p < 0.01$, 1df; for dung, $\Sigma\chi^2$ comparing 4 and 6 hours' heat shock for homogeneity = 160.72, $p < 0.01$, 1df). 59.1% of NG*w*18 ascospores germinated on dung with 4 hours' heat shock while 41.6% germinated with only 2 hours' heat shock, as opposed to 10.9% with 6 hours' heat shock. All these germination frequencies are significantly different (for dung, $\Sigma\chi^2$ comparing 2 and 4 hours' heat shock for homogeneity = 31.45, $p < 0.01$, 1df; for dung, $\Sigma\chi^2$ comparing 4 and 6 hours' heat shock for homogeneity = 183.21, $p < 0.01$, 1df). Nevertheless, it remains possible that 4 hours' heat shock does not produce optimum germination of NG*w*18 ascospores, in which case the optimum heat shock period might be more likely to be between 2 and 4 hours.

One cautionary note should be sounded in the case of BB9*w*9 germination data. White mutant spores of this genotype were plated from a mixed suspension of wild-type and white ascospores. Wild-type germinating ascospores were found on the dung plates subjected to 2 and 4 hours' heat shock. It is conceivable that these wild-type germlings could have stimulated the germination of BB9*w*9 ascospores. This might have been likely if BB9*w*9 ascospores had apparently germinated optimally with 2 hours' heat shock (the optimum conditions for wild-type spores), but this was not so. In all probability the presence of wild-type germlings has at most only a minimal effect on BB9*w*9 germination.

After the novel BB9*w*9 ascospore germination conditions had been established, it was decided to see whether BB9*w*8, BB*w*11 and BB*w*21 ascospores responded to these same conditions. In Chapter 2 of PART ONE of this thesis, data were accumulated which suggested that these 3 mutations were re-isolations of mutation BB9*w*9. Limited germination data are available for all 3 mutations, but these data do suggest that BB9*w*8, BB*w*11 and BB*w*21 mutant ascospores show relatively high germination frequencies (40-55%) on dung medium with 4 hours' heat shock. This is further circumstantial evidence that the 3 mutations in question may be re-isolations of BB9*w*9, but it should be remembered that NG*w*18 ascospores also germinate well under these conditions, although no other ascospore genotypes are known to do so.

Conclusions

1. The optimum conditions for ascospore germination were genotype (or more truly phenotype)-dependent and those optima established in this work were:

UV*w*M7 ascospores - plain agar and no heat shock;

*w*BHj ascospores - plain agar and ½ hour heat shock at 50°C;

NG*w*18 and BB9*w*9 ascospores - dung medium and 4 hours' heat shock at 50°C.

2. These optimum conditions seem correlated with the apparent thickness of the ascospore wall:

UV*w*M7 spores are "collapsing" and thin-walled (like *w*78). Such ascospores require little stimulus to germinate and indeed germination seems to be inhibited by high osmotic pressure, salt concentrations and heat shocks;

*w*BHj ascospores are "non-collapsing" but do not have noticably thick spore walls. They require a slight stimulus to achieve optimum germination, but still germinate well without this, although significantly less so. These ascospores germinate equally well on plain and dung media. Hence germination is not inhibited by the high osmotic pressure and salt concentrations of the dung medium, at least with zero or ½ hour heat shock treatments;

NG*w*18 and especially BB9*w*9 ascospores appear to be thick-walled. They require the greatest stimulus to germinate - both long periods of heat shock and dung germination medium. The latter requirement is particularly needed in order to germinate BB9*w*9 ascospores, which appear to have thicker walls than NG*w*18 ascospores.

Chapter 2: Changes in gene conversion frequency with the time of dehiscence from a cross

Introduction

In earlier studies the gene conversion frequencies of the *w*10 and *w*78 locus I mutations generally declined with the time of dehiscence from any particular HCF cross, although in some instances there was a small initial rise in conversion frequency prior to the subsequent decline (Wickramaratne, 1974; Helmi, 1980; Lamb, personal communication). Two hypotheses were put forward to explain this phenomenon: either all apothecia produced more conversion octads early in their period of dehiscence or those apothecia which matured earlier produced relatively more conversion octads. These hypotheses were not thought of as being mutually exclusive. Helmi (1980) showed that the general trend (at least in the case of mutation *w*78) was for all apothecia to produce more conversion octads early in their period of dehiscence. He found no evidence for a special type of apothecium producing only, or a majority of, conversion octads. In the same study Helmi showed that the conversion frequency of locus I mutation *w*3C1 also decreased with the time of dehiscence in HCF crosses. Lamb (personal communication) observed a similar decline in the conversion frequency of mutations *w*10 and *w*78 with the time of dehiscence from LCF crosses, although the pattern was not so clearcut, perhaps because of sampling effects on the relatively small numbers of conversion octads which were being scored in such crosses. Indeed, Helmi (1980) failed

to observe such a phenomenon in further LCF crosses involving mutations *w*78 and *w*3C1. Lamb (personal communication; some of these data were communicated by Helmi (1980)) also investigated the changes in conversion frequency with time of dehiscence for the non-locus I mutation *w*62. No steady decrease in conversion frequency with time was observed in either 2 HCF or 3 LCF crosses. Instead, the data showed only slight, random fluctuations in conversion frequency with time. Helmi (1980) investigated the changes in conversion frequency with time for a further non-locus I mutation, BB*w*11. This mutation showed random fluctuations in conversion frequency with time in 3 replicates of the same HCF cross. More interestingly, in these same crosses, changes were observed in the relative frequencies of the most frequent conversion classes (the 6:2 and 5:3) with time. In all 3 replicates of the same cross the 5:3/6:2 ratio decreased with the time of dehiscence, although statistical analyses were not performed to demonstrate whether this change was significant. This change in the 5:3/6:2 ratio appeared largely attributable to decreases in the absolute frequency of 5:3 octads. The 6:2 frequency showed no clear pattern of change with the time of dehiscence. A similar clear decrease in the 3:5/2:6 ratio with time could not be demonstrated. If such a phenomenon does occur, it might not have been apparent due to sampling effects on the small numbers of 3:5 and 2:6 conversion octads produced in such crosses. Nor were such changes in the 5:3/6:2 ratio observed in LCF crosses involving the same mutation. Again this could have been due to conversion octads

being rare in LCF crosses, with the frequency of 5:3 octads particularly reduced in comparison with HCF crosses. Furthermore, LCF crosses involving mutation BB*w*11 showed even more irregular changes in conversion frequency with time than seen in HCF crosses. There was also greater heterogeneity between replicates of the same cross, perhaps illustrative of sampling effects on small numbers of conversion octads.

In this study the aim was to investigate the changes in conversion frequency with time in crosses involving non-locus I mutations BB9*w*9, *w*BHj, NG*w*18 and *w*62 and newly isolated mutation UV*w*M7 which is closely linked to locus I and may even be part of it (see Chapter 3, PART TWO). It was hoped to investigate this phenomenon in LCF and HCF crosses involving each mutation, subject to the availability of suitable data. Mutation *w*62 was included in this survey since Lamb (personal communication) had obtained insufficient data to conclusively demonstrate that this mutation showed no clear-cut changes in conversion frequency with time. Since it is now thought likely that mutations BB9*w*8, BB*w*11 and BB*w*21 are re-isolations of mutation BB9*w*9, it was decided to investigate whether all 4 mutations showed similar changes in conversion frequency and 5:3/6:2 ratio with time of dehiscence. Contrary findings would weaken the hypothesis that all 4 mutations are, in fact, identical, while positive correlations would strengthen the hypothesis and provide more data on conversion frequency changes with time for what may be a single mutation. A further aim was to look for any changes in the 3:5/2:6 ratio in the crosses concerned, since Helmi (1980) had insufficient data to determine this. Changes in PMS/MS

ratios with time were also looked for in crosses involving other mutations. NG*w*18 was the most likely candidate, since, like BB*w*11 (BB9*w*8, BB9*w*9 and BB*w*21) the most frequent conversion class was the 6:2, followed by the 5:3. In crosses involving all the other mutations studied, the most frequent conversion class was the 6:2, followed by the 2:6.

Methods

Consecutive collecting lids from altogether 51 + × *w* crosses were scored: all crosses were incubated under the same conditions as described in GENERAL MATERIALS AND METHODS. Crosses involving each particular mutation were divided into HCF and LCF categories, according to the already known conversion behaviour and genetic conversion control system acting on that mutation. It should be noted that for a particular mutation (e.g. *w*62) its conversion frequency in a HCF cross may be similar to the conversion frequency of another mutation (e.g. *w*BHj) in a LCF cross. The time of dehiscence for each cross was divided into 3 periods - early, middle and late. Because the crosses differed in the length of the period for all octads to dehisce, this classification was made by dividing the dehiscence period, regardless of its length, into 3 approximately equal periods.

Results

The 51 crosses of the 7 mutants under investigation were comprised of a mixture of individual crosses and replicates of the same cross. The time from inoculation to cross matura-

tion varied from 9 to 19 days and the time during which individual crosses continued spore dehiscence varied from 4 to 12 days. The data obtained from these 51 crosses are displayed in Table 46. It can be seen from this Table that rarely were the numbers of octads dehisced in each of the early, middle and late periods even approximately equal. Generally the largest numbers of octads were dehisced in the early or middle periods, but there were some exceptions to this. From a consideration of Table 46 and Figures 8, 9, 10 and 11, the data relating to each of the 7 mutations will be dealt with in turn:

Mutation BB9*w*9: of the 14 crosses studied 7 were of high (9-15%) and 7 were of low (1.5-3.5%) conversion frequency. The 7 HCF crosses (of the *ccf*-5(*A*) homozygous type) showed irregular, heterogeneous changes in conversion frequency, with no overall patterns easily discernible (Fig.8(a)). In Cross 1 the changes in conversion frequency were only slight, while in Cross 3 the variation was more than 6%. Replicates of the same cross (Crosses 4 and 5) also showed heterogeneity in the patterns of conversion frequency change with time. If the mean conversion frequency data from all 7 HCF crosses are considered, there is an apparent rise in conversion frequency from 10.6% in the early period, to 11.8% in the middle period, to 12.9% in the late period. However, such a phenomenon was only observed in 3 out of the 7 HCF crosses studied. This rise in mean conversion frequency was paralleled with a rise in the frequency of the most common conversion class - the 6:2 - with time. Application of a 2-way analysis of

TABLE 46: Changes in conversion frequency with time of dehiscence, in +x*w*crosses involving white ascospore mutations BB9*w*9, BB9*w*8, BB*w*21, *w*BHj, NG*w*18, *w*62 and UV*w*M7

	Dehiscence Period				Dehiscence Period		
	E	M	L		E	M	L
Mutation: BB9*w*9							
Cross 1 (RS8-1- x BB9*w*9+)HCF		%		Cross 2 (RS17-1- x BB9*w*9+)HCF		%	
NRCF	15.2	14.7	15.4	NRCF	12.6	12.8	14.9
6:2	4.0	8.0	10.0	6:2	5.4	7.3	9.9
5:3	8.1	5.0	4.0	5:3	5.7	4.9	3.7
2:6	1.0	1.3	0.9	2:6	0.5	0.6	0.7
3:5	2.0	0.5	0.6	3:5	1.0	0.0	0.7
Total octads	99	401	350	Total octads	738	507	463
Cross 3 (18P- x BB9*w*9+)HCF		%		Cross 4 (RS17-3- x BB9*w*9+)HCF		%	
NRCF	12.1	11.3	5.9	NRCF	8.7	12.0	10.1
6:2	5.1	5.8	5.9	6:2	4.0	5.8	6.9
5:3	4.7	3.8	0.0	5:3	3.1	5.5	2.3
2:6	1.2	0.5	0.0	2:6	1.7	0.7	0.9
3:5	1.2	1.3	0.0	3:5	0.0	0.0	0.0
Total octads	257	399	51	Total octads	424	292	218
Cross 5 (RS17-3- x BB9*w*9+)HCF		%		Cross 6 (7R7- x BB9*w*9+)HCF		%	
NRCF	8.3	10.6	15.3	NRCF	10.6	11.0	12.9
6:2	3.5	7.3	9.1	6:2	6.6	7.5	9.5
5:3	3.2	2.3	4.7	5:3	3.6	2.3	1.5
2:6	0.6	0.4	1.0	2:6	0.3	1.0	1.5
3:5	1.0	0.6	0.3	3:5	0.1	0.4	0.5
Total octads	867	1028	594	Total octads	899	845	402

TABLE 46: (continued)

	Dehiscence Period				Dehiscence Period		
	E	M	L		E	M	L
Cross 7 (7R7- x BB9w9+)HCF				Cross 8 (92- x BB9w9+)LCF			
		%				%	
NRCF	11.8	12.4	8.1	NRCF	2.4	3.7	3.7
6:2	3.5	7.7	4.8	6:2	0.5	1.7	2.3
5:3	6.0	2.9	2.8	5:3	0.0	1.7	1.4
2:6	1.0	1.0	0.2	Total octads	1075	713	215
3:5	1.3	0.8	0.2				
Total octads	400	596	497				
Cross 9 (BB92-92- x BB9w9+)LCF				Cross 10 (BB92-92- x BB9w9+)LCF			
		%				%	
NRCF	2.7	2.1	3.9	NRCF	1.9	6.3	5.6
6:2	2.1	0.4	2.7	6:2	1.7	3.5	3.4
5:3	0.4	1.3	1.2	5:3	0.2	2.1	1.1
Total octads	524	239	259	Total octads	416	284	179
Cross 11 (BB92-94- x BB9w9+)LCF				Cross 12 (9225-14- x BB9w9+)LCF			
		%				%	
NRCF	1.6	2.3	2.9	NRCF	2.1	3.1	2.3
6:2	1.2	1.9	2.6	6:2	1.3	1.7	1.9
5:3	0.4	0.4	0.3	5:3	0.4	0.5	0.0
Total octads	247	259	342	Total octads	779	753	214
Cross 13 (9225-14- x BB9w9+)LCF				Cross 14 (9225-51- x BB9w9+)LCF			
			%			%	
NRCF	3.3	2.8	5.8	NRCF	2.7	1.1	2.9
6:2	1.8	2.0	3.8	6:2	1.6	0.8	2.2
5:3	0.1	0.3	1.9	5:3	0.6	0.2	0.4
Total octads	843	1341	313	Total octads	513	536	273

TABLE 46: (continued)

Mutation: BB9*w*8

	Cross 1 (9225-16- x BB9*w*8+)HCF			Cross 2 (9225-27- x BB9*w*8+)HCF		
	Dehiscence Period			Dehiscence Period		
	E	M	L	E	M	L
	%			%		
NRCF	9.2	7.4	11.6	12.4	8.7	9.5
6:2	5.2	4.8	6.5	4.0	4.7	5.6
5:3	3.0	1.8	3.8	4.7	2.8	2.3
2:6	0.7	0.7	1.3	2.9	1.1	1.6
3:5	0.3	0.1	0.0	0.7	0.1	0.0
Total octads	1230	1368	525	679	1498	305

Mutation BB*w*21

	Cross 1 (9225-5- x BB*w*21+)HCF			Cross 2 (9225-27- x BB*w*21+)HCF		
	Dehiscence Period			Dehiscence Period		
	E	M	L	E	M	L
	%			%		
NRCF	17.3	14.6	14.6	11.7	13.3	13.4
6:2	6.1	6.4	8.3	5.7	8.3	8.8
5:3	8.1	4.4	4.9	2.9	2.8	2.0
2:6	2.4	3.5	0.7	2.2	1.9	2.2
3:5	0.8	0.3	0.7	1.0	0.3	0.4
Total octads	248	343	144	512	324	501

	Cross 3 (9225-27- x BB*w*21+)HCF		
	Dehiscence Period		
	E	M	L
	%		
NRCF	10.7	11.1	11.0
6:2	5.7	8.3	6.7
5:3	2.8	2.2	2.4
2:6	2.1	0.6	2.0
3:5	0.1	0.0	0.0
Total octads	718	362	255

TABLE 46: (continued)

Mutation: *w*BHj

Cross 1 (7P- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	13.2	12.0	10.7
6:2	9.2	8.3	5.5
2:6	2.0	0.8	3.6
Total octads	304	399	253

Cross 2 (17P- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	13.7	16.1	12.8
6:2	9.6	11.5	6.6
2:6	1.5	1.7	3.5
Total octads	481	663	290

Cross 3 (17P- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	14.4	13.7	11.3
6:2	6.4	9.7	7.1
2:6	2.4	1.6	2.4
Total octads	949	953	617

Cross 4 (BH3- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	17.1	12.6	10.6
6:2	9.5	6.0	4.9
2:6	3.1	2.3	4.2
Total octads	738	650	858

Cross 5 (BH3- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	15.0	12.6	7.8
6:2	8.8	9.8	5.1
2:6	2.9	1.0	1.3
Total octads	798	701	475

Cross 6 (BH13- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	14.1	15.0	11.5
6:2	6.9	9.5	7.4
2:6	3.5	3.6	1.1
Total octads	491	527	269

Cross 7 (BH43- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	14.3	11.7	19.4
6:2	9.4	7.4	13.8
2:6	1.3	1.2	3.6
Total octads	700	649	412

Cross 8(BH43- x *w*BHj+)HCF

	Dehiscence Period		
	E	M	L
		%	
NRCF	14.6	15.5	10.7
6:2	9.3	9.5	6.9
2:6	2.2	3.2	3.1
Total octads	724	772	159

TABLE 46: (continued)

	Dehiscence Period				Dehiscence Period		
	E	M	L		E	M	L
Cross 9 (BH5- x *w*BHj+)LCF				Cross 10 (BH7- x *w*BHj+)LCF			
		%				%	
NRCF	6.8	9.5	6.3	NRCF	7.8	10.9	6.4
6:2	4.6	4.2	4.6	6:2	4.1	5.3	3.5
2:6	0.7	2.1	0.6	2:6	2.6	3.4	1.7
Total octads	593	381	350	Total octads	807	925	653
Cross 11 (BH7- x *w*BHj+)LCF				Cross 12 (BH9- x *w*BHj+)LCF			
		%				%	
NRCF	6.7	8.0	4.9	NRCF	9.6	11.5	4.9
6:2	3.0	3.5	2.7	6:2	5.7	6.5	2.4
2:6	2.6	3.0	1.4	2:6	2.2	3.4	1.9
Total octads	465	373	367	Total octads	784	816	627
Cross 13 (BH22- x *w*BHj+)LCF				Cross 14 (BH22- x *w*BHj+)LCF			
		%				%	
NRCF	7.5	8.9	6.8	NRCF	8.6	7.1	6.2
6:2	3.2	5.6	2.5	6:2	4.6	3.9	4.5
2:6	2.1	1.3	1.1	2:6	1.6	2.0	0.8
Total octads	438	1103	279	Total octads	616	1219	625
Cross 15 (BH45- x *w*BHj+)LCF							
		%					
NRCF	7.0	6.8	8.3				
6:2	3.4	4.1	6.3				
2:6	1.6	1.4	1.5				
Total octads	614	1108	336				
Mutation: NG*w*18							
Cross 1 (92- x NG*w*18+)HCF				Cross 2 (257R-8- x NG*w*18+)HCF			
		%				%	
NRCF	5.7	1.9	2.2	NRCF	2.7	2.2	1.3
6:2	3.1	1.3	1.4	6:2	1.5	1.2	1.1
5:3	1.4	0.2	0.2	5:3	0.5	0.3	0.1
Total octads	1915	2321	1652	Total octads	401	591	749

TABLE 46: (continued)

	Dehiscence Period				Dehiscence Period		
	E	M	L		E	M	L
Cross 3 (257R-47+ x 3NGω18-)HCF				Cross 4 (257R-115- x NGω18+)HCF			
		%				%	
NRCF	2.9	3.8	4.4	NRCF	4.7	4.2	3.7
6:2	1.5	2.9	3.4	6:2	1.7	2.5	2.2
5:3	0.5	0.4	0.0	5:3	1.3	0.4	0.4
Total octads	1179	984	619	Total octads	235	479	268
Cross 5 (257R-154+ x 3NGω18-)HCF				Cross 6 (257R-158+ x 3NGω18-)HCF			
		%				%	
NRCF	3.1	3.0	4.0	NRCF	4.6	1.6	2.6
6:2	2.3	3.0	4.0	6:2	3.0	1.0	1.3
5:3	0.5	0.0	0.0	5:3	0.7	0.0	0.7
Total octads	393	234	225	Total octads	899	578	304
Cross 7 (257R-194+ x 3NGω18-)HCF				Cross 8 (257R-210+ x 3NGω18-)HCF			
		%				%	
NRCF	2.6	1.5	2.8	NRCF	4.2	2.5	3.6
6:2	1.8	0.9	2.0	6:2	2.8	1.7	3.1
5:3	0.5	0.2	0.0	5:3	0.7	0.2	0.2
Total octads	986	885	255	Total octads	427	590	448
Mutation: *w62*							
Cross 1 (7114- x 207-5-*w*62+)HCF				Cross 2 (7114- x 5*w*62+)HCF			
		%				%	
NRCF	7.8	5.5	3.8	NRCF	4.3	4.7	5.4
6:2	4.9	3.4	2.5	6:2	3.0	3.5	3.7
2:6	1.2	1.3	0.6	2:6	1.1	1.0	1.1
Total octads	680	3638	1253	Total octads	924	1057	700

TABLE 46: (continued)

	Dehiscence period				Dehiscence period		
	E	M	L		E	M	L
Cross 3 (7114- x 12ω62+)HCF				Cross 4(1114+ x 4ω62-)LCF			
		%				%	
NRCF	6.6	5.9	4.0	NRCF	0.6	1.0	1.2
6:2	4.4	4.0	2.7	6:2	0.6	1.2	0.6
2:6	1.4	1.4	0.9	2:6	0.0	0.2	0.3
Total octads	564	697	226	Total octads	629	901	342
Cross 5 (1114+ x 7ω62-)LCF				Cross 6 (1114+ x 8ω62-)LCF			
		%				%	
NRCF	3.7	0.5	1.4	NRCF	2.2	1.5	0.7
6:2	3.1	0.3	1.4	6:2	1.4	1.0	0.4
2:6	0.2	0.1	0.0	2:6	0.7	0.4	0.2
Total octads	1137	746	500	Total octads	558	1016	842
Cross 7 (1114+ x 10ω62-)LCF							
		%					
NRCF	1.4	1.8	2.1				
6:2	0.6	0.7	1.8				
2:6	0.4	0.4	0.3				
Total octads	862	1343	385				

Mutation: UVωM7

Cross 1 (92- x UVωM7+)HCF				Cross 2 (7114- x UVωM7+)HCF			
		%				%	
NRCF	2.7	2.6	2.2	NRCF	1.8	1.7	1.2
6:2	1.2	1.0	0.9	6:2	1.0	1.2	0.4
2:6	1.0	0.7	0.7	2:6	0.6	0.3	0.5
Total octads	934	1388	554	Total octads	1454	2173	1565

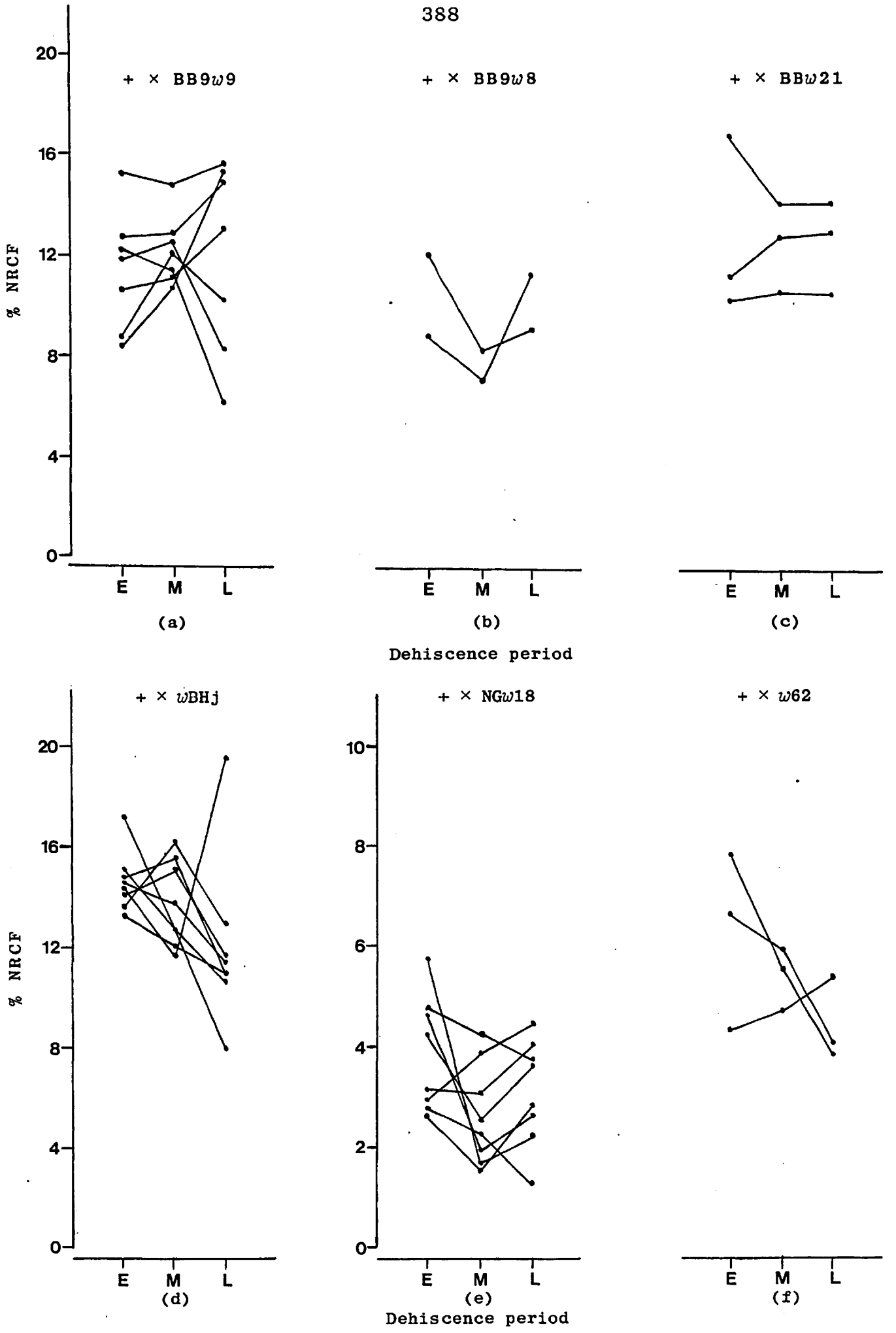


Fig. 8: Changes in narrower ratio conversion frequencies with time of dehiscence in + × ω HCF crosses involving 6 mutants. E, M and L are early, middle and late respectively in Figs. 8, 9, 10 and 11.

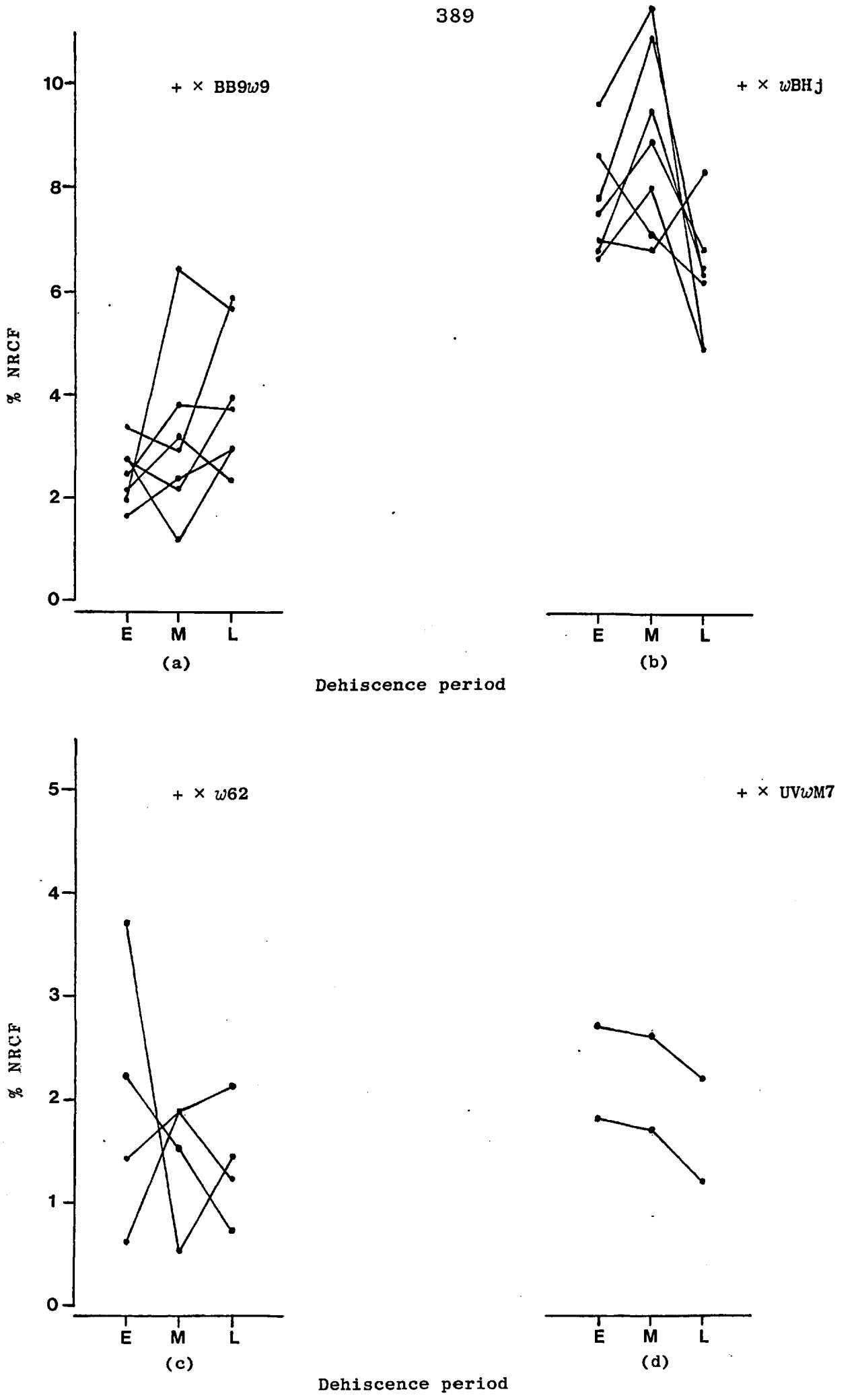


Fig. 9: Changes in narrower ratio conversion frequencies with time of dehiscence in + × ω LCF crosses involving 3 mutants and + × ω "HCF" crosses involving mutant UVωM7.

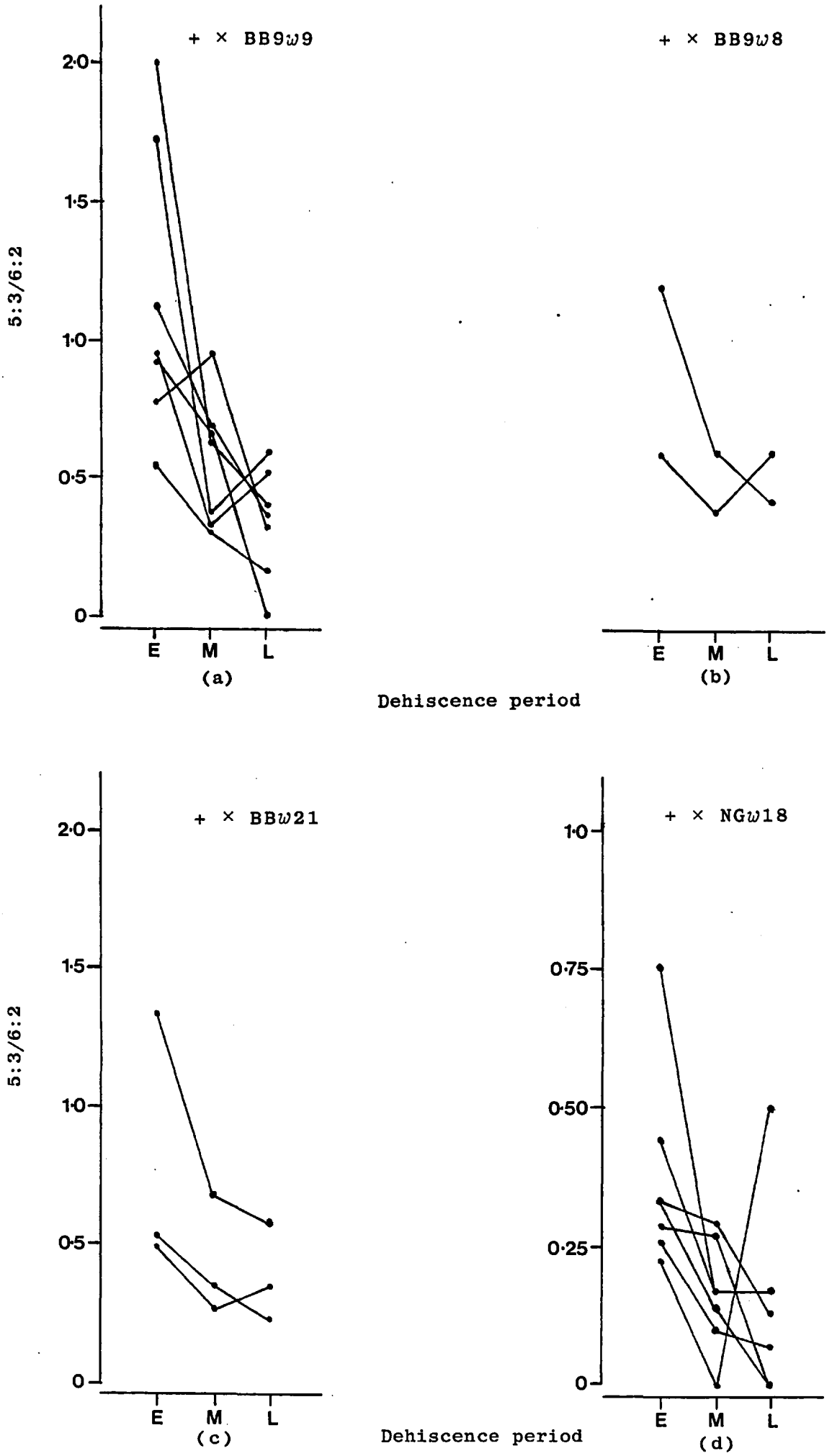
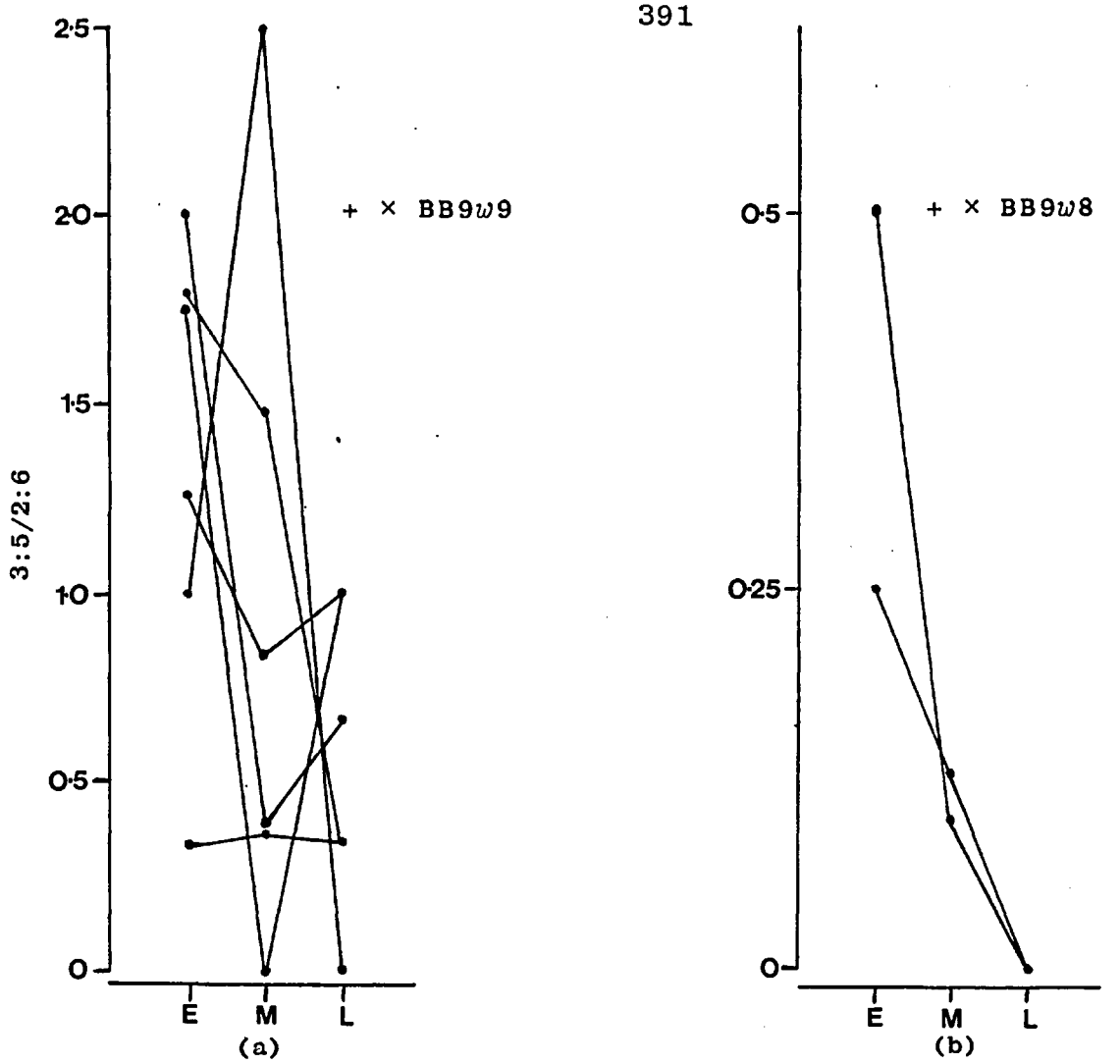


Fig. 10: Changes in the 5:3/6:2 ratio with time of dehiscence in + × *ω* HCF crosses involving 4 mutants.



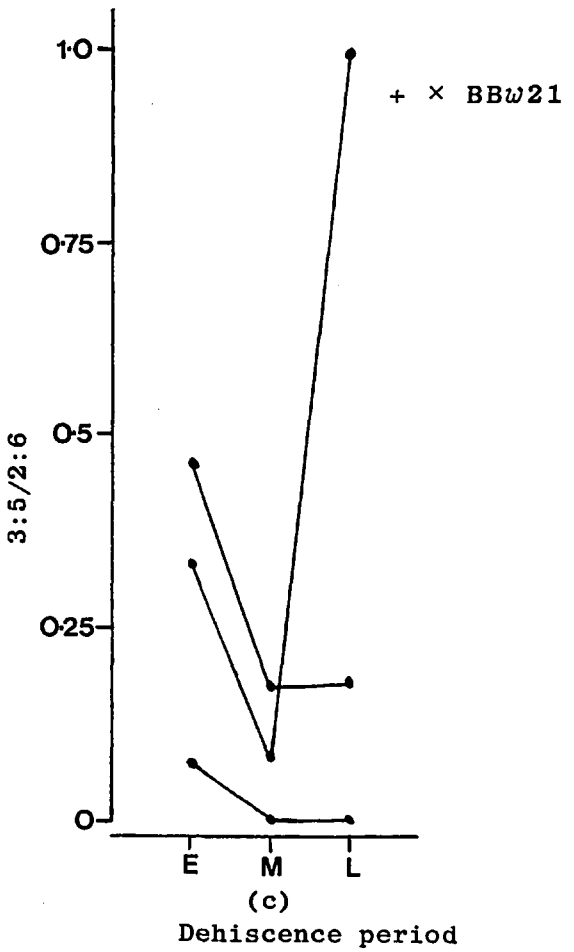


Fig. 11: Changes in the 3:5/2:6 ratio with time of dehiscence in + × ω HCF crosses involving 3 mutants.

variance test to the % NRCF data revealed no significant differences in conversion frequencies either between the periods of dehiscence or between the 7 crosses themselves (for the periods - calculated F ratio = 0.19, for 2 and 12 dfs, while tabulated F ratio (for p = 0.05) = 3.9; for the crosses - calculated F ratio = 1.86 for 6 and 12 dfs, while tabulated F ratio (for p = 0.05) = 3.0; since both calculated F ratios are less than the tabulated F ratios, there are no significant effects of either dehiscence period or cross on the % NRCF). In the 7 LCF crosses (of the +, *ccf*-5(*B*) × *w*, *ccf*-5(*A*) type), some apparent slight pattern of conversion frequency change with time was discernible. In all of the 7 crosses the conversion frequency was higher in the late period than in the early period (Fig.9(a)). However, the behaviour of the conversion frequency in the middle period was heterogeneous. The mean conversion frequencies based on all 7 crosses were 2.5% in the early period, 3.0% in the middle period and 3.8% in the late period. This rise in mean conversion frequency was paralleled with a rise in the mean frequencies of the 2 most frequent conversion classes - the 6:2 and 5:3 with time. Application of a 2-way analysis of variance test to the % NRCF data revealed no significant differences in conversion frequencies either between the periods of dehiscence or between the 7 crosses themselves (for the periods - calculated F ratio = 3.15, for 2 and 12 dfs, while tabulated F ratio (for p = 0.05) = 3.9; for the crosses - calculated F ratio = 1.98 for 6 and 12 dfs, while tabulated F ratio (for p = 0.05) = 3.0; since both calculated F ratios are less than the tabulated F ratios,

there are no significant effects of either dehiscence period or cross on the % NRCF). Most interestingly, in all HCF crosses except one (Cross 7) there was a consistent rise in 6:2 frequency with time (6:2s being the most frequent conversion class), while in all HCF crosses except two (Crosses 4 and 5) there was a consistent decrease in 5:3 frequency. Accordingly, it can be seen from Fig.10(a) that the 5:3/6:2 ratio tends to decrease with the time of dehiscence. This decrease was not consistent in all 7 HCF crosses - in only 4 crosses did the ratio decrease steadily with the time dehiscence, while in the remaining 3 crosses the 5:3/6:2 ratio was merely less in the late than the early period. The mean 5:3/6:2 ratio for all 7 crosses was 0.90 in the early period, 0.46 in the middle period and 0.39 in the late period. Applying a $\Sigma\chi^2$ homogeneity test to the overall numbers of 5:3 and 6:2 octads in each of the 3 periods, these data were shown to be heterogeneous between the periods ($\Sigma\chi^2 = 30.32$, $p < 0.01$, 2df). Thus it can be concluded that the 5:3/6:2 ratio does tend to decline with the time of dehiscence in HCF crosses involving BB9*w*9. This agrees with the similar findings of Helmi (1980) from data involving BB*w*11 in HCF crosses, although Helmi did not demonstrate whether the 5:3/6:2 ratio changes with time for mutation BB*w*11 were statistically significant. As already noted, mutations BB9*w*9 and BB*w*11 may be identical and these results strengthen that hypothesis. It should be remarked that high postmeiotic to meiotic ratios (on different models of gene conversion and H-DNA formation) indicate less correction of the mispairs formed at the mutant site in the H-DNA

(Wickramaratne and Lamb, 1978; Helmi and Lamb, unpublished). Therefore in these HCF crosses involving BB9ω9, the decline of the 5:3/6:2 ratio with the time of dehiscence could indicate that correction becomes more efficient, or has a longer period in which to operate, as the crosses reach later stages of spore liberation. This feature was not observable in LCF crosses involving the same mutation. In these crosses (8 to 14) the 6:2 frequency does tend to increase with time, consistently so in 4 crosses, while in all 7 crosses the 6:2 frequency is higher in the late period than in the early. However, the 5:3 frequency shows no consistent pattern of decrease, showing random fluctuations - indeed, the mean 5:3 frequency from all 7 crosses tends to increase with time. This may simply be due to sampling effects on small numbers of 5:3 conversion octads. Thus, although the data show no evidence of a decrease in the 5:3/6:2 ratio with time of dehiscence in BB9ω9 LCF crosses, this cannot be ruled out. Such crosses should be repeated, using large crossing plates to produce large sample sizes for scoring. This might overcome any sampling effects. The data also provide some evidence for a decrease in the 3:5/2:6 ratio with time in HCF crosses, but any pattern is by no means clear. Data are available for 6 crosses. In 5 of these crosses the 3:5/2:6 ratio was less in the late period than the early, but the decrease was not consistent through the middle period (see Fig.11(a)). The mean 3:5/2:6 ratio for all 6 crosses was 1.0 in the early period, 0.7 in the middle and 0.48 in the late period. Applying a $\Sigma\chi^2$ homogeneity test to the overall numbers of 3:5 and 2:6 octads in each of the 3 periods these data were still shown to be homogeneous among the

periods ($\Sigma\chi^2$ = 2.61, p = 0.20-0.30, 2df). This might have been due to the relatively small numbers of 3:5 and 2:6 octads scored in each period, even amalgamating data from 6 crosses.

Mutation BB9*w*8: This mutation was thought to be a re-isolation of BB9*w*9 and if this is so it should show similar changes (or lack of changes) in conversion frequency with the time of dehiscence. Data are only available for 2 HCF crosses (of the *ccf*-5(*A*) homozygous type) involving this mutation. From Fig.8(b) it can be seen that in both of these crosses the conversion frequency was lowest in the middle period. Considering the overall data from both crosses the mean conversion frequency in the early period was 10.3%, 8.1% in the middle period and 10.8% in the late period. These changes in % NRCF were paralleled and largely caused by similar changes in the frequencies of the 2 most frequent conversion classes - the 6:2 and the 5:3. Application of a 2-way analysis of variance test to the % NRCF data revealed no significant differences in conversion frequencies either between the periods of dehiscence or between the 2 crosses themselves (for the periods - calculated F ratio = 1.98 for 2 and 2 dfs, while tabulated F ratio (for p = 0.05) = 19.0; for the crosses - calculated F ratio = 0.27 for 1 and 2 dfs, while tabulated F ratio (for p = 0.05) = 18.5; since both calculated F ratios are <u>less</u> than the tabulated F ratios there are no significant effects of either dehiscence period or cross on the % NRCF) These results are in agreement with the results concerning mutation BB9*w*9, supporting the hypothesis that both mutations

are, in fact, the same. It can be seen from Fig.10(b) that in 1 cross the 5:3/6:2 ratio decreased with time, while it did not in the other cross. Considering the overall data from both crosses, the 5:3/6:2 ratio was 0.76 in the early period, 0.49 in the middle period and 0.53 in the late period, i.e. the decline in 5:3/6:2 ratio was not consistent. Furthermore, these differences in 5:3/6:2 ratio were not significant at the $p = 0.05$ level, as was shown by applying the $\Sigma\chi^2$ homogeneity test to the overall numbers of 5:3 and 6:2 octads in each of the 3 dehiscence periods ($\Sigma\chi^2 = 4.16$, $p = 0.10\text{-}0.20$, 2df). In both HCF crosses involving mutation B8qω8, the 3:5/2:6 ratio was observed to consistently decrease with time (see Fig.11(b)). Considering the overall data from both crosses, the 3:5/2:6 ratio was 0.32 in the early period, 0.12 in the middle period and 0.0 in the late period. However, these ratio differences were not quite significant at the $p = 0.05$ level ($\Sigma\chi^2 = 5.02$, $p = 0.05\text{-}0.10$, 2df). It would seem quite likely that if further BB9*w*8 HCF crosses could be analysed, and large sample sizes scored, such decreases in the 3:5/2:6 ratio might be shown to be significant. This finding of an apparent, but non-significant, decrease in the 3:5/2:6 ratio with time in HCF BB9*w*8 crosses agrees with similar findings in HCF BB9*w*9 crosses, again supporting the hypothesis that these mutations are identical.

Mutation BB*w*21: this mutation was also thought to be a re-isolation of BB9*w*9 and might be expected to show similar changes (or lack of changes) in conversion frequency with

time of dehiscence. Data are only available for 3 HCF crosses (of the *ccf*-5(*A*) homozygous type) involving this mutation. From Fig.8(c) it can be seen that all 3 crosses show a different pattern of conversion frequency change with time, i.e. the data are heterogeneous as were those involving BB9*w*9. Changes in conversion frequency with time would appear to be random. If the mean conversion frequency data from all 3 HCF crosses are considered, there is little overall change in conversion frequency with time, with % NRCFs of 12.2% in the early period and 12.9% in both the middle and late periods. Application of a 2-way analysis of variance test to the % NRCF data revealed no significant differences in conversion frequencies between the periods of dehiscence (calculated F ratio = 0.03 for 2 and 4 dfs, while tabulated F ratio (for p = 0.05) = 6.9; since the calculated F ratio is less than the tabulated F ratio, there is no significant effect of dehiscence period on the % NRCF) while the conversion frequencies between the crosses were significantly different at the p = 0.05 level (calculated F ratio = 9.50 for 2 and 4 dfs, while tabulated F ratio (for p = 0.05) = 6.9; since the calculated F ratio is more than the tabulated, there is a significant effect of cross on the % NRCF). This lack of a significant difference in conversion frequency between the 3 dehiscence periods is in agreement with similar results involving mutations BB9*w*8 and BB9*w*9, supporting the hypothesis that all 3 mutations are the same. In 2 of the 3 HCF crosses involving BB*w*21 the frequency of 6:2 octads (the most frequent conversion class) rose consistently with time, while in only 1 cross did the 5:3 frequency decrease in such a manner. However, considering the mean conversion frequencies

from all 3 crosses, there was a consistent rise in 6:2 frequency throughout the period of spore dehiscence and a consistent fall in 5:3 frequency. As a result, the mean 5:3/6:2 ratio fell from 0.65 in the early period to 0.41 in the middle period to 0.32 in the late period. These differences in the 5:3/6:2 ratio were significant at the $p = 0.05$ level, as was shown by applying the $\Sigma\chi^2$ homogeneity test to the overall numbers of 5:3 and 6:2 octads in each of the 3 dehiscence periods ($\Sigma\chi^2 = 6.80$, $p = 0.02$-0.05, 2df). In 2 out of the 3 crosses analysed, this 5:3/6:2 frequency showed a consistent decline (Fig.10(c)). In 2 crosses the 3:5/2:6 ratio was seen to decrease with the time of dehiscence, while the mean values from all 3 crosses showed no such decline (3:5/2:6 E = 0.25; M = 0.10; L = 0.18). Furthermore, these overall changes in the 3:5/2:6 ratio were not significant at the $p = 0.05$ level ($\Sigma\chi^2 = 1.28$, $p = 0.50$-0.70, 2df).

Mutation *w*BHj: of the 15 crosses studied, 8 were of high (12-14.5%) and 7 were of low (6-8%) conversion frequency. The 8 HCF crosses (of the *ccf*-6(*A*) homozygous type) showed a tendency for the conversion frequency to decline with the time of dehiscence (Fig.8(d)). In 4 of the 8 crosses (1, 3, 4 and 5) this decrease in conversion frequency was consistent throughout the whole period of dehiscence, while in 3 crosses (2, 6 and 8) the conversion frequency rose in the middle period before decreasing to values in the late period lower than those in the early. In only 1 cross (Cross 7) was the conversion frequency higher in the late period than in the early. If the mean conversion frequency data from all 8 crosses are considered, there is an apparent fall in conver-

sion frequency from 14.7% in the early period, to 13.8% in the middle period, to 11.7% in the late period. This fall in mean conversion frequency was not paralleled by a clear fall in the frequency of either of the 2 most frequent conversion classes - the 6:2 and the 2:6 - with time. Application of a 2-way analysis of variance test to the % NRCF data revealed no significant differences in conversion frequencies either between the periods of dehiscence or between the 8 crosses themselves (for the periods - calculated F ratio = 2.54, for 2 and 14 dfs, while tabulated F ratio (for p = 0.05) = 3.7; for the crosses - calculated F ratio = 0.60 for 7 and 14 dfs, while tabulated F ratio (for p = 0.05) = 2.8; since both calculated F ratios are less than the tabulated F ratios, there are no significant effects of either dehiscence period or cross on the % NRCF). In the 7 LCF crosses (of the +, *ccf*-6(*B*) × *w*, *ccf*-6(*A*) type) an apparently much clearer pattern of conversion frequency change with time of dehiscence was observable. In 5 out of the 7 crosses the *w*BHj conversion frequency rose from the early to the middle period and then declined in the late period (Fig.9(b)). In all 5 of these crosses (9, 10, 11, 12 and 13) the conversion frequency was at its lowest in the late period. This was also true of 1 of the 2 "non-conforming" crosses. If the mean conversion frequency data from all 7 crosses are considered, there is an apparent rise in conversion frequency from 7.8% in the early period to 9.1% in the middle period, followed by a fall to 6.1% in the late period. These changes in % NRCF were paralleled by similar changes in the frequencies of the 2 most frequent conversion classes - the 6:2 and 2:6 - with time. Application of a 2-way analysis of variance test

to the % NRCF data revealed that the observed differences in conversion frequency between the dehiscence periods were significant at the p = 0.05 level (calculated F ratio = 6.03 for 2 and 12 dfs while tabulated F ratio (for p = 0.05) = 3.9; since the calculated F ratio is greater than the tabulated F ratio, there are significant differences in conversion frequency between the periods of dehiscence). Furthermore, the same 2-way analysis of variance revealed no significant differences in conversion frequency among the 7 crosses (calculated F ratio = 0.71 for 6 and 12 dfs, while tabulated F ratio (for p = 0.05) = 3.0; since calculated F ratio is less than tabulated F ratio, there are no significant differences in conversion frequency among the 7 crosses). This initial rise and subsequent fall in conversion frequency with the time of dehiscence from a cross is a novel pattern among all the spore colour mutations studied in the Pasadena strains of *Ascobolus immersus*, although in some HCF crosses involving locus I mutations *w*10 and *w*78, this phenomenon was occasionally seen, but only as a deviation from the normal pattern of continuous conversion frequency decline with time (Wickramaratne, 1974; Lamb, personal communication). Furthermore, in these cases initial increases in conversion frequency occurred very early in the overall dehiscence period.

Mutation NG*w*18: Data are available for 8 HCF crosses involving locus II mutation NG*w*18. It can be seen from Fig.8(e) that there is no apparent clearcut pattern in the change of conversion frequency with the time of dehiscence from a cross. However, a closer consideration of the data in Table 46 reveals

that in 6 crosses (1, 4, 5, 6, 7 and 8) conversion frequency decreased from the early to the middle period and then rose again in the late period - to a value higher than that seen in the early period in 3 of these crosses. If the mean conversion frequency data from all 8 crosses are considered, the conversion frequency decreased from 4.1% in the early period to 2.4% in the middle period, rising again to 2.7% in the late period. This is exactly opposite to the pattern exhibited by mutation *ω*BHj in LCF crosses. However, application of a 2-way analysis of variance test to the % NRCF data revealed that these differences in conversion frequency between the 3 periods of dehiscence just failed to reach significance. Furthermore, the differences in conversion frequency between the 8 crosses were also shown to be non-significant (for the periods - calculated F ratio = 3.22 for 2 and 14 dfs while tabulated F ratio (for p = 0.05) = 3.7; for the crosses - calculated F ratio = 1.58 for 7 and 14 dfs while tabulated F ratio (for p = 0.05) = 2.8; since both calculated F ratios are <u>less</u> than the tabulated values, there are no significant effects of dehiscence period or cross on the % NRCF). In 7 of the 8 crosses the frequency of the second most frequent conversion class - the 5:3 - decreased throughout the period of dehiscence, while the frequency of by far the most abundant conversion class - the 6:2 - showed no such clear cut pattern of change. Notwithstanding this, it can be seen from Fig.10(d) that there is a clear tendency for the 5:3/6:2 ratio to decrease with the time of dehiscence - this occurred in 7 of the 8 crosses. 5:3 octads were relatively infrequent and in 3 crosses none was observed in the late

period while they were absent from the middle period in 2 of the crosses. The mean 5:3/6:2 ratio for all 8 crosses was 0.35 in the early period, 0.15 in the middle period and 0.09 in the late period. Applying a $\Sigma\chi^2$ homogeneity test to the overall numbers of 5:3 and 6:2 octads in each of the 3 periods, these data were shown to be heterogeneous between the periods ($\Sigma\chi^2 = 17.77$, $p < 0.01$, 2df). Thus it can be concluded that the 5:3/6:2 ratio does tend to decline with the time of dehiscence in HCF crosses involving NG*w*18. This is in agreement with the conversion behaviour of mutation BB9*w*9 (and mutations BB*w*11 and BB*w*21 if they are not re-isolations of BB9*w*9). For both mutations 6:2s are the most frequent conversion octads, followed by 5:3s, although the latter class is relatively scarcer in the case of NG*w*18 conversion. In passing it is interesting to note that not only do both mutations exhibit roughly similar conversion spectra, but they also seem to confer an unusually thick ascospore wall with similar optimum ascospore germination conditions. It was not possible to investigate changes in the 3:5/2:6 ratio with time since these conversion octads - particularly the 3:5s - were relatively rare and therefore their frequencies highly variable during all 3 periods of ascospore dehiscence, probably due to sampling effects on small numbers. Changes in NG*w*18 conversion frequency in LCF crosses were not investigated since the NRCF was very low (*c*. 0.14%) and it had been shown that the bulk of 6:2 and 5:3 octads were phenocopies and not genuine convertants (Chapter 4, PART ONE). The data could only have related to phenocopy frequency changes and not genuine conversion frequency changes.

Mutation *w*62: of the 7 crosses studied, 3 were of high (5-6%) and 4 were of low (1.3-2.2%) conversion frequency. The HCF crosses (of the *ecf*-1(*K*) homozygous type) showed no regular pattern of conversion frequency change with the time of dehiscence (Fig.8(f)). In 2 crosses (1 and 3) the conversion frequency decreased steadily with time, while in the third cross it increased. If the mean conversion frequency data of all 3 HCF crosses are considered, there is an overall decrease in conversion frequency from 6.0% in the early period, to 5.4% in the middle period, to 4.4% in the late period. This decrease in % NRCF with time is paralleled and largely caused by a decrease in the frequency of the commonest conversion class - the 6:2 - with time. The second most abundant conversion class - the 2:6 - does not show such a straightforward decrease with time. Application of a 2-way analysis of variance test to the % NRCF data revealed no significant differences in conversion frequencies either between the periods of dehiscence or between the 3 crosses themselves (for the periods - calculated F ratio = 1.39 for 2 and 4 dfs while the tabulated F ratio (for p = 0.05) = 6.9; for the crosses - calculated F ratio = 0.37 for 2 and 4 dfs while tabulated F ratio (for p = 0.05) = 6.9; since both calculated F ratios are <u>less</u> than the tabulated values, there are no significant effects of either dehiscence period or cross on the % NRCF). The 4 LCF crosses involving mutation *w*62 showed completely irregular changes in conversion frequency with time, with no overall pattern discernible (Fig.9(c)). If the mean conversion frequency data of all 4 LCF crosses are considered there is an apparent slight decrease

in conversion frequency from 2.2% in the early period to 1.5% in the middle period and to 1.2% in the late period. However, application of a 2-way analysis of variance test to the % NRCF data revealed no significant differences in conversion frequencies either between the periods of dehiscence or between the 4 crosses themselves (for the periods - calculated F ratio = 0.44 for 2 and 6 dfs while the tabulated F ratio (for p = 0.05) = 5.1; for the crosses - calculated F ratio = 0.25 for 3 and 6 dfs while tabulated F ratio (for p = 0.05) = 4.8; since both calculated F ratios are less than the tabulated values there are no significant effects of either dehiscence period or cross on the % NRCF). These results are in agreement with the data of Lamb (personal communication, but partly communicated by Helmi (1980)) who observed only slight, random fluctuations in *w*62 conversion frequency in 2 HCF and 3 LCF crosses. No overall pattern in conversion frequency change with the time of dehiscence was discernible in either type of cross.

Mutation UV*w*M7: this mutation was UV-induced and isolated during the course of the present work. It is closely linked to locus I and may even be part of this locus. However, in *ccf*-2(*P*) homozygous crosses it shows much lower conversion frequencies (of 2-4%) than any other locus I mutations apart from UVK*w*8, which does not seem to respond to the *ccf*-2 conversion control system (unlike all other locus I mutations). UV*w*M7 does seem to respond to *ccf*-2, giving higher conversion frequencies in *ccf*-2(*P*) homozygous crosses than in *ccf*-2(*P*) × *ccf*-2(*91*) crosses (here it gives a NRCF of *c*. 0.5%). In this experiment data are only available for 2 HCF crosses involving mutation UV*w*M7. It can be seen from Fig.9(d) that

in both of these crosses the conversion frequency decreased with the time of dehiscence. If the mean conversion frequency data from both HCF crosses are considered there is an apparent fall in conversion frequency from 2.1% in the early period, to 2.0% in the middle period and to 1.5% in the late period. This decrease in % NRCF with time of dehiscence is paralleled by a decrease in the frequency of the most abundant conversion class - the 6:2, but the same phenomenon is not observed for the second most abundant conversion class - the 2:6. Application of a 2-way analysis of variance test to the % NRCF data reveals that not only are the conversion frequency differences between the dehiscence periods significant, but so are the differences between the 2 crosses (for the periods - calculated F ratio = 103.0 for 2 and 2 dfs while tabulated F ratio (for p = 0.05) = 19.0; for the crosses - calculated F ratio = 784.0 for 1 and 2 dfs while tabulated F ratio (for p = 0.05) = 18.5; since both calculated F ratios are greater than the tabulated values there are significant effects of both dehiscence period and cross on the % NRCF). Thus in HCF crosses involving mutation UV*w*M7 it would seem that conversion frequency declines with the time of dehiscence as has been demonstrated for nearby locus I mutations *w*10, *w*78 and *w*3C1 (Wickramaratne, 1974; Helmi, 1980; Lamb, personal communication). However, it would be most worthwhile to obtain additional data from other HCF crosses involving UV*w*M7.

Discussion

The above experiments have shown that for most mutations studied there were no significant changes in conversion frequency with the time of dehiscence, unlike the decreases observed for locus I mutations in HCF crosses at least.

Considering the data involving each mutation in turn it was shown that in both HCF and LCF crosses mutation BB9*w*9 showed no significant changes in conversion frequency with time. The data were highly variable among crosses of the same type. When mean conversion frequencies for both HCF and LCF crosses were considered there was an apparent increase in conversion frequency with time, but among all the crosses these increases were not statistically significant. Similarly, in HCF crosses involving mutations BB9*w*8 and BB*w*21 no significant patterns of conversion frequency changes with time of dehiscence could be demonstrated. These results agree with the findings of Helmi (1980) on conversion frequency changes with time in HCF and LCF crosses involving mutation BB*w*11. Such changes were shown to be irregular and variable between different crosses involving this mutation. When considering the 3 mutations BB9*w*8, BB9*w*9 and BB*w*21, the most interesting data on conversion frequency changes with time involve changes in PMS/MS ratios - 5:3/6:2 and 3:5/2:6. Much better data are available relating to the 5:3/6:2 ratio. This showed a significant decrease with the time of dehiscence in HCF crosses involving mutations BB9*w*9 and BB*w*21. Data were only available for 2 HCF BB9*w*8 crosses and in only 1 of these did the 5:3/6:2 ratio decrease with time. These changes in 5:3/6:2 ratio were

apparently attributable to changes in the absolute frequency of both 5:3 and 6:2 conversion octad classes: in all BB9*w*9 HCF crosses except 1 there was a consistent increase in 6:2 frequency with time while in all HCF crosses except 2 there was a consistent decrease in 5:3 frequency. In 2 of the 3 HCF crosses involving BB*w*21, the 6:2 conversion frequency rose with time while in only 1 cross did the 5:3 frequency decrease in such a manner. However, if the data from all 3 crosses are considered together, the mean 6:2 frequency showed a consistent increase with time while the mean 5:3 frequency showed a similar decrease. Although the 2 HCF BB9*w*8 crosses did not show a significant decrease in the 5:3/6:2 ratio with time of dehiscence, in both crosses the 6:2 frequency increased with time, while in only 1 cross did the 5:3 frequency show such a decrease. Helmi (1980) had largely attributed similar changes in the 5:3/6:2 ratio in HCF crosses involving mutation BB*w*11 to decreases in the 5:3 class only. However, in this work involving mutations BB9*w*8, BB9*w*9 and BB*w*21 (which along with BB*w*11 are either closely linked or more probably are re-isolations of the same mutation) absolute changes in the 6:2 frequency are also implicated. Indeed, in this work the evidence for increases in the 6:2 frequency with time was stronger than evidence for decreases in the 5:3 frequency. Some evidence was also accumulated for similar decreases in the 3:5/2:6 ratio with the time of dehiscence, but this was far less strong and in all cases failed to reach statistical significance. In the 2 HCF crosses involving BB9*w*8, the 3:5/2:6 ratio decreased sharply with time, but when the overall data from both crosses were considered, these differences in 3:5/2:6 ratio between the 3 dehiscence periods

just failed to reach statistical significance at the $p = 0.05$ level. It would be extremely valuable to obtain and analyse data from further HCF BB9*w*8 crosses to see whether a statistically significant decline in the 3:5/2:6 ratio with time could be demonstrated. In 5 of the 7 HCF crosses involving BB9*w*9 the 3:5/2:6 ratio was smaller in the late period than in the early period of spore dehiscence, but this decrease was not always continuous through the middle period. The mean 3:5/2:6 ratio for all BB9*w*9 HCF crosses decreased with the time of dehiscence, but this decrease was not statistically significant. In 2 out of 3 HCF crosses involving mutation BB*w*21 the 3:5/2:6 ratio decreased with time of dehiscence, but when the mean ratio for all 3 crosses was considered this was not so. Furthermore, these overall changes in the 3:5/2:6 ratio with time were not statistically significant. Thus some evidence was obtained for changes in the 3:5/2:6 ratio with time, but this was only suggestive and not statistically significant. It is likely that one of the main reasons for this is that 3:5 and 2:6 octads (particularly the former) are much rarer than 5:3 and 6:2 octads. The small numbers scored in each period would be much more likely to show random, sampling variations which would tend to mask any real tendency for 2:6s to increase with time and 3:5s to decrease. Probably for similar reasons no changes in the 5:3/6:2 and 3:5/2:6 ratios with time were detected in LCF crosses involving mutation BB9*w*9. No suitable LCF cross data were available for analysis involving mutations BB9*w*8 and BB*w*21. Overall, the similarity of data from crosses involving BB9*w*8, BB9*w*9, BB*w*11 and BB*w*21 makes it more likely

that all 4 mutations are in fact re-isolations of the same mutation. If this is so, any effects on their conversion frequency changes with time could either be allele specific or locus specific. The latter explanation is favoured since all 3 locus I mutations investigated prior to this work all showed roughly similar decreases in conversion frequency with time (Wickramaratne, 1974; Helmi, 1980; Lamb, personal communication). However, it should be remembered that locus II mutation NG*w*18 (unlinked to BB9*w*9, etc.), which shows a roughly similar conversion spectrum to BB9*w*9 in HCF crosses, also showed similar changes in conversion frequency (at least in terms of the 5:3/6:2 ratio) with time in this experiment. Thus, for those mutations studied in which 6:2 octads were the most frequent conversion class, followed by the 5:3s, a similar decrease in the 5:3/6:2 ratio with time was observed. It would be most interesting to investigate whether this feature is observed in crosses involving other mutations with similar conversion spectra, or whether decreases in the 3:5/2:6 ratio with time might be clearly seen in crosses involving mutations with 2:6s and 3:5s as their most frequent conversion classes. It is conceivable that both ratio changes always occur, but where the relevant octad classes are rare, cannot easily be detected. This would occur as a general phenomenon if the mispair correction process in H-DNA becomes more efficient or has a longer period in which to operate as crosses reach late stages of maturity and spore liberation.

Changes in the conversion frequency of mutation *w*BHj with time had not been investigated prior to this work. In HCF crosses there was an apparent tendency for *w*BHj conver-

sion frequency to decrease with time of dehiscence, particularly during later stages of cross fertility. There was a definite suggestion that this overall decrease was preceded by an initial increase in conversion frequency in some crosses. However, statistical analysis revealed that this apparent general decrease in conversion frequency with time was not statistically significant. In LCF crosses involving mutation *w*BHj there was a much more definite pattern of conversion frequency change with time. In 5 out of 7 such crosses the conversion frequency sharply rose from the early to the middle periods, followed by an even sharper decrease. This statistically significant pattern of conversion frequency change was quite novel, not having been observed as a general pattern for any other mutations in the Pasadena strains of *Ascobolus immersus*.

No significant changes in *w*62 conversion frequency with the time of dehiscence in both HCF and LCF crosses were observed in this work. In the HCF crosses the mean conversion frequency and the mean frequency of the most abundant conversion class (the 6:2) decreased with time, but these changes were not significant. Similar non-significant conversion frequency decreases with time were observed in the LCF crosses. These results are in full agreement with the data of Lamb (personal communication; partly communicated by Helmi (1980)).

In the 2 HCF crosses involving mutation UV*w*M7, conversion frequency was observed to decrease with time. This change in conversion frequency was statistically significant, as were the conversion frequency differences between

the 2 crosses. The decrease in % NRCF with time was paralled by a decrease in the frequency of the most abundant conversion class, the 6:2, but no clearcut trend in the frequency of the second most abundant class - the 2:6 - was discernible. Mutation UV*w*M7 is closely linked to locus I and may even be part of it. From the limited data available in this experiment it would seem that UV*w*M7 shows the same pattern of decrease in conversion frequency with time already demonstrated for the locus I mutations *w*10, *w*78 and *w*3C1 in HCF crosses (Wickramaratne, 1974; Helmi, 1980; Lamb, personal communication). Confirmatory data from further crosses would be most valuable. These decreases in NRCF are closely paralleled by decreases in the frequency of the most frequent conversion class - the 6:2 in the case of *w*10, *w*78 and UV*w*M7 and the 2:6 in the case of *w*3C1. Accordingly, it would seem that the tendency for conversion frequency to decrease with time is a trait which applies generally to locus I. From this it should not be concluded that this phenomenon will not be observed for any non-locus I mutations. Indeed, in this work there was a tendency for *w*BHj conversion frequencies to decrease with time of dehiscence in HCF crosses, but this was not statistically significant, while in LCF crosses there was a significant initial increase in conversion frequency followed by a decrease.

In conclusion, there was no overall pattern in conversion frequency change with time for mutations BB9*w*9 (BB9*w*8 and BB*w*21), NG*w*18 and *w*62 while distinct patterns were observable for *w*BHj (at least in LCF crosses) and UV*w*M7. However, for those mutations showing frequent PMS there were

changes in the 5:3/6:2 and to a lesser extent, the 3:5/2:6 ratios with time. Therefore, the pattern, or lack of one, in conversion frequency change with time differed between loci in the same organism. The pattern observed for locus I mutations is not general for all mutations in the Pasadena strains of *Ascobolus immersus*.

Changes in recombination frequency with time have been observed in other ascomycete fungi. In an analysis of linearly ordered asci in *Neurospora crassa* and *Sordaria fimicola* Lamb (1967) observed significant changes in second division segregation frequency and the relative frequencies between ordered classes 1 and 2 and between classes 3, 4, 5 and 6. He showed that these phenomena were due to a differential maturation and bursting of asci with different spore arrangements, rather than to real changes in crossover frequencies or spindle orientations during development. With maturation, second division segregation frequencies showed a significant decrease (*N.crassa, asco*), no change (*N.crassa, tan* spore) or significant increases (*S.fimicola, gray, hyaline*). Lamb explained some of these phenomena in terms of the metabolic activities of wild-type nuclei and their position or distribution in the ascus. MacDonald and Bond (1974) found in all crosses studied in *Sordaria brevicollis* a significant decline in the frequency of 2:2:2:2 asci with increasing age of the cross, implicating preferential dehiscence or production of this type of ascus. They concluded that the distribution of wild-type spores within the ascus is not the cause of the preferential maturation and consequent discharge of certain types of asci. Wickramaratne (1974) and Lamb (personal communication) observed decreases

in the frequency of both 6:2 and 2:6 asci with the time of dehiscence for crosses involving locus I mutations *w*10 and *w*78 in the Pasadena strains of *Ascobolus immersus*. Helmi (1980) showed that the main conversion classes in + × *w* HCF crosses - whether they be 6:2s or 2:6s - decreased with the time of dehiscence. This indicates that the number of wild-type spores in an ascus can be irrelevant to the observed changes in the frequency of conversion asci. In this work the observed decline in the 5:3/6:2 ratio with time of dehiscence in HCF crosses involving BB9*w*9, BB*w*21 and NG*w*18 and the similar observation for HCF crosses involving BB*w*11 made by Helmi (1980), suggest an increase in the correction efficiency at these sites with the time of maturity of the cross. Alternatively, correction may simply have longer in which to operate as the cross matures. In this work there was also some evidence for decreases in the 3:5/2:6 ratio with time, indicating that +/*w* mispairs can be repaired more efficiently/frequently to *w* as well as +. If the conversion parameter of correction frequency is variable during the dehiscence period of a cross, other parameters could vary also, accounting for the changes in the frequencies of different conversion classes. In an unpublished analysis, Lamb and Helmi showed that, on different H-DNA models, an increase of a conversion parameter could increase, decrease or not affect the frequencies of certain conversion asci. One possible explanation of the differences in conversion frequency change with time between loci is that one parameter changes value in the same direction for all loci, but their different initial

values for this parameter could result in an increase, decrease or no change in the conversion behaviour monitored. Alternatively, more than one "effective compound" is changing in concentration during apothecial maturation, with different compounds affecting different loci. A third possibility is that only one compound is changing concentration, but this affects conversion parameters at different loci in different ways. These explanations are not mutually exclusive, nor do they exclude the possibility of mutations in different loci showing the same sort of changes in conversion behaviour with time of dehiscence.

Similar variations in other factors during the spore discharge or release period have been observed in other fungi, e.g. Duncan and Galbraith (1973) reported that the dimensions of the basidiospores of certain fungi increased significantly, reached a maximum and finally decreased during the course of the dehiscence period; in *Panaeolus semiovatus* the dimensions of basidiospores produced on successive days were very different.

An important conclusion from these experiments and earlier studies (Wickramaratne, 1974; Helmi, 1980; Lamb, personal communication) is the need to harvest and score all asci produced during the complete dehiscence period of any cross if a true determination of the gene conversion frequency of any mutation segregating in that cross is to be obtained. The conversion data from a single cross may thus sometimes be a result of the pooling of non-homogeneous data from different maturity periods. Helmi (1980) pointed out that this could affect the accuracy of quantitative analyses based on such pooled data.

Chapter 3: Mutation and reversion studies

Mutagenesis and the mutants

Introduction

The main aim of these mutagenesis experiments was to induce and isolate white ascospore colour mutations of different mutagenic origins closely linked to the three mutations whose conversion control systems were investigated in PART ONE of this thesis. Such a range of mutations at the three loci concerned would facilitate studies leading to a more complete understanding of the genetic controls of gene conversion at these sites. Such studies would involve an investigation of the action of a given conversion control factor on a set of linked ascospore colour mutations of different mutagenic origins (i.e. of different molecular natures); the frequency and extent of co-conversion; the occurrence of polarity in conversion and the nature of any such polarity. The conversion behaviour of those spore colour mutations not linked to any of the sites under study is also of interest in that if the conversion behaviour of any particular mutation differs significantly in crosses to different derived wild-type strains, this might provide preliminary evidence for further genetic conversion control systems.

A further aim was to obtain outside markers flanking the BB9*w*9, *w*BHj and NG*w*18 mutations studied in PART ONE. Such flanking mutations would also facilitate studies leading to a better understanding of the genetic controls and mechanism of gene conversion at the sites concerned,

by allowing an investigation of the relationship between gene conversion at a particular site and the associated recombination frequency in adjacent intervals. The most suitable flanking marker mutations would map from 2 to 10 (or even 20) recombination units from the site in question and would be those whose segregations can be scored relatively easily, e.g. morphological, auxotrophic, drug-resistant and some categories of granular ascospore colour mutations (in the latter case the wild-type red pigment is localized in granules rather than being evenly distributed over the spore wall). Granular mutations are usually less useful than the other categories since, for most of the mutations studied, white is epistatic to granular so that granular can only be visualized in w^+ genotype spores. However, such mutations are useful in the detection of wider ratio conversion octads (Lamb, 1972) and other unique conversion classes, such as 6+:2*w* and 2+:6*w* octads arising from postmeiotic segregation and in the detection of ab4+:4*w* octads (Ghikas and Lamb, 1977).

Ascospore colour mutants were detected by scanning collecting lids from wild-type x wild-type crosses (which may have been subjected to mutagenesis) for octads containing 4 wild-type and 4 ascospore colour mutant spores. Single mutant spores from such octads were isolated, germinated and crossed to wild-type to check for one-gene segregation, namely the production of 4+:4*m* octads as the main pattern. Auxotrophic and drug resistant mutants were screened for in appropriate ways, which are described below, while morphological mycelium growth mutants were detected

by observing their growth pattern shortly after ascospore germination.

Spontaneous mutation frequency

In *Ascobolus immersus* a relatively high frequency of spontaneous forward mutation from wild-type (red) to white ascospore colour has been reported by different authors, and the same phenomenon was observed in this work. The data from all these studies, involving both the Pasadena and European strains, are shown in Table 47. This high frequency of spontaneous mutation (0.06% in this work) probably results from there being a large number of genes specifying spore colour in *Ascobolus immersus*. Indeed, in European strain *28*, 75 loci specifying ascospore colour have been mapped (Nicolas et al. 1981). In calculating the spontaneous mutation frequency it should be remembered that both parental strains are subjected to mutagenesis such that a 4+:4*w* octad from a + × + cross, may result from a mutation present in either parental nucleus which, through fusion, produces the ascus. The spontaneous mutation frequency is therefore defined as the proportion of mutated nuclei among a population of nuclei and is equal to one half the frequency of 4+:4*w* octads observed in a cross. This value is only an estimate of the true spontaneous mutation frequency since there may be selection for or against the mutated nuclei and each 4+:4*w* octad harvested from a cross may not arise from an indeperdent event, due to nuclear multiplication following mutagenesis. For induced mutations, where only one parental strain is subjected to mutagenesis, the induced mutation frequency is equal to the frequency of

4+:4ω octads minus the spontaneous mutation frequency for that cross. Again, the value obtained is only an estimate for the same reasons as outlined above.

TABLE 47: Estimates of the frequency of spontaneous white ascospore colour mutation in *Ascobolus immersus*.

Wild-type strains	Author	Estimated frequency (%)
European	Lissouba et al.(1962)	0.05
"	Leblon (1972a,b)	0.001
Pasadena	Stadler, Towe and Rossignol (1970)	0.02
"	Wickramaratne (1974)	0.01-0.8
"	Ghikas (1978)	0.07-0.1
"	Helmi (1980)	0.07-0.7
"	Webb, B.Sc. Project (1981)	0.04-0.06
"	Present work	0.06

In this work, the frequencies of spontaneous granular, morphological and auxotrophic mutations were not estimated, but Helmi (1980) gave estimates of 0.001 to 0.07% for granular mutations and 1.0% for morphological mycelium growth mutants.

Mutagenesis experiments

1. Induction with chemicals.

The three chemical mutagens used were N-methyl-N'-nitro-N-nitrosoguanidine (NG), ethyl methanesulphonate (EMS) and

acridine mustard (ICR-170), with concentrations of 100 µg/ml, 12.1 mg/ml (0.1M) and 40 µg/ml respectively. All three mutagens were dissolved in pH 7.0 phosphate buffer (41.3% (v/v) of 1/15 Molar $KH_2\ PO_4$ and 58.7% (v/v) of 1/15 Molar $Na_2\ HPO_4.2H_2O$). The same basic method was followed for all three mutagens: one derived wild-type parental strain was grown on crossing medium in a 9 cm diameter petri dish, while the other strain (to be mutagenised) was grown on cellophane overlaying crossing medium in a 4 cm diameter petri dish. All plates were incubated at 17.5°C until the cellophane discs became covered with the growing mycelium, usually 2-3 days. The cellophane with the culture was peeled off and immersed (mycelium up) in a dish which contained the mutagenic solution, in the dark, for 10, 180 or 120 minutes, for NG, EMS and ICR-170 respectively. The cellophane bearing the treated culture was washed three times with liquid complete medium and transferred on to the crossing medium (mycelium down) where the other parent was growing, on the opposite side of the petri dish. The cellophane was removed after about 8 hours, leaving behind the treated culture. The crossing plates were then incubated normally at 17.5°C until the crosses were fertile and ascospores could be harvested.

It is important to note that all these mutagenesis experiments were carried out in a fume cupboard. All of the mutagens are highly dangerous and EMS is particularly volatile. The mutagen solutions were also prepared in a fume cupboard.

NG treated crosses showed a 14-fold higher mutation frequency than control crosses (see Table 48). This difference in mutation frequencies was shown to be highly

TABLE 48: Frequency of NG induced ascospore colour mutations

Cross 25-5R+ × 7R7-	Total octads	No. of 4+:4w octads	%4+:4w octads	Mutation frequency (%)	
				Spont-aneous	Induced
Control	17,785	21	0.12	0.06	-
NG treated (1 parent)	6,340	60	0.95	0.06*	0.83

* presumed frequency

significant by applying the χ^2 homogeneity test ($\Sigma\chi^2 = 95.83$, $p < 0.01$, 1 df). This 14-fold increased value is in line with the findings of Ghikas (1978) who observed that NG-treated crosses exhibited a 10-14-fold higher mutation frequency than control crosses. However, Ghikas (1978) used different derived wild-type strains in her experiments (9r+ and 61-), only half the NG concentration used in this work, and a slightly different mutagenesis method (the cellophane + mycelium discs were floated in mutagen, rather than being immersed as in this work). No estimates of EMS or ICR-170 mutation frequencies were obtained.

2. Induction with UV.

A similar method of preparing the mycelium of one parent for mutagenesis was used in these experiments, i.e. growing the culture on a cellophane disc overlaying crossing medium in a 4 cm diameter petri dish for 2-3 days. The

cellophane plus growing culture was then peeled off and exposed to 254 nm UV light for 30 seconds, using a CC-22 Chromato Vue UV cabinet (the dose rate at the position of the exposed culture was about 800 ergs/sec/cm^2). This dose was chosen because it gave the highest yield of ascospore colour mutations (Mafi, personal communication). The frequency of such mutations was higher at greater UV doses, but the crosses were far less fertile, while the converse was true at lower UV doses. After mutagenesis the treated culture was transferred (mycelium down) to one side of a 9 cm diameter crossing plate which contained the growing mycelium of the other parental strain. After 8 hours the cellophane disc was peeled off, leaving the mutagenized culture in place. The crossing plates were incubated at 17.5°C until the crosses were fertile and the ascospores could be harvested.

In these experiments, UV mutagenized crosses showed a 3-4-fold higher mutation frequency than control crosses (see Table 49). These differences in mutation frequencies were shown to be highly significant by applying the χ^2 homogeneity test ($\Sigma\chi^2 = 18.21$, $p < 0.01$, 1 df). As already mentioned, higher UV doses would have given a higher mutation frequency, but the overall yield of ascospore colour mutants would have been lower.

TABLE 49: Frequency of UV induced ascospore colour mutations

Cross 25-5R+ 7R7-	Total octads	No. of 4+:4w octads	%4+:4w octads	Mutation frequency (%)	
				Spon-taneous	Induced
Control	17,785	21	0.12	0.06	-
UV treated (1 parent)	13,352	46	0.34	0.06	0.22

No direct comparisons can be drawn with the estimated UV induced frequency of ascospore colour mutations (0.34%) obtained by Ghikas (1978) because she used a different method of mutagenesis and a different UV dose.

The above methods of mutagenesis by treating the mycelium were efficient and straightforward for obtaining ascospore colour mutants, because these mutants could be seen and isolated directly from the collecting lids of the treated crosses. However, these methods have one main disadvantage in that mutated nuclei may divide mitotically for quite a long period prior to crossing. Accordingly, a large number of the isolated mutations can, in fact, be isolations of the same mutation, increasing the labour of isolating different mutations. Furthermore, if some of the hyphae containing mutated nuclei are included in the formation of the subhymenium, these mutations will remain undetectable.

Isolation of morphological and auxotrophic mutants

The ascospores of *Ascobolus immersus* are among the largest fungal spores, ranging from about 50 to 60 µm long and 30 to 35 µm wide (Lamb, 1981). Such large spores make the isolation of auxotrophic mutants difficult, because the quantity of nutrients carried over from the parents into the spores is large enough to support the growth of mutant germlings on minimal medium for long enough to make immediate selection impossible. Asynchronous ascospore germination complicates the matter still further. Furthermore, the filamentous widely spreading growth of the wild-type colonies of *Ascobolus* is a particular disadvantage when attempting to apply selective plating techniques. Helmi (1980) found that nutrients contained within the ascospore were sufficient to support growth of the germling in distilled water for up to two days after germination, and that there was no difference in size between colonies growing in distilled water or liquid complete medium during this period. Accordingly, the total isolation method was found to be the most straightforward and efficient (though the most laborious) method to use for the isolation of both morphological and auxotrophic mutants. This procedure consisted of plating spores from a mutagenized cross, at a suitable dilution, on plates of the appropriate germination medium. Morphological growth mutants were easily distinguishable under the dissecting microscope soon after germination, and were isolated into small tubes of stock culture medium. The remaining germinating spores were individually isolated and inoculated, widely spaced, on to

minimal medium. After incubating at 37°C for 24 hours, any further morphological or slow growing mutants were isolated. The colonies growing on minimal medium were subcultured again on to fresh minimal medium and incubated for a further 24 hours at 37°C. Following this, any relatively small colonies were isolated and transferred into small tubes of stock culture medium for later confirmatory tests of auxotrophy and final characterization of growth requirements by inoculating on to plates of minimal medium supplemented by the Holliday "pools" of nutrients (Holliday, 1956). Any colonies which failed to grow on incubation at 37°C were incubated at 25°C overnight to detect any temperature sensitive mutants.

An attempted application of the filtration enrichment technique to obtain auxotrophic mutants

In *Ascobolus immersus*, the asynchronous germination of ascospores and the large amount of nutrients that such spores contain, has hindered the application of selective methods of obtaining auxotrophic mutants, such as filtration enrichment. Furthermore, *Ascobolus immersus* does not produce any asexual spores, which are generally far smaller than sexual spores, thus avoiding most of the problems of parental carry over of nutrients. However, Dr. Lamb (personal communication) suggested breaking off the first few cells soon after septation of the germination tubes, separating these cells, and proceeding as usual, treating the cells as conidia in *Neurospora crassa*. The basic

filtration enrichment method involves the suspension in minimal medium of germinating fungal spores (or hyphal fragments, as in this experiment) from a suitably mutagenized cross, incubating and passing this liquid culture through a filter with a suitable pore size to remove growing prototrophic colonies. As a result, the medium contains an enriched concentration of auxotrophic mutant propagules, as well as other slow growing mutants. After a suitable number of periodic filtrations, the remaining propagules can be plated on to solid complete medium to be isolated and characterized. This method was first applied to the filamentous fungus *Ophiostoma multiannulatum* by Fries (1947), but has since been successfully applied to *Neurospora crassa* (Catcheside, 1954; Woodward et al. 1954), *Saccharomyces cerevisiae* (Takahashi, 1959) and *Coprinus lagopus* (Day and Anderson, 1961). Helmi (1980) first attempted to apply the filtration enrichment method to *Ascobolus immersus*, but did not meet with success. In this work, Helmi's original method was modified and was as follows: wild-type ascospores were harvested from UV mutagenized wild-type × wild-type crosses. These ascospores were germinated in 200 ml liquid horse dung medium by applying 2 hours' heat shock at 50°C and incubating the liquid culture overnight at 25°C. This incubation time allowed the ascospore germination tubes to septate. At this stage the hyphae were fragmented by blending for 1 - 2 minutes at speed 7 in a Sorval omni-mixer. The resulting culture, which contained a mixture of non-germinated spores, small germinating colonies and hyphal fragments, was

centrifuged for 5 minutes at 6000 r.p.m. to remove the non-germinated spores, large hyphal fragments and germinated spores with hyphal fragments still attached. The supernatent, which contained the hyphal fragments (usually 2 or 3, but sometimes 1-celled), was filtered through a Whatman No.1 filter paper (sometimes this was replaced by a 0.8 μm pore-sized Metricel membrane filter). The filter and hyphal fragments were washed through with 400 ml liquid minimal medium (to remove any traces of the horse dung medium and nutrients leaked from any broken cells) and the hyphal fragments resuspended in 250 ml liquid minimal medium. This suspension was gently shaken for 24 hours at laboratory temperature (22° - 27°C). By this time, growing colonies could be observed in the culture and were filtered off by passing through a 500 μm or 305 μm pore-sized nylon filter (Simon Products Ltd.), depending on colony size. The filtrate was incubated again with continuous gentle shaking for a further 24 hours; a second filtration was performed, followed by a third after a further 24 hours' incubation. All incubations were carried out in a sterile UV-illuminated cabinet to reduce the risk of contamination. Subsequent to the final filtration through nylon, the filtrate was passed through a Whatman No.1 filter paper (or 0.8 μm pore-sized Metricel membrane filter) and the hyphal fragments collected on this filter resuspended in 20 ml liquid complete medium. The resulting suspension of hyphal fragments was plated out on solid complete medium and incubated overnight at 37°C. The above method was repeated many times and each step carefully monitored and

modified accordingly. Despite this, few colonies could be recovered at the final stage, and none of them turned out, after testing on minimal medium, to be auxotrophic. Initially contamination was a problem, but this was overcome without adding any antibiotics to the liquid cultures by carrying out all incubations in a UV-illuminated cabinet and all filtrations and platings under aseptic conditions. Reasons for the lack of success of the filtration enrichment technique in this work could include starvation death of any auxotrophic propagules by prolonged incubation in liquid minimal medium. There was also a general problem of the lack of regrowth of hyphal fragments on solid complete medium at the end of the experiment. In later trials, such hyphal fragments were incubated in liquid complete medium for 6 hours at 25°C prior to plating on solid medium. This did stimulate further growth on solid medium to some degree. Since these experiments, other workers (including Dr. S. Helmi, Dr. S. Hiremath, Dr. B.C. Lamb and various 3rd-year B.Sc. project students) have continued with trials of the filtration enrichment method and it seems likely that with careful refining of each step it will ultimately prove successful.

Isolation of drug resistant mutants

An experiment was carried out to obtain strains of *Ascobolus immersus* resistant to the drug DL-p-fluorophenylalanine. A drug concentration of 1.25mg/250ml medium was used, this concentration having originally been used

in similar studies by Stadler, Towe and Rossignol (1970). The experimental method was as follows: wild-type ascospores were harvested from UV- or NG-mutagenized wild-type × wild-type crosses and germinated in liquid horse dung medium. Germinating spores were filtered off by passing this liquid culture through a Whatman No.1 filter paper and resuspended in 20 ml liquid complete medium. Warm (37°C) complete agar medium containing the drug was poured into clean, sterile petri dishes each containing a few mls of the germinating spore culture. These plates were incubated overnight at 37°C after which time drug resistant mutant colonies could be seen growing on the surface of the medium, while the wild-type germlings were apparently prevented from growing by the drug at the concentration used in this experiment. Thus drug resistant mutant colonies could easily be isolated from the surface of the solid medium.

The mutants

1. White ascospore colour mutants.

Altogether 21 mutants of this type were isolated, the majority resulting from UV-induction, but some arose spontaneously while others were NG-induced. Apparently white ascospores were isolated from 96 4+:4ω octads, but it was only possible to germinate a fraction of these ascospores, despite trying a whole range of germination treatments on each spore. This was only to be expected since in Chapter 1 of PART TWO of this thesis it was shown that the optimum germination conditions for a small range of white ascospore

colour mutants were radically different. Sometimes it was possible to germinate an ascospore on a second or third different germination treatment, but this was frequently not so. It is quite probable that exposing a particular ascospore to unfavourable germination conditions could render it less likely to germinate under appropriate conditions. This category of mutants was the most sought after in this study, since it was hoped to obtain white ascospore colour mutants of various mutagenic origins closely linked to the white sites whose conversion control characteristics were investigated in PART ONE of this thesis. All of the white mutations induced and/or isolated were autonomously expressed in the ascospore, i.e. all the white mutations segregated as simple Mendelian factors in + × *w* crosses, giving 4 red:4 white ratios in the ascus. The selection procedure employed in this experiment would be unable to detect non-autonomous white ascospore colour mutants. However, in + × *w* crosses involving such mutations, the zygote is a diploid, heterozygous at the *w* site in question. Accordingly, it might be expected that the + allele could make up for the missing product of the *w* allele, producing 8+:0*w* asci. Since no non-autonomous mutations have been found in the Pasadena strains, it is likely that the white spore phenotype is not the result of blocking any of several steps in the biochemical pathway of wild-type pigment synthesis, but a result of mutations affecting the ascospore wall layers (probably the episporium, according to Van Brummelen (1968)) required for the binding of the red pigment. This

is supported by the fact that most white ascospores are smaller in size than the wild-type and in some genotypes, e.g. *w*78, UV*w*M7 and NG*w*11 the ascospores collapse shortly after dehiscence. It would seem that such genotypes lack an ascospore wall layer, probably the episporium. In this work, certain white ascospore genotypes, e.g. BB9*w*9 and NG*w*18 had abnormally thick spore walls. Again, the mutations concerned would seem to be producing abnormal spore wall layers unable to bind the wild-type red pigment.

2. Granular ascospore colour mutants.

Altogether 6 mutants of this type were isolated, 2 having arisen spontaneously while 4 were UV-induced. Apparently granular ascospores were isolated from 14 octads but it was not possible to germinate over one half of the spores isolated. It is interesting to note that some mutants were isolated as white ascospore colour but were subsequently shown to be granular in crosses to derived wild-type strains. The red pigmentation in granular ascospore mutants is, as the name implies, restricted to a number of particles which appear attached to the spore wall. These granules were variable in size and number, not only among different granular mutants, but certain mutants were also variable in the expression of the mutation, some ascospores having many small granules with almost a wild-type appearance, while other ascospores contained only a few, large or small granules, appearing almost white. All of the granular mutants isolated in this work behaved like those described by Ghikas (1978), in that they were

hypostatic to all white mutations tested, i.e. spores of genotypes *w*, *gr*$^+$ and *w,gr* could not phenotypically be distinguished from each other since both types appeared white and smooth spore walled. It is possible that in the case of such hypostatic granular mutations the missing or abnormal spore wall layer due to the epistatic white mutation is the same one from which these granular particles originate or bind. It would be interesting to investigate whether a particular granular mutation is hypostatic to a whole range of different white mutations. It is conceivable on the basis of the above theory that the same granular mutation could be expressed in the presence of certain white mutations but not of others. This would apply in the case of white mutations which affected different spore wall layers, or the same layers in different ways (e.g. absence of the layer or abnormality of its structure).

3. Pink ascospore mutants.

4 mutants of this type were isolated, all having resulted from UV-induction. When initially isolated, 2 of these mutants appeared to be white, only revealing their true nature in subsequent crosses to derived wild-type strains. The expressivity of all 4 mutations studied in this work was variable, and particularly so in the case of 3 mutations, i.e. in + × pink crosses the 8 spores of some octads showed a continuous gradient from wild-type red to almost white, with no clear segregation discernible,

while in other octads the segregation of 4 red:4 pink was clearly scorable. In the case of only 1 of the 4 pink mutant strains were clear segregations scorable in most octads arising from + × pink crosses. Due to this variable expression of most pink ascospore colour mutations, it is best to avoid their use in gene conversion studies since octad classes cannot reliably be scored. It is interesting to note that Lamb (1976) observed a phenomenon of competition for pigment between ascospores in the same ascus: in + × *w*78 crosses the 6 red spores in 6+:2*w* octads were paler than the 4 red spores in 4+:4*w* octads, while the 2 red spores in 2+:6*w* octads were the darkest of all. This phenomenon was seen in this work in octads arising from + × *w*BHj crosses, but did not seem to apply universally to crosses involving other white mutations.

4. Clock morphological mycelial growth mutants.

3 UV induced mutants of this type were isolated. These mutants form concentric growth rings, the first one appearing approximately 24 hours after inoculation with germinating spores or small pieces of mycelium. The growth rings are composed of highly branched, dense hyphae alternating with thinner and faster growing hyphae. These mutants resemble the clock mutant of *Neurospora crassa* described by Sussman, Lowry and Durkee (1964) and were first observed in *Ascobolus immersus* by Yu-Sun (1964b)

5. Compact morphological mycelial growth mutants.

5 UV induced mutants of this type were isolated. All exhibited a very restricted, dense growth habit, growing at less than one-tenth the rate of wild-type strains. Only 2 of these mutants produced fertile crosses, enabling them to be used in mapping studies.

6. Drug resistant mutants.

2 mutants resistant to the drug DL-p-fluorophenylalanine were isolated. Both mutants proved to be rather unstable and "leaky" and thus were of limited value in mapping studies.

7. Auxotrophic mutants.

2 auxotrophs were isolated, but both were highly "leaky" and thus would not make reliable markers in any experiments. Due to the "leaky" nature of both mutations it was not possible to determine their growth requirements using minimal medium supplemented with the pools devised by Holliday (1956).

Mapping the mutants

In the preceding experiments 21 white, 6 granular and 4 pink ascospore colour mutants were isolated, together with 8 morphological, 2 drug resistant and 2 auxotrophic mutants. The white ascospore colour mutants were of primary interest since the main aim of these experiments was to induce and isolate mutations closely linked to the

white ascospore colour mutations whose conversion behaviour was investigated in PART ONE of this thesis. However, it was also of interest to determine whether any of the other ascospore colour mutations were closely linked to the same sites. Initially each mutant strain was crossed to derived wild-type strains of both mating types to confirm the genotypic spore colour mutant nature of each strain. In this way it was discovered that some mutants which had initially appeared phenotypically white, were shown to be genotypically granular or pink ascospore mutants. Subject to the availability of strains of appropriate mating type, all white mutant strains were intercrossed, as were all granular and pink mutant strains. This was to determine which, if any, mutations were re-isolations of the same mutation. Only about half of all the crosses performed were fertile: rather surprisingly, no mutations turned out to be re-isolations of the same mutation. Furthermore, none of the mutations appeared linked to each other. The tests for linkage were carried out by tetrad analysis, scoring the numbers of parental ditype (PD), non-parental ditype (NPD) and tetratype (T) octads (Plates 3 to 6) and obtaining the recombination frequency from the formula:

$$\text{R.F.} = \frac{(\text{NPD} + \frac{1}{2}\text{T}) \times 100}{\text{P} + \text{NPD} + \text{T}} \quad \%$$

All the ascospore colour mutations isolated in this work were tested for their linkage, not only to the BB9*w*9, *w*BHj and NG*w*18 (locus II) mutations studied in PART ONE of this thesis, but also to mutations of loci I, III and IV. Ascospore colour mutant strains isolated by Helmi

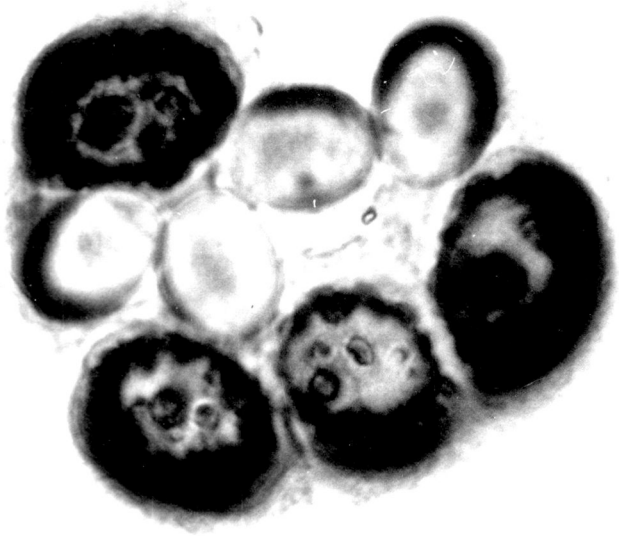


Plate 3: A 4*gr*:4*w* (PD) octad from cross BH27*gr*+ × 3*w*IDEC-. Mag. × 400

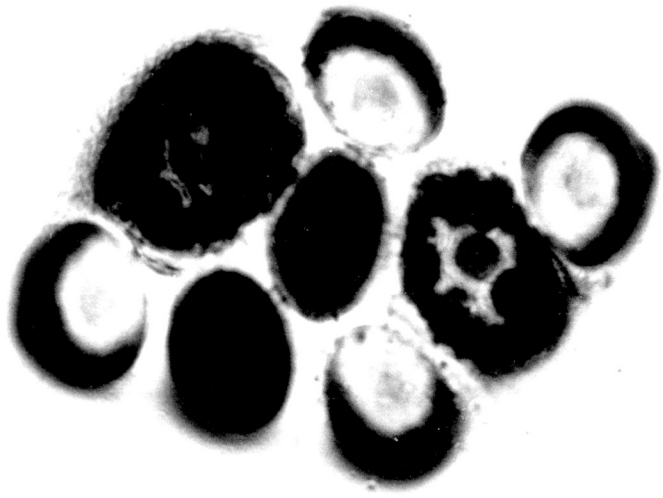


Plate 4: A 2+:2*gr*:4*w* (T) octad from cross BH27*gr*+ × 3*w*IDEC-. Mag. × 350.

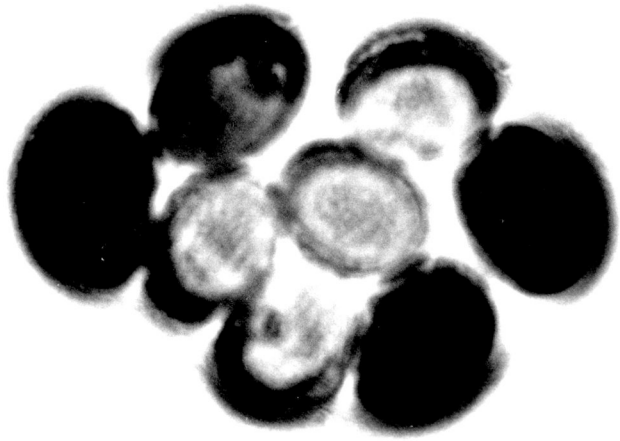


Plate 5: A 4+:4*w* (NPD) octad from cross UV*w*M7+ × *w*BHj-. Mag. × 400.

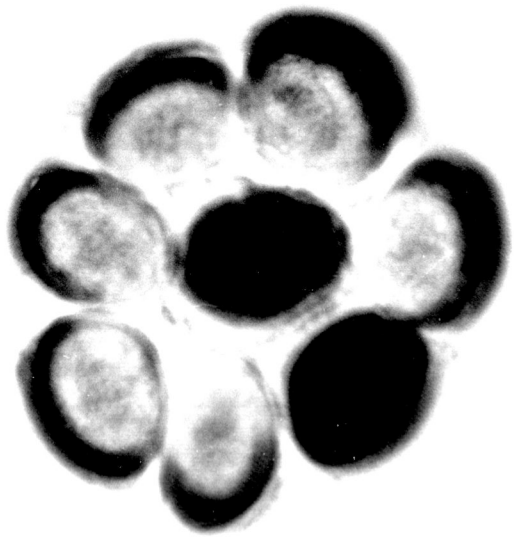


Plate 6: A 2+:6*w* (T) octad from cross UV*w*M7+ × *w*BHj-. Mag. × 400.

(1980) and Shirin Mafi were kindly supplied and were also used in these mapping experiments (the mutations carried by these strains had already been tested for their linkage to locus I). Again recombination frequencies were obtained from tetrad analysis, with less than 500 octads being scored in cases of non-linkage, but the largest possible sample sizes were scored when linkage was suggested. None of the mutations tested turned out to be linked to BB9*w*9, *w*BHj, NG*w*18 (locus II) or loci III and IV. However, mutation UV*w*M7 was shown to be very closely linked to locus I and may indeed be part of this locus. This mutation was UV-induced from a cross between derived wild-type strains 25-5R+ × 7R7-, both carrying *ccf*-2(*P*). This *ccf* is closely linked to locus I and is involved in the conversion control of locus I mutations. UV*w*M7 showed a recombination frequency of 5.12% with locus I mutation *w*78 in a *ccf*-2(*P*) homozygous cross, and a recombination frequency of 1.75% with mutation *w*3C1 in a *ccf*-2(*P*) × *ccf*-2(*91*) cross. These recombination frequencies probably largely differ because of the different *ccf*-2 genotypes of each cross, rather than the relative position of *w*78 and *w*3C1 in locus I, although this must make some contribution.

The 8 morphological mutants were tested for linkage to BB9*w*9, *w*BHj and NG*w*18 only, since these sites were of primary interest. It was not possible to use the drug resistant and auxotrophic mutants in these mapping studies, due to their unstable and leaky nature. In these mapping experiments, recombination frequencies were obtained by random spore analysis. Flanking markers 2-10 recombina-

tion units away from any of the 3 mutant sites were not detected. Indeed, all of the morphological mutants which produced fertile crosses were unlinked to all of the 3 mutations under study.

Conversion behaviour of newly isolated ascospore colour mutations

Each ascospore colour mutant strain isolated in this experiment was crossed to at least 2 different derived wild-type strains. This was to obtain preliminary evidence for any further conversion control systems not hitherto detected. Such evidence could be in either of two forms: a particular ascospore colour mutant strain might show different conversion frequencies or spectra in crosses to different derived wild-type strains. Alternatively, if a mutant strain gave a fertile cross with only 1 derived wild-type strain and this cross showed a high conversion frequency (say, of at least several percent in a cross with a reasonable sample size of dehisced octads), this might be due to the action of a particular conversion control factor. Since it was known that all the ascospore colour mutations isolated in this work (except UV*w*M7) were unlinked to BB9*w*9, *w*BHj, NG*w*18 (locus II) and locus I, if any mutations did show HCF it is more than probable that fresh conversion control systems could be responsible. Alternatively, one of the known systems could be non-specific to one target region after all. The most likely candidate might be the "Super" system (*ccf*-3, *ccf*-4) since this is the only conversion control system known to have an unlinked, or loosely linked target region in the Pasadena strains of *Ascobolus*

immersus (of course, in other organisms systems are known which seem to be specific to v.few unlinked targets, e.g. the *rec* genes in *Neurospora crassa* (Catcheside, 1977)).

Accordingly, all the ascospore colour mutant strains isolated in this experiment were crossed to at least 2 different derived wild-type strains. The data obtained from these crosses are displayed in Table 50.

From this Table it can be seen that 5 mutant strains gave fertile crosses with 2 or more derived wild-type strains, while a further 5 mutant strains gave fertile crosses with only one derived wild-type strain. The conversion data involving each ascospore colour mutant strain will be considered in turn:

S*w*M1. This mutation arose spontaneously and showed a low conversion frequency (of 0.15 and 0.22%) in crosses to both derived wild-type strains. It is difficult to accurately determine the conversion spectrum of mutations showing such low conversion frequencies (due to random, sampling variations in the small numbers of convertant octads and possible variations in phenocopy and false cluster frequencies). However, both crosses showed similar conversion spectra, with very frequent PMS (66.67 and 81.82%) and conversion almost equally to wild-type (50.00 and 53.33%) and to mutant. These data provide no evidence for any further conversion control systems.

NG*w*M1. This mutation was NG-induced and showed low conversion frequencies (of 0.20 and 0.32%) in crosses to both derived wild-type strains. The conversion spectra exhib-

TABLE 50: Conversion data from crosses involving newly isolated ascospore colour mutations and various derived wild-type strains

Mutant strain	Crossed to	Octad classes (+:*w*),%					%NRCF	Relative % PMS	%Conv. to +	Total octads
		4:4	6:2	2:6	5:3	3:5				
S*w*M1+	7R7-	99.57	0.04	-	0.07	0.11	0.22	81.82	50.00	2780
"	18P-	99.66	0.03	0.03	0.05	0.05	0.15	66.67	53.33	6549
NG*w*M1+	7R7-	99.75	0.05	0.05	0.10	0.05	0.20	75.00	75.00	4341
"	18P-	99.68	0.08	0.04	0.15	0.05	0.32	62.50	71.88	8644
UV*w*M3+	7R7-	99.76	-	0.05	0.10	0.10	0.24	83.33	41.67	2092
UV*w*M4-	25-5R+	99.59	0.20	0.10	0.10	-	0.41	24.39	73.17	3076
"	1114+	99.63	0.18	0.09	0.10	-	0.37	27.03	75.68	9982
UV*w*M7+	7114-	98.31	0.97	0.44	0.17	0.11	1.69	11.24	67.46	8326
"	16P-	97.70	1.09	0.19	0.82	0.17	2.28	43.42	83.77	4659
"	18P-	96.87	1.45	0.66	0.83	0.17	3.11	32.15	73.31	8191
"	7R7-	95.71	1.43	0.48	2.38	-	4.29	55.48	88.82	210
"	92-	97.77	0.94	0.77	0.41	0.09	2.20	22.73	61.36	6903
"	421-	99.50	0.26	0.06	0.12	0.04	0.48	33.33	79.17	5029
UV*w*M8+	7R7-	99.29	-	0.71	-	-	0.71	0.0	0.0	564
BH27*gr*+	18P-	98.74	-	0.63	-	-	0.63	0.0	0.0	559
UV*gr*M1+	7R7-	78.57	21.43	-	-	-	21.43	0.0	100.0	420
"	18P-	74.89	22.13	0.11	1.25	0.46	23.97	7.13	97.54	872
*2*gr*2S-	25-5R+	91.75	4.05	0.30	2.25	1.65	8.25	47.27	76.36	667
UV*p*M1+	7R7-	88.24	3.92	7.84	-	-	11.76	0.0	33.33	502

* Strain isolated by Helmi (1980)

ited by both crosses were also similar, with very frequent PMS (62.50 and 75.00%) and conversion predominantly to wild-type (71.88 and 75.00%). These data provide no evidence for any conversion control system acting on this mutation. However, it is interesting to note that the conversion spectrum of this mutation in both crosses performed is typical of a base substitution mutation (i.e. PMS is frequent) and it is widely held that NG largely induces such mutations (Leblon, 1972 a,b). Again, it should be noted that the observed conversion spectra for mutations showing LCF should not be regarded as very reliable.

UV*ω*M3. This mutation was UV-induced and showed a low conversion frequency of 0.24% in a sole fertile cross. Apparent PMS was extremely frequent (83.33%) while conversion occurred slightly more frequently to mutant than to wild-type (41.67%). The data from this single cross provide no insight into possible conversion control systems acting on this mutation.

UV*ω*M4. This mutation was also UV-induced and low converting (with % NRCFs of 0.37 and 0.41%) in both fertile crosses, one of which was to a "Super" derived wild-type strain, showing no evidence of conversion enhancement by the "Super" conversion control system. In both crosses this mutation showed less frequent, but still common, PMS (24.39 and 27.03%) and conversion overwhelmingly to wild-type (73.17 and 75.68%). The conversion data from both crosses showed good agreement, with no evidence for genetic differences in UV*ω*M4 conversion behaviour.

UV*w*M7. This mutation was UV-induced and shown to map very close to locus I, perhaps even forming part of that locus. Thus it was of great interest to determine its conversion behaviour in response to *ccf*-2 and the "Super" *ccfs*, all of which modify the conversion behaviour of locus I mutations (with the possible exception of UVk*w*8). The UV*w*M7 strain used in these crosses carries *ccf*-2(*P*) and in crosses to 4 *ccf*-2(*P*) carrying derived wild-type strains (16P-, 18P-, 7R7- and 92-) gave a conversion frequency range of 2.28 to 4.29%. The latter value was an extreme, coming from a cross giving a very small sample of dehisced octads. Excluding this cross, the conversion frequency range was 2.28 to 3.11%. This is far lower than the conversion frequencies of other locus I mutations (with the exception of aberrant UVk*w*8, which may be a large deletion (Ghikas, 1978)) in *ccf*-2(*P*) homozygous crosses. These range from 11-12% for *w*78, 10-11% for *w*3C1 to around 8% for NG*w*5 (Lamb and Helmi, 1978; Ghikas, 1978). However, UV*w*M7 does seem to be responding to the closely linked *ccf*-2 system since it showed a much lower conversion frequency of 0.48% in a cross to a *ccf*-2(*91*) carrying strain (421-). Again, this conversion frequency is much lower than usual for other locus I mutations in +, *ccf*-2(*91*) × *w*, *ccf*-2(*P*) crosses, but the important fact is that the conversion frequency is lower than that observed in *ccf*-2(*P*) homozygous crosses. It is probable that UV*w*M7 shows lower conversion frequencies in its apparent response to the *ccf*-2 factors than all other (except one) locus I mutations because it is not truly part of locus I and more importantly, is further away from the site of H-DNA formation at

this locus (presumed to be *ccf*-2; Helmi (1980)), so that H-DNA reaches the UV*w*M7 site less frequently than it reaches other, closer, locus I mutations. Accordingly, in all types of cross, UV*w*M7 would have less opportunity to convert although this opportunity would still be under the control of the *ccf*-2 factors. The same UV*w*M7 strain was also crossed to a "Super" derived wild-type strain (7114-), this cross giving a conversion frequency of 1.69%. This frequency is slightly lower than any frequencies observed in crosses to *ccf*-2(*P*) wild-type strains (2.28-4.29%) but is the same order of magnitude. Certainly there is no evidence for enhancement of the UV*w*M7 conversion frequency by the "Super" factors in this cross. This could be for two reasons: either the "Super" conversion control system is unable to enhance UV*w*M7 conversion frequencies, or the UV*w*M7 strain used in these experiments carries the dominant reducer "allele" of *ccf*-4 (one of the two "Super" factors) so that conversion could not be enhanced in crosses to any "Super" strains. The latter explanation is favoured, since "Super" is known to enhance the conversion frequencies of all locus I mutations tested, with the exception of aberrant mutation UVk*w*8. Experiments should be undertaken to distinguish between the two possible explanations of the lack of "Super" action on UV*w*M7 offered here. It is also of interest to note that mutation UV*w*M7 showed broadly the same conversion spectrum in all the *ccf*-2(*P*) homozygous crosses undertaken, together with the +, *ccf*-2 (*91*) × *w*, *ccf*-2(*P*) cross. Conversion was predominantly to wild-type (61.36 to 88.82%) and PMS was fairly frequent

(22.73 to 55.48%). However, in the cross to the "Super" derived wild-type strain, the conversion spectrum was rather different: conversion was still predominantly to wild-type (67.46%) but PMS was much rarer (11.24%). This reduction in PMS was largely due to a reduction in the relative frequency of 5:3 octads. If this effect has a genetic basis - which seems likely since large sample sizes were scored for all crosses except one - this could be due to the action of one of the "Super" factors (which seems unlikely) or another modifier of conversion behaviour, which may or may not be novel. Nearby cryptic heterozygosity in the "Super" cross would result in a reduction in the frequency of PMS, since heterozygous cryptic mutations can increase, but not decrease the efficiency of correction of a closely linked, observable mutation (Leblon and Rossignol, 1973; Lamb and Ghikas, 1979). This apparent variation in the conversion spectrum of UV*w*M7 merits further investigation.

UV*w*M8. This mutation was UV-induced and showed a low conversion frequency of 0.71% in a sole fertile cross. Since only a relatively small sample size of dehisced octads was scorable from this cross, the conversion spectrum (and indeed frequency) cannot be determined with much certainty. In the single, fertile cross all conversion octads were 2:6s. These data do not provide any information relating to possible conversion control systems acting on UV*w*M8.

BH27*gr*. This granular ascospore mutation arose spontaneously and in a single fertile cross showed a low conversion

frequency of 0.63%. Again, only a relatively small sample size was scorable from this cross and all apparent conversion octads were 2+:6*grs*. It is more difficult to determine granular conversion frequencies because some granular mutations show variable expressivity. However, this makes identification of octads with a majority of genuine wild-type spores difficult, since some granular spores may be so heavily granulated as to appear wild-type dark red. In this experiment, all conversion octads had an excess of granular spores and so were more likely to be true convertant octads or false clusters of spores. The data contain no evidence for the existence of a conversion control system.

UV*gr*M1. This mutation was UV-induced and showed very high apparent conversion frequencies (of 21.43 and 23.97%) in crosses to two derived wild-type strains. Most of the apparent conversion octads in each cross were 6+:2*grs*. Thus it is quite possible that many of these octads could genotypically be 4+:4*grs* for the reasons outlined above. However, the apparent conversion frequencies of UV*gr*M1 in both crosses show very close agreement. The possible conversion behaviour of this mutation might thus merit further attention. It would be most valuable to isolate the six apparently wild-type spores from a number of 6+:2*gr* octads and determine the true genotypic nature of these isolates by crossing to derived wild-type (or even white mutant) strains. If most octads were demonstrated to be genuine 6+:2*gr* octads, this would be the first demonstration of a high converting granular mutation in the Pasadena

strains of *Ascobolus immersus*. Furthermore, if conversion octads could be scored with reasonable certainty, the system governing the conversion behaviour of this mutation should be further investigated.

2*gr*2S. This mutation arose spontaneously and was isolated by Helmi (1980). In a sole fertile cross, the granular mutation showed a high conversion frequency of 8.25%. Like mutation UV*gr*M1, the most frequent conversion class was the 6+:2*gr*, but 5+:3*gr* and 3+:5*gr* octads were also fairly frequent. Again it would be informative to determine the true genotypic nature of these apparent conversion octads. If found to be genuine, this would be a demonstration of a second granular mutation that could show a high conversion frequency in the same strains of *Ascobolus immersus*.

UV*p*M1. This pink ascospore colour mutation was UV-induced and showed an apparent high conversion frequency of 11.76% in a single fertile cross. Most apparent conversion octads were 2+:6*ps*, but 6+:2*ps* were also frequent. It is extremely unlikely that most of these octads are true convertants because the segregation of pink mutations is notoriously difficult to score and this cross was no exception. This is due to the highly variable expression of most pink mutations complicated by competition for pigment among the spores of the same ascus, as described by Lamb (1976). It is best to avoid using most pink mutations in gene conversion studies.

In summary it can be said that 5 out of the 6 white ascospore colour mutations used in this study showed low conversion frequencies (of well below 1%) in crosses to one

or more derived wild-type strains. The sixth mutation was closely linked to locus I and was shown to apparently respond to the locus I closely linked *ccf*-2 factors, although with reduced conversion frequencies compared to all locus I mutations (except UVk*w*8). Further experiments should be performed to conclusively demonstrate this response to *ccf*-2 in crosses with all possible *ccf*-2 genotypes. A positive response of this mutation to the "Super" conversion control system was not proven. UV*w*M7 also showed conversion spectrum differences in the crosses performed which may have been due to genetic conversion modifiers and merits further attention. 3 of the 6 mutations showed conversion predominantly to wild-type, 1 mutation showed rough parity in conversion direction, while a further mutation exhibited slightly more frequent conversion to mutant and the remaining mutation showed conversion entirely to mutant (in a cross with a small sample size). In earlier studies involving both the Pasadena and European strains of *Ascobolus immersus* the direction of conversion ranged from overwhelmingly to wild-type to overwhelmingly to mutant, with no overall bias in conversion direction when considering the conversion behaviour of large numbers of mutations (Leblon, 1972a; Ghikas, 1978; Lamb and Ghikas, 1979). Only in the study of Yu-Sun (1966) was a significant overall bias in conversion direction to wild-type observed, but in this work the behaviour of relatively few mutations was investigated. 2 of the 3 granular mutations showed apparent high conversion frequencies which, if shown to be genuine, might indicate the presence of novel

conversion control systems. A single pink ascospore colour mutation showed an apparent high conversion frequency, but the true conversion frequency was almost certainly much lower than this. It would be unwise to use this mutation in further conversion studies.

Reversion studies

Introduction

One of the most certain methods of ascertaining the molecular nature of a mutation is by amino acid sequence determination of the polypeptide products of both wild-type and mutant genes. Such an approach was outside the scope of this thesis. A second method, which is less certain but more generally applicable, is based on the different frequencies of reversion of a mutation to wild-type obtained with different chemical mutagens. A "true" reverse mutation requires a particular change in the specific site which originally mutated, restoring the wild-type allele. Apparent back-mutation to wild-type may also be caused by "suppression" of the first mutation. Such suppressor mutations may be intragenic, e.g., when the primary mutation is the deletion of a base pair, the wild-type phenotype may be restored by a secondary mutation (the suppressor) such as the insertion of another base or the deletion of two more nearby. Suppressor mutations can also be intergenic (external) such as the *amber*, *ochre* and *opal* suppressor mutations.

Base substitution mutations tend to be reverted by mutagens that specifically cause base substitutions, while

addition frameshift mutations will be reverted by mutagens that tend to cause deletion mutations, and vice-versa. Large deletion mutations will not be reverted by any class of mutagen (Auerbach, 1976; Leblon, 1972a; Yu-Sun et al. 1977). The accuracy of the results obtained from reversion analysis is improved if the mutations under investigation are highly stable. This is generally true of white ascospore colour mutations in *Ascobolus immersus* where the spontaneous reversion frequencies are very low (Lissouba et al. 1962; Rossignol, 1969; Paszewski and Prazmo, 1969; Wickramaratne, 1974).

The main interest in this experiment was to determine the molecular nature of the mutations whose conversion control was studied in PART ONE of this thesis. This would be very useful since Lamb and Ghikas (1979) showed that it was not possible to infer the molecular nature of a mutation from its conversion spectrum alone, as was postulated by Leblon (1972a, b). The conversion control systems acting upon 3 unlinked mutations, were investigated in this thesis. These mutations were BB9*w*9 and *w*BHj, which were both UV-induced and thus could have any of a whole range of molecular types (Auerbach, 1976). The third mutation was NG*w*18, which was NG-induced and thus most likely to be a base substitution (Leblon, 1972a; Auerbach, 1976; Yu-Sun et al. 1977). Of these mutations it would have been most interesting to determine the molecular nature of BB9*w*9 since this shows a conversion spectrum characteristic of a frameshift mutation in LCF crosses (i.e. PMS is infrequent) and of a base substitution in HCF crosses, according to the criteria of Leblon (1972a, b). However, as outlined in

Chapter 2 of PART ONE, it was impossible - despite much labour - to obtain fertile self-crosses involving this mutation, and so such reversion analysis was not attempted. One possible alternative method suggests itself and this involves mutagenizing the white parent in + × *w* crosses. Any reversion of the white mutation would produce 8+:0*w* octads. Unfortunately such octads can also arise in + × *w* crosses from corresponding site conversion events, where all 4 chromatids are involved in H-DNA formation at exactly corresponding sites. Such 8+:0*w* conversion octads occurred at a frequency of 0.05% in HCF crosses involving BB9*w*9 in this work, but were much rarer in LCF crosses. Thus in the latter type of cross it might just be possible to distinguish conversion 8+:0*w*s from reversion 8+:0*w*s since the latter might show clustering on the collecting lid due to mitotic propagation of a reverted white nucleus in the mycelium prior to crossing. This mitotic propagation could be encouraged by performing the crosses in large dishes rather than 9 cm Petri dishes. Nevertheless, the procedure could be highly unreliable in estimating reversion frequencies and much wasted labour could be involved in attempting to determine the nature of any suspected revertants. Thus, in this experiment it was decided to concentrate upon reversion analysis of the UV-induced mutation *w*BHj, since this gave good, fertile self-crosses. The conversion spectrum of this mutation in both LCF and HCF crosses was supposedly typical of a base substitution mutation, according to the criteria of Leblon (1972a, b).

Methods

The methods of treating the mutant self-crosses were based on those of Leblon (1972a, b) and were fully described in the Mutagenesis section of this Chapter. Three chemical mutagens were used: NG, which is believed to primarily induce base substitution mutations; ICR-170, which is thought to primarily induce addition frameshifts, and EMS, which probably induces some deletion frameshifts, together with mainly base substitutions (Leblon, 1972a, b; Auerbach, 1976; Yu-Sun et al. 1977). The untreated control crosses were performed in the same way as the mutagenized crosses, except that the cultures growing on cellophane discs were immersed in pH7.0 phosphate buffer without mutagen. Both treated and untreated crosses were incubated at 17.5°C until fertile, and during the whole period of octad dehiscence. The octads were harvested on collecting lids and the total numbers collected scored, while screening for any octads which contained wild-type spores.

Results

The data obtained from the *w*BHj reversion experiments are displayed in Table 51, with reversion data concerning the same mutation obtained by Lamb and communicated by Helmi (1980). From this Table it can be seen that no octads containing *w*BHj revertant spores were observed either in this experiment or in the earlier experiments performed by Dr. Lamb. This could be for one of two reasons: either the sample sizes scored were not large enough, or mutation *w*BHj could be a large deletion which

TABLE 51: *w*BHj reversion data

Experiment No.	Spontaneous reversion	Reversion treatment Mutagen, concentration, time of exposure to mutagen		
		NG	ICR-170	EMS
		100µg/ml, 10 mins.	40µg/ml 2 hours	12.1 mg/ml 3 hours
1	0 revertants in 13,734 octads	0 revertants in 14,403 octads	0 revertants in 18,614 octads	0 revertants in 15,961 octads
		50µg/ml, 10 mins.		
2*	0 revertants in 12,727 octads	0 revertants in 12,479 octads	--	--
TOTALS	0 revertants in 26,461 octads	0 revertants in 26,882 octads	0 revertants in 18,614 octads	0 revertants in 15,961 octads

* Data obtained by Lamb and communicated by Helmi (1980)

could not be reverted by any of the mutagens used. Much larger sample sizes will need to be scored to distinguish between these possibilities. However, it would seem more likely that *w*BHj could be reverted if larger sample sizes were scored - in further data obtained by Lamb and communicated by Helmi (1980), no revertants of the spontaneous locus I mutation *w*78 were obtained from mutagenized crosses giving similar sample sizes. Mutation *w*78 is believed to be a base substitution, although this is by no means certain. Furthermore, in the same series of experiments, Lamb was unable to observe any revertants of the NG-induced locus IV mutation NG*w*11 in almost 40,000 octads

scored from an NG-treated self-cross.

Is it possible to obtain any information as to the molecular nature of mutation *w*BHj from the data obtained in this experiment? It is interesting to note that no *w*BHj revertants were obtained with the frameshift-inducing mutagens ICR-170 and EMS in sample sizes of over 18,000 and 15,000 octads for each treatment respectively. However, in the data of Lamb communicated by Helmi (1980), 4 4+:4*w* revertant octads involving mutation *w*3C1 were observed in 33,000 octads scored from an EMS mutagenized cross. In at least one of these octads, *w*3C1 reversion was due to the induction of a second intragenic frame-shift (suppressor) mutation, of opposite sign to the original *w*3C1 mutation. This suppressor mutation, also conferring a white mutant spore phenotype, was designated *w*Su$^+$. Mafi (personal communication) was able to revert this mutation by treatment with ICR-170. Again the reversion was due to the induction of an intragenic suppressor mutation which also conferred a white mutant spore phenotype. She was able to obtain such *w*Su$^+$ revertants from ICR-170 mutagenized crosses producing smaller samples of dehisced octads than were scored in this experiment, using comparable mutagen doses. Thus Lamb and Mafi were both able to induce reversion of frameshift mutations with relative ease. Since such revertants were not observed in this experiment, it seems far less likely that *w*BHj is a frameshift mutation than a base substitution or a larger deletion or addition. The results of the experiments performed by Lamb and Mafi are in agreement with the conclusion of Leblon and Paquette (1978) that ICR-170 and EMS

induce frameshifts predominantly in opposite directions in *Ascobolus immersus*, each commonly reverting mutations induced by the other but not mutations induced by itself. A further point to note is that no spontaneous revertants of *w*BHj were observed in more than 26,000 octads scored. This illustrates the stability of *w*BHj and supports the validity of gene conversion data involving this mutation. Although time did not permit reversion studies of mutation NG*w*18, in data obtained from other experiments, no spontaneous revertants of this mutation were observed in 32,486 octads scored. Thus NG*w*18 is also a stable mutation giving valid gene conversion data.

In summary, it can be said that although *w*BHj revertants were not observed in these experiments, from the data obtained it would seem more likely that *w*BHj is a base substitution mutation or an addition or deletion of at least several bases. The data were also valuable in demonstrating the stability of mutations *w*BHj and NG*w*18.

Conclusions

1. White ascospore colour mutations linked to BB9*w*9, *w*BHj or NG*w*18 were not isolated, while one such mutation proved to be closely linked to locus I.

2. No outside markers flanking mutations BB9*w*9, *w*BHj or NG*w*18 were isolated.

3. The conversion behaviour of newly isolated ascospore colour mutations was determined. The white mutation closely linked to locus I (UV*w*M7) apparently responded to

the *ccf*-2 factors. This mutation showed a variable conversion spectrum in crosses to different derived wild-type strains. 2 apparently high converting granular mutations were isolated. If genuine, these conversion frequencies could be under the control of novel *ccfs*. The white ascospore colour mutations showed a wide range of conversion direction, from overwhelmingly to wild-type to overwhelmingly to mutant.

4. Due to lack of fertile self-crosses, it was not possible to determine the molecular nature of mutation BB9*w*9 by reversion analysis, although in the absence of such fertile self-crosses an alternative method might be possible. Although no *w*BHj revertants were detected, the reversion data so obtained provide some evidence that *w*BHj is unlikely to be a frameshift mutation.

5. Mutations *w*BHj and NG*w*18 were shown to be highly stable since no spontaneous revertants were observed in the experiments undertaken.

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THE IDENTIFACTION AND PROPERTIES OF CONVERSION CONTROL FACTORS 5 and 6 IN THE PASADENA STRAINS OF *ASCOBOLUS IMMERSUS*

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Genetic controls of gene conversion in the Pasadena strains of *Ascobolus immersus* have been described for white (*w*) mutations at ascospore pigmentation locus I (Lamb and Helmi, *Genet. Res. 32*, 67-78, 1978; Helmi and Lamb, *Heredity*, 1981, in press) and for an unlinked white mutation, *w*-62 (Emerson and Yu-Sun, *Genetics, 55, 39-47, 1967).* Conversion of *w*-62 is affected by a closely linked conversion control factor (*ccf*) now called *ccf*-1, while three separate *ccfs* (numbers 2, 3 and 4) affect conversion at locus I.

Data of Lamb and Ghikas (*Genetics, 92, 49-65*, 1979) suggested the existence of further *ccfs* affecting other white ascospore mutations. We have identified and studied the properties of *ccf*-5, affecting mutation BB9*w*9, and *ccf*-6, affecting *w*-BHj. Both *ccfs* are closely linked to the mutations whose conversion they control. Each factor has two forms, heterozygosity for the *ccf* reducing gene conversion of the white mutation. *ccf*-1 acts similarly on *w*-62. This factor and *ccfs* 5 and 6 have properties in common with the *cv* conversion modifiers (Girard and Rossignol, *Genetics, 76*, 221-243, 1974) in the European strains of *Ascobolus immersus*. Considerations of the relative changes in frequency of the different conversion classes in crosses homozygous and heterozygous for the *ccf* in question, and whether the *ccfs* co-convert with the *w* site controlled, provide some insight into the likely mechanism of *ccf* action involved.