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THE CONTROL OF GENETIC RECOMBINATION IN NEUROSPORA CRASSA

AN INVESTIGATION INTO THE CONTROL OF GENETIC RECOMBINATION IN SOME STRAINS OF NEUROSPORA CRASSA

By

ANTHONY JOHN FREDERICK GRIFFITHS, B. A. (HONS.)

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AUTHOR: Anthony John Frederick Griffiths, B.A.(Hons.) Keele

SUPERVISOR: Professor S.F.H. Threlkeld

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The understanding of basic cellular processes has been greatly facilitated through investigation of the behaviour of mutant forms. In a similar way the mechanisms of genetic recombination may be clarified by a study of strains which are known to show inherited differences in recombination behaviour at meiosis. The haploid fungus Neurospora crassa is particularly well suited to such an investigation since recombination frequency heterogeneity has been extensively reported in that organism, and the differences are believed to be, to a large extent, under genetic control. Strains showing recombination frequency heterogeneity over a marked genetic region have been extensively analysed in the present work and the mode of action of the factors controlling recombination frequency has been investigated by combining differing strains in heterokaryons.

PREFACE

This dissertation describes studies carried out in the Research Unit in Biochemistry, Biophysics and Molecular Biology at McMaster University, from September 1963 to January 1967. Except where others are specifically mentioned, it consists entirely of my own original work, and no similar dissertation has been submitted to any other University.

I would like to thank my supervisor, Dr. S.F.H. Threlkeld, for his invaluable advice and encouragement throughout the research, and Dr. G.R. Morrison of the McMaster Psychology Department for his helpful suggestions concerning the statistical analysis.

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A.J.F. Griffiths

Research Unit in
Biochemistry, Biophysics &
Molecular Biology.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The stocks of Neurospora crassa which are used in laboratory investigation today constitute an extremely diverse group with respect to genetic endowment. This is partly due to the fact that strains of this haploid fungus have been collected in the field from widely differing geographical habitats, and have consequently been subjected to different selection pressures. Furthermore, diversity has been introduced secondarily into the gene pool of the fungus, through the widespread use of mutagenic agents for the purpose of obtaining mutant forms.

Consequently, a great deal of variability is expected in the behaviour of Neurospora crassa with respect to basic cellular processes. One such process in which variability has been extensively described is recombination frequency at meiosis. This thesis represents an attempt to understand the ways in which differential genetic endowment can affect this process.

The frequency at which recombinant genotypes are produced at meiosis has long provided the basis for genetic mapping. The underlying assumption of mapping is that recombination frequency is a function of the physical distance between the markers concerned on their appropriate linkage group. Whereas this tenet has been largely upheld in practice, inconsistencies in gene order and linkage arrangements are by no means uncommon, recombination appearing in fact to be also a function of the ancestry of the strains used. Such inconsistencies seem to have

been minimised where all the markers used were induced into the same strain.

Hence the importance of the "genetic background" of a strain became acknowledged in dealing with linkage. Barratt (1954) was the first to issue a word of caution in this respect. He pointed out that a large number of Neurospora strains are derived from either C.C. Lindegren's A and a wild-type stocks or from E.V. Abbott's 4A and 12a wild-types. His compilation of available data on centromere distances of markers on most of the linkage groups, led him to the conclusion that if one of the parents in a cross is of predominantly Abbott ancestry, then centromere distances significantly different from distances in Lindegren x Lindegren crosses can be expected.

In crosses of Abbott 4A x Lindegren, he found significant decreases in centromere distances for the markers thre-1, mt, al-1, chol-1, nt and asco.

In crosses of Abbott 12a x Lindegren, he found significant decreases for the markers thre-1 and me-5, but not for arg-6 or me-3.

Crosses which were found to give centromere distances greater than in a Lindegren x Lindegren cross for the markers mt, pab-1, aur and al-2, were found to be essentially backcrosses in ancestry, of the type (Abbott x Lindegren) x Lindegren or (Abbott x Lindegren) x Abbott.

No appreciable ascospore abortion was noticed in any of these crosses, which suggests that a failure to detect recombinants because of an elimination of crossover chromatids is an unlikely explanation of the findings. It had been suggested by Singleton (1948) that failure of synapsis at prophase 1 might account for the lowered recombination.

The failure to synapse would be due to some sort of heterozygosity between Abbott and Lindegren strains, either at a genic level or at a chromosomal level. This heterozygosity could then be decreased by backcrossing and restored recombination frequencies might be expected. However, frequencies in excess of the Lindegren x Lindegren cross (such as those observed), are not expected on this model. Possibly the Abbott stocks contain genes which are able to increase recombination in a situation where some degree of synapsis (or homozygosity) exists, as in the backcrosses.

Thus the data in Barratt's review posed the question which was to pervade future work on the control of recombination; that is, are the heterogeneous recombination data due to quantitative gene control, or to heterozygosity per se? Stadler (1956) designed experiments to decide between the two hypotheses. He had observed heterogeneity of second division segregation frequencies of the asco locus on linkage group VI, and two strains were selected which gave markedly different frequencies when crossed to asco (10% & 36%). These two strains were then intercrossed and the progeny from four asci from the cross were individually backcrossed to asco and scored for second division frequency for asco. If the second division frequency differences were due to a single gene difference, one might expect to see the segregation of this gene in all the asci. However, no such patterns were evident, each ascospore giving a frequency lying between the limits set by the original crosses. Although this experiment assumes that the asco stocks used are isogenic except for mating type, the results suggest that if there are genes controlling recombination, the control is a complex one, possibly involving several interacting gene products.

Stadler went on to explore the possibility that heterozygosity of the parents in a cross could lead to lowered recombination frequencies. A cross of rib-1;A (riboflavin) to asco; a gave a low second division frequency for asco of 12.6%. Progeny from this cross of the genotype rib-1; A were crossed to the original asco, a and second division segregations ranging from 12% to 34% were observed. A cross showing a 24% frequency was selected and rib-1; A progeny from this cross were again backcrossed to asco. The range shown by these progeny varied from 30% to 37%. The cross showing the highest frequency of the original range (34%) showed a range of 36% to 48% on backcrossing. These results indicate that heterozygosity of some kind is responsible for the original low frequency, and that the reduction of heterozygosity due to backcrossing is facilitating recombination. In no case did the frequency of second division segregation fall with backcrossing. This behaviour of asco on backcrossing was confirmed by Towe (1958) who was able to compare the linkage map of the asco - centromere region in the parent cross, with that produced by the first backcross. The parent cross was asco, a x cys,ylo,ad;A, and the backcross was of one of the cys,ylo,ad;A progeny to the asco,a parent. (The backcross was selected for its high second division segregation frequency for asco.)

Parent cross: asco - 6.02 - cys - 3.46-ylo-3.61-ad-0.60-centromere

Total 13.69 map units

First backcross: asco-7.48-cys-7.13-ylo-8.37-ad-0.62-centromere

Total 23.60 map units

(The figures quoted here are not from Towe 1958, but from revised data (Stadler & Towe (1962)). It is of interest to note that not all marked

regions are affected to the same degree by backcrossing: there is a more than twofold increase in the cys-ad region, while there is little if any increase in adjacent regions.

Frost (1961) has extended the type of analysis found in Barratt's review. In a survey of published data on crosses of various types of ancestry, with each type individually showing homogeneity of data, he finds that the mating type (mt) to centromere distances fall roughly into three groups of values.

A4 x L	2.31 units
L x L (A4 x L) x L A12 x L C x L (A4 x L) x C (A4 x C) x L	4.07 - 8.59 units
(A4 x L) x A4 (A12 x L) x L (A12 x L) x (A12 x L) (A4 x L) x A12	11.43 - 16.27 units

(A4 = Abbott 4 A ancestry: A12 = Abbott 12a ancestry: L = Lindegren ancestry: C = Chilton ancestry.)

From these data, he concludes that only A4 x L can give reduced distance compared with the L x L control, whereas either A4 or A12 can give rise to increased distances in a manner that defies interpretation on the basis of a simple recombination gene hypothesis or a heterozy-

gosity hypothesis. (Frost has evidence that Chilton strains behave similarly to Lindegren wild types.) Frost had no data on A x A crosses, but later work (Lavigne & Frost 1964) has shown that these crosses give lower distances than L x L. These data largely confirm Barratt's findings on the mt locus, but Frost also enlarged on the data on other linkage groups. Although the latter data are considerably less extensive than that for mt, it appears that where data are available, other markers in general confirm the mt findings. (It appears that the reductions of distances found by Barratt in A12 x L crosses are exceptions rather than the general rule.) However, several exceptions to the mt patterns exist which make overall generalisation difficult. An interesting exception is that in A4 x L crosses, markers which are close to the centromere show a significant reduction, whereas distal markers show no effect. Also in (A4 x L) x L crosses distal markers show an increase whereas proximal ones do not. (The role of the centromere in governing recombination frequency will be discussed below.)

As a simple heterozygosity explanation seemed to be ruled out, Frost explored the possibility of the segregation of factors controlling centromere distance. One ascus was chosen from an A12a x LA cross and each spore pair was used in a backcross to one of the parental strains. Due to the segregation of the mt locus in the ascus, not all spore pairs could be crossed to the same parent; however, the results proved interesting:

Spore pair 1 (A) x Al2a mt - centromere 7.02 units

Spore pair 2 (A) x Al2a mt - centromere 14.37 units

Spore pair 3 (a) x LA mt - centromere 7.69 units

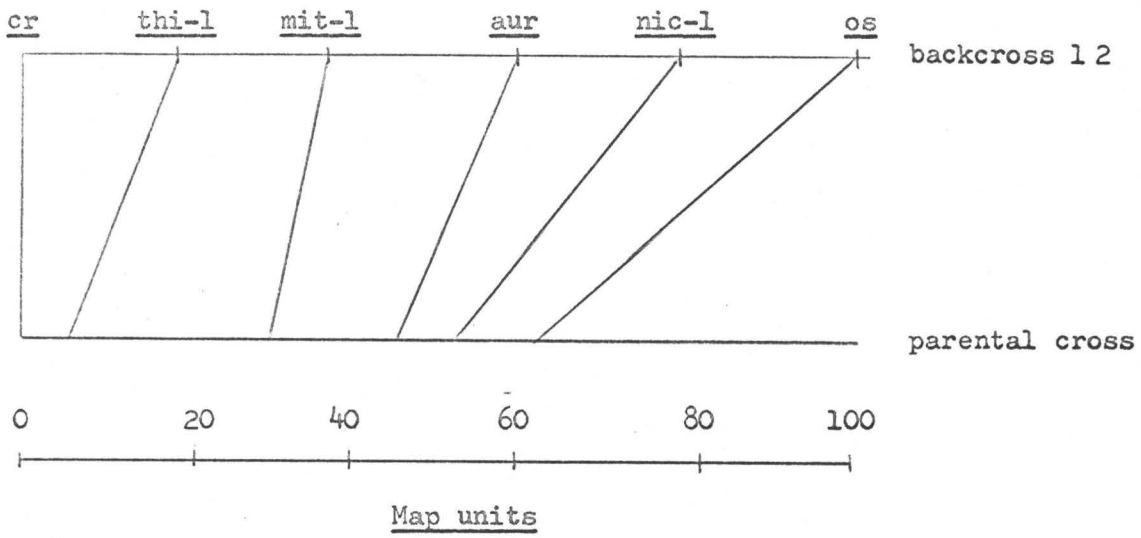
Spore pair 4 (a) x LA mt - centromere 14.49 units

The differences between spore pairs 1 and 2, and between pairs 3 and 4 are significant at the 5% level. Hence, it appears as though a genetic factor responsible for centromere distance is segregating at the second division in this ascus. Furthermore, spore pairs 1 and 2 also showed different centromere distances for asco when an asco,a strain was crossed to them.

1 A x asco,a, 11.67 units)
) χ^2 gives
2 A x asco,a, 16.72 units) P < 1%

All the data on mt - centromere distances cannot be explained by a one factor difference such as the above, and Frost suggests that at least three genetic factors are involved since the mt distances fall broadly into three groups. However, this conclusion must be regarded as speculative at this stage.

Further data on the mating type linkage group has been provided by Cameron, Hsu & Perkins (1966). Map distances after twelve generations of backcrosses to wild type have been compared with those in the original cross to the same wild type. A multiply marked linkage group I was used and selected for in each backcross. The results can be summarised diagrammatically as follows.



Here too the marked regions are not all affected to the same degree, although the differential is not as marked as in the results of Towe (Stadler & Towe 1962). The data show a further resemblance to those of Stadler & Towe in that a hint of a correlation of relaxed chiasma interference with backcrossing (and, presumably, decreased heterozygosity) was observed.

Analogous backcrossing experiments have been performed by Nakamura (1966). He observed the second division segregation frequency of ts (tan ascospore) on linkage group V on backcrossing. ts gives different second division segregation frequencies when crossed to different wild type strains;

ts x P2a 21.3%

ts x 8a 47.0%

ts x LT2a 58.0%

Nakamura performed backcrosses to ts in each of these three lines for three or four generations by selecting ts^+ progeny that gave the highest

centromere distance, in a way analogous to Stadler's experiments on asco (Stadler 1956).

P2a line

Generation of backcrosses	Range of second division frequencies for ts
First	22.0 to 45.3 mean 31.9
Second	32.1 to 52.9 mean 42.5
Third	34.4 to 52.7 mean 43.7
Fourth	28.6 to 45.9 mean 39.6

It will be remembered that Stadler in no instance observed distances lower than the previous generation, whereas in Nakamura's data the ranges overlap after the first backcross generation. An analysis of variance applied to the second, third and fourth backcross generations reveals no significant difference, suggesting that an equilibrium level of second division frequency has been attained after the first backcross.

8a line

Generation of backcrosses	Range of second division frequencies for ts
First	53.0 to 57.1 mean 55.0
Second	47.8 to 57.5 mean 54.0
Third	54.0 to 69.9 mean 61.2
Fourth	55.4 to 71.2 mean 65.0

LT2a line

Generation of backcrosses	Range of second division frequencies for ts
First	60.0 to 68.5 mean 63.4
Second	58.6 to 73.4 mean 67.7
Third	64.2 to 80.1 mean 72.0

In these two lines there is a certain amount of overlap of the ranges, and it seems as though equilibrium is attained less rapidly. However, the general increase in map distance on decreased heterozygosity seems to be borne out by the results. Furthermore, the data cannot be explained by the hypothesis of a single locus controlling recombination which might predict two modes in the second division segregation frequency range in the first backcross generation, one of which should coincide with the frequency of the parental cross. Here again, then, a choice cannot be made between the two hypotheses of multilocus control of recombination and heterozygosity per se.

In both Stadler's and Nakamura's data it is possible to test whether the factors affecting crossing over are actually in the genetic region under study or outside that region. In Nakamura's cross P2a x ts, second division segregation frequency was about 20%; hence, if one assumes only single crossovers in the centromere - ts region, then 90% of the progeny from that cross would be expected to be parental for the ts - centromere region. As the scored progeny were selected for ts^+, 90% of the scored progeny should be identical in the ts - centromere region to the P2a parent, and consequently 90% of the progeny should give centromere distances of the ts x P2a type. This was by no means found

to be true. Similarly, in Stadler's asco asci, the moderately close linkage of asco to the centromere should result in several asci of parental ditype with respect to recombination-controlling factors if they are actually in the asco-centromere region. However, no asci of such a type were found by Stadler; in fact no segregation patterns were found at all in his data with respect to centromere distance. Thus, it appears that the genes concerned are outside the regions studied.

De Serres (1958) performed experiments which throw light on the problem of heterozygosity in the region under study. He made crosses between heteroalleles of the ad-3 locus on linkage group I, and found that different prototroph frequencies were produced depending on the wild type strain in which the ad-3 mutations were induced. The rest of the genome in these crosses was maintained more or less isogenic from cross to cross by repeated backcrossing of mutants to a 74A wild type. His results are summarised in the following table.

Cross	Recombination Percentage	
1. x his-2 + B / + A +	0.09 - 0.11	
2. x his-2 + B / + A +	0.14 - 0.18	Homozygous / Heterozygous
3. x his-2 + B / + A +	0.35 - 0.39	
4. x his-2 A + / + + B	0.64 - 0.72	
5. x his-2 + B / + A +	0.63 - 0.69	

(A and B represent heteroalleles of the ad-3 region.

▬▬ = 74A ancestry.

----- = Lindegren ancestry.

his-2 is an outside marker which remained constant.)

Thus it appears that heterozygosity in or near the ad-3 region can stimulate recombination between ad-3 alleles, since the heterozygous crosses 3, 4 and 5 give significantly higher recombination values than crosses 1 and 2.

De Serres also performed crosses between alleles induced in the same wild type background, but outcrossed into a variety of backgrounds. Thus, purportedly, the ad-3 region remains constant while the rest of the genome varies. De Serres does not record the precise ancestry of these crosses but it is of interest to observe that the frequencies fall into three groups in the same manner showed by Frost's survey of mt - centromere distances. This set of data suggests that in addition to intra-region heterozygosity, extra-region differences are important too.

Cooke (1967) has attempted to resolve the seemingly contradictory data on intra- and extra-region heterozygosity, by varying both the region under study (the ylo - tryp-2 region on linkage group VI) and the rest of the genome. His crosses may be diagrammatically summarised in this manner.

Parents of cross	Approximate recombination frequency
1. [—]——— x [—]———	12.7%
2. [—]——·····x [—]——·····	14.9%
3. [—]—·····x [—]——·····	28.8%
4. ([—]——— x [-]·····) ([—]——·····x [-]·····) ([—]—·····x [-]·····)	24.5%

St. Lawrence ancestry. ———

Lindegren ancestry. --------

In the diagram, the area of the genome in square brackets represents the ylo - tryp-3 region, and the region to the right of the brackets represents the rest of the entire genome. For example, the parent on the left in cross 3 has been produced by selecting for the ylo - tryp region induced in a St. Lawrence background in a series of crosses represented by (St. Lawrence x Lindegren) x Lindegren. Hence in that parent the background of the rest of the genome is roughly 75% of Lindegren ancestry. Cooke has demonstrated statistically that there is no difference between groups 1 and 2 or between 3 and 4. However, all other comparisons are significantly different. Cooke concludes that heterozygosity, whether localised in the region studied, or generalised, is not adequate per se to explain the results of these experiments. He believes that a small number of interacting genes in the Lindegren strain, all of which must be present to produce a high recombination

value, is probably the best explanation of his data, although heterozygosity cannot be ruled out completely as an hypothesis.

De Serres' data on intra-region heterozygosity is the only work which points to the importance of this type of heterozygosity. It should, however, be pointed out that his results could also be due to heterozygosity of a small region closely linked to the ad-3 region.

The work reviewed so far has on the whole pointed out the importance of extra-region heterozygosity in the control of recombination over large stretches of chromosome. The nature of this heterozygosity remains in doubt, although some evidence has been presented which suggests that a small number of interacting loci controlling recombination may be responsible. The first demonstrations in Neurospora of genes affecting recombination in specific genetic regions are attributable to Catcheside and his co-workers (Jessop & Catcheside (1966), Smith (1966), Catcheside (1966)). These workers have been successful in observing in crosses the segregation of single genetic factors which affect recombination in the regions under study. These observations represent success where other workers have failed. The reason for this success may be partly due to the fact that the Catcheside group have mostly investigated fluctuations in intra-locus recombination, whereas previous workers had concentrated on relatively larger regions of the genome between separate loci. However, Smith (1966) has pinpointed a gene controlling recombination in the pyr-3 - leu-2 region of linkage group IV which is a region about ten map units long. Nevertheless it would appear empirically that there is a better chance of finding recombination genes segregating if one examines a small genetic region.

To date, three recombination genes have been identified; *rec-1* controlling prototroph frequency between *his-1* heteroalleles, *rec-2* controlling recombination between the *pyr-3* and *leu-2* loci, and *rec-3* controlling recombination at the *am* locus. It appears that all three genes have a similar mode of action, such that in all three cases a cross involving rec^+ in a heterozygous or a homozygous state at meiosis will produce a low recombination value in the region affected, whereas homozygous rec^- produces high frequency of recombination. Thus, rec^+ is considered to be dominant to rec^-.

The way in which these *rec* genes are identified can be illustrated with reference to the *his-1* experiments (Jessop & Catcheside 1966). A cross of $am^+, his\text{-}1^{K83}; a$ x $am, his\text{-}1^+; A$ was used to produce two progeny, both of the genotype $am, his\text{-}1^{K83}; a$, called *am*.K83 *a*(1) and *am*.K83 *a*(2). These two strains, when crossed to various *his-1, inos.A* tester strains bearing a selection of *his-1* alleles, produced highly divergent results as seen in the table following.

his-1, inos.A tester parent	*am*.K83 *a*(1) # prototrophs per 10^5 ascospores (a)	*am*.K83 *a*(2) # prototrophs per 10^5 ascospores (b)	a ÷ b
K 566	4.2	0.2	21.0
K 627	4.3	0.3	14.3
K 90	17.3	1.6	10.8
K 141	45.7	2.76	16.6
K 616	41.9	3.9	10.7
K 651	27.0	1.7	15.9
K 625	3.1	3.7	0.8
K 83	0	0	-

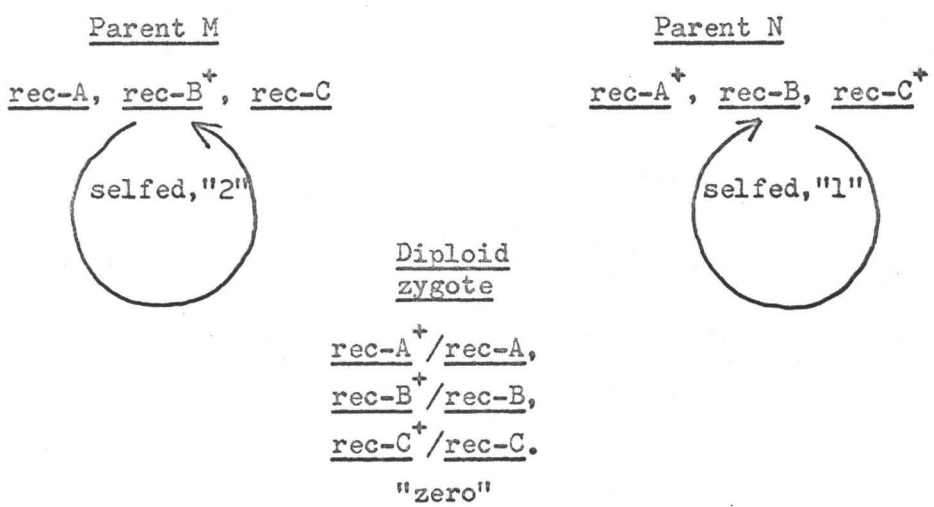
Tester K 625 is the only case where no difference is manifest between the columns. On the basis of the evidence in the table, *am*.K83 *a*(1) may be classified as *rec*$^-$ and *am*.K83 *a*(2) as *rec*$^+$. Also, strains K 566 to K 651 must be *rec*$^-$, and K 625 must be *rec*$^+$. Subsequent test showed that most strains can be classified into either the *rec*$^+$ or *rec*$^-$ group with little ambiguity. A consideration of these tests suggests another reason why other workers have failed to identify *rec* genes in segregation, in that, in order to observe segregations of *rec*$^+$ and *rec*$^-$, the segregants have to be crossed to *rec*$^-$ testers. The *rec-1* locus does not appear to have an effect on recombination between the flanking markers *am* and *inos*, but both *rec-1* and *rec-3* appear to be specific in the sense that the *his-1* locus is insensitive to *rec-3* and the *am* locus insensitive to *rec-1*. As seen from the table, *rec-1* has a 10- to 20-fold effect on recombination, and a similar difference is observed in the effect of *rec-3* on the *am* locus. However, the single inter-locus *rec* gene (*rec-2*) seems to have only a two-fold effect. The action of the *rec* genes does not seem to be dependent on close linkage to the loci they control: *rec-1* has been located some eighteen map units from *his-1*, but it has been shown that *rec-3* might be on a separate linkage group to *am*. Jessop and Catcheside (1966) and Smith (1966) suggest that it would be unexpected if the *rec* genes were to show specificity of action solely in the regions studied. However, the three studies they have undertaken have revealed three *rec*-type genes, and it seems likely that an intensified search at many other loci could be expected to reveal additional similar controls. Furthermore, the fact that other workers have failed to detect segregation of *rec* genes in studies over

larger genetic regions might mean that several rec-type genes may be acting on these regions, whose combined segregation patterns might give progeny showing a wide range of recombination behaviour when backcrossed to the parental generation, rather than a simple bimodal distribution such as the identified rec genes show. If this is the case, Smith must be considered fortunate in having found a large region controlled by a single rec gene.

Whether rec genes are specific or not, they provide a possible explanation of the correlation of heterozygosity with low recombination frequency. A simple model can be constructed in which the centromere distance of a gene X is under the control of three separate rec-type loci (rec-A, rec-B and rec-C), each of which controls crossing over in exactly one third of the centromere - X region. It can also be assumed that in a cross involving X and its wild-type allele, heterozygosity exists at all three rec loci in the diploid fusion nucleus and throughout prophase 1 of meiosis. With respect to recombination genes the diploid can then be represented rec-A^+/rec-A, rec-B^+/rec-B and rec-C^+/rec-C. This will mean that the whole centromere to X region is under the influence of dominant inhibiting rec^+ genes. Consequently, recombination in this heterozygote should be mimimal, and can be represented by "zero" to imply that no region is free from inhibition. If the rec genes are unlinked, the cross should produce eight combinations of rec alleles. If each of these progeny are backcrossed to the parental strains, the number of regions between the centromere and X which are free of rec^+ inhibition are shown in the following table. It is assumed that a cross with two regions free of rec^+ dominance will give

higher recombination frequency than a cross with one region free of rec$^+$, and the latter higher than a cross with a complete rec$^+$ inhibition.

It is seen from the table that three classes of recombination frequency should result if the progeny are backcrossed to parent M, giving a trimodal distribution, and two classes when backcrossed to N. The number of classes expected depends on the number of regions in meiosis which are under the control of a rec gene in a heterozygous state (rec$^+$/rec$^-$), and if these regions are numerous, a continuous amodal distribution is expected ranging from the value obtained in the heterozygous cross up to the value obtained in the parent showing the highest frequency of recombination when selfed. (In the diagram, the limiting classes are the "zero" and "2" classes.)



Progeny classes. (A=rec-A etc.)	# regions free from rec$^+$ domination when crossed to M	# regions free from rec$^+$ domination when crossed to N
A B$^+$ C ✓	2	0
A$^+$ B C$^+$ ✓	0	1
A B$^+$ C$^+$ ✓	1	0
A$^+$ B C ✓	1	1
A B C$^+$ ✓	1	1
A$^+$ B$^+$ C ✓	1	0
A. B C	2	1
A$^+$ B$^+$ C$^+$	0	0

Much, although not all, of the reviewed data can be accounted for on the basis of the above model. Nevertheless, other suggestions have been put forward to account for heterogeneity of recombination values, and these will be considered here.

The centromere has been hypothesised as a possible source of variation. Fincham (1951) compared the linkage map of chromosome 1 in Neurospora crassa with that of the closely related species Neurospora sitophila, and demonstrated that in both arms, markers proximal to the centromere showed an elevated second division segregation frequency in the latter species. Also, map distances between distal markers remained essentially unchanged. Scott-Emuakpor (1966) extended Fincham's observations to other linkage groups and demonstrated that the differentials between the two species are not always in the same direction. As mentioned above, Frost (1961) and Stadler & Towe (1962) have shown effects on recombination due to proximity to the centromere. Beadle (1932) has shown in Drosophila that a homozygous translocation which brought a marked region closer to the centromere resulted in the suppression of crossing over in that region.

Proximity of a region to heterochromatin has been proposed as a contributing factor in recombination heterogeneity (Mather, 1939). However, evidence concerning this suggestion is unavailable in Neurospora.

Chromosomal aberrations in Drosophila cause significant changes in recombination in adjacent regions (Sturtevant & Beadle, 1936) and in other chromosomes (for example, Schultz & Redfield (1933), Williamson (1966)). The effect of translocations on recombination has been ob-

served in Neurospora by St. Lawrence (1953). However, patterns of ascospore abortion which would be expected from heterozygous aberrations have not been observed in the reviewed experiments.

Heterozygosity in the form of minor base sequence differences or minor structural differences as a result of radiation treatment remains a feasible explanation of the results given here. Presumably the primary effect of these factors would be to reduce the efficiency of homologous pairing at meiosis, and thereby to decrease the efficiency of exchange between the homologues.

Thus, in summarising the above discussion, it may be stated that heterozygosity of some kind outside the region under study does seem to depress recombination frequency. The discovery of rec-type genes opens up an attractive way of explaining many of the phenomena attributed to heterozygosity, but structural heterozygosity cannot be ruled out as an explanation of the findings. It is difficult to see how the latter type of heterozygosity, being present outside a region, can affect the recombination behaviour within that region, yet unexplained interchromosomal effects on recombination do exist (as mentioned above for Drosophila).

The work to be reported in this thesis was initiated primarily to discern the relative importance of recombination-affecting genes and "structural" heterozygosity on inter-locus recombination in Neurospora. The rationale behind the experiments performed is as follows. Two strains known to differ in recombination frequency over a specific region when crossed to a common conidial parent, may be combined in a heterokaryon. The heterokaryon may then be crossed to the same conidial

parent. If the original nuclear components of the heterokaryon are identifiable through the presence of appropriate marker loci, then the recombination behaviour of the component strains inside the heterokaryon may be compared with their behaviour when they are used independently. If the recombination frequency associated with a component nucleus of the heterokaryon differs significantly from the recombination frequency of that nucleus when present in a cross as a homokaryotic strain, it may be assumed that structural heterozygosity was not the cause, or was not the only cause, of the original diversity between the strains. The conclusion would be that the changes in recombination frequency reflect the activity of gene products, and the internuclear exchange of such products in the heterokaryon. Although these gene products may not be strictly recombination-specific in their action, they may nevertheless be presumed to be under the influence of natural selection at least in part because of their action on recombination.

A failure to demonstrate depression or elevation of recombination frequency of any given nucleus while inside the heterokaryon, cannot be conclusively attributed to the absence of recombination-concerned gene product exchange, but may rather reflect the lack of continuous cytoplasmic contact between the nuclei at the time of formation of these gene products. Hence, the only result of this experiment which can be meaningful is the demonstration of recombination change of the nuclear components due to heterokaryosis.

Neither can cytoplasmic effects be ruled out (as a heterokaryon is also a heteroplasmon (McDougall & Pittenger, 1966), unless it can be shown that the recombination frequencies of the homokaryotic strains remain individually constant when these homokaryotic strains are used as conidial parents in crosses to the common parent. However, in the many reciprocal crosses that have been performed in Neurospora, it appears that no marked cytoplasmic effects on recombination have been reported. Furthermore, the nature of cytoplasmic mutants which have been detected in fungi make them unlikely candidates for a role in the control of chromosome exchange.

If no internuclear interaction occurs, the heterokaryon can be expected to show a recombination frequency somewhere between the extremes set by the component strains, its precise value depending on the nuclear ratio, and, consequently, on the ratio of the component meioses occurring in the heterokaryon cross. (It should be pointed out that, although a heterokaryon is a mixture of nuclei on a common cytoplasm, if the heterokaryon is crossed, any one meiosis can involve only one heterokaryotic nuclear component. Thus the ratio of two types of meiosis occurring should presumably reflect to some degree the nuclear ratio of the heterokaryon.) If an interaction does occur, the recombination frequency of the heterokaryon cross could conceivably be intermediate, higher than either component, or lower than either component, depending on, again, the nuclear ratio, and also on the mode and strength of the interaction. An analysis of the ratio of different types of meiosis occurring, via the use of nuclear, and hence meiotic, markers, makes it possible to differentiate between these alternative possibilities.

CHAPTER 2

MATERIALS AND METHODS

Materials

1. Markers used

The mutants used as markers in this study are shown below.

Mutant	Phenotype	Isolation #	Mutagen	Linkage group	Ancestry
ad-1	Requires adenine	3254	X-rays	VI L	L ci M
al-2	Albino conidia	15300	X-rays	I R	L ci SL
arg-6	Requires arginine	29997	spontaneous	I R	L ci M
arom-1	Requires aromatic amino acids	Y7655	N-mustard	II R	L ci SL
cys-2	Requires cysteine	80702	Neutrons	VI L	A X L ci M
leu-3	Requires leucine	R156	UV	I L	M ci SL
pan-2	Requires pantothenic acid	Y154M64	?	VI L	M
thi-2	Requires thiamin	9185	X-rays	III R	L ci M
ylo	Yellow conidia	Y30539y	N-mustard	VI L	M ci M

(L=Lindegren: M=mixed: A=Abbott: SL=St. Lawrence: ci=crossed into)

Several of these mutants were obtained already in the form of multiply-marked stocks. These were:-

Strain genotype	Mating type	Fungal Genetics Stock Centre #	Linkage group
ad-1, pan-2	a	467	VI
arg-6, al-2	a	272	I R
arg-6, al-2	A	313	I R
cy-2, ylo	a	489	VI L

All stocks were obtained from the Fungal Genetics Stock Centre, Dartmouth College, Hanover, New Hampshire, U. S. A.. Most of the mutants have been investigated in detail, and references to these investigations are found in Neurospora Newsletter #9 (June 1966).

2. Media

The media used consisted of a carbon source, inorganic salts, trace elements, the vitamin biotin, distilled water plus appropriate growth supplements. Media used for different purposes differed only in the carbon sources, as described below. Where solid medium was needed, 2% Difco agar was added. Inorganic salt solution was made up in 4x strength as follows, according to Westergaard and Mitchell (1947).

KNO_3	4.0g.
KH_2PO_4	4.0g.
$MgSO_4.7H_2O$	2.0g.
$CaCl_2.6H_2O$	0.4g.
NaCl	0.4g.
Biotin	16.0µg.
Trace element solution	1.0ml.
Distilled water up to	1000ml.

Trace element solution consisted of:

$Na_2B_4O_7.10H_2O$	0.01g.
$CuSO_4.5H_2O$	0.1g.
$FePO_4.2H_2O$	0.2g.
$MnSO_4.4H_2O$	0.02g.
$ZnSO_4.7H_2O$	2.00g.
$Na_2MoO_4.2H_2O$	0.02g.
Distilled water up to	250 ml.

Four different carbon source combinations were used according to the purpose of the medium. For crosses, 2% sucrose was used; for vegetative growth 2% glucose; for testing auxotrophic markers 0.4% sorbose plus 0.2% sucrose; and for assays of prototroph frequency 1% sorbose plus 0.05% glucose plus 0.05% fructose. Brockman & de Serres (1964) have shown that the sorbose-fructose-glucose (SFG) medium minimises the variability of "sorbose toxicity" effects, which are due to differing autoclave times.

Biochemical supplements needed as growth requirements were added as follows.

adenine	100mg./litre
L-arginine HCl	500mg./litre
L-leucine	200mg./litre
L-Methionine	500mg./litre
Ca-pantothenate	100mg./litre
shikimic acid	100mg./litre
thiamin	100mg./litre

The pH of various media has been checked using coloured pH indicator papers, and seems to vary between about 6.2 and 6.6 depending on supplementation, on whether or not the medium has been autoclaved, and on the source of distilled water.

Methods

Stock cultures were maintained on solid glucose medium in a 7°C incubator, and subcultured at 3 month intervals. Subcultures were grown up at 25°C prior to storage at 7°C.

Crosses were performed in large test-tube slopes at 25°C. The strain to be used as the protoperithecial parent was inoculated and left at 25°C for 4 to 6 days before a few drops of conidial suspension from the conidial parent were added. Where the results of different crosses were to be compared, the cross technique was kept constant in terms of fertilisation time and as many other factors as possible. It was found that crosses are more likely to produce abundant perithecia if most of the aerial conidia and mycelium are removed from the cross tube before fertilisation. In relation to this problem, if a dilute solution of Tween 80 (0.01% by volume) is used to suspend the conidial parent (Stephens, V. R., personal communication), perithecia production is often enhanced. Presumably the reduction of surface tension by the Tween 80 facilitates contact between the fertilising conidia and the trichogynes. Mature ascospores are harvested from the walls of the cross tube at periods ranging from four weeks onwards, depending on the cross. It has been found that ascospores recently shot from the perithecia are very often immature, and sometimes have to be left a further four weeks to ensure high germination percentages.

Mature ascospores are removed from the cross tube with a microspatula and spread in a small drop of 1.5% sodium hypochlorite over the surface of a $1cm^2$. block of 4% agar. The spores are usually in an ideal state for isolation after 30 minutes. They are picked off one at a time under a dissection microscope, using tungsten wire needles, which have been sharpened in molten sodium nitrite. The spores are deposited individually into small slopes of vegetative medium. These random spore isolates are heat shocked immediately in a 60°C water bath for 40 minutes to induce germination. The heat shock also kills any conidia that have managed to survive the hypochlorite treatment. Germination tubes may be observed under the microscope after several hours, and the cultures are usually fully grown by 4 to 6 days. Ungerminated isolates sometimes germinate after a second heat shock.

Cultures (such as those derived from random spore isolates) can be tested for their biochemical requirements by inoculating appropriately supplemented Petri plates of sorbose-sucrose medium. Cultures were periodically tested in this way to check the possibility of reversion at the marker loci. Morphological markers were scored by observing the culture tube directly.

Mating-type tests were made in the following way. A dilute suspension of conidia from a tester strain of known mating type was spread over the agar surface in an appropriately supplemented Petri plate. After one day, a mycelial lawn has formed, and at this stage a large square is cut in the agar, and the medium around this square is removed. (This procedure prevents the excessive build-up of conidia on the agar square remaining.) After another 3 days, conidia of the strains of unknown

mating type are jabbed onto the mycelial surface with a wire twist. Fertilisation by opposite mating types is scored by observing the appearance of black perithecia on the agar surface. In this way, up to 20 strains can be tested on one Petri plate. As a rule, unknown mating types should be crossed to tester strains of both mating types as a double check.

Heterokaryons were made by two methods. The first method was to inoculate both the component strains at the same spot on a slope of appropriately supplemented vegetative medium. The second method was to make conidial suspension of each contributing strain and to add one drop of each suspension to an unsloped tube of vegetative medium. The second method is faster, and more convenient for tests of heterokaryon compatibility where large numbers of combinations have to be tested. No attempt was made to make heterokaryons from strains of opposite mating types. The successful establishment of heterokaryosis is detected by complementation of forcing auxotrophic markers, and a secondary check is the complementation of morphological markers. In both cases a wild type phenotype results. Heterokaryons must, of course, be made and maintained on a medium which is "minimal" with respect to complementing loci.

A perithecial analysis is a random spore analysis in which all the spores come from one perithecium. This means that a perithecium must be isolated before the spores are shot. The perithecium is placed on a block of 4% agar and rolled around to remove foreign ascospores from its surface, although this manipulation is unnecessary if none of the perithecia in the cross tube have dehisced. A drop of 1.5% sodium hypochlorite is added to kill any conidia that may be adhering to the

perithecium, and the whole perithecium is squashed to expel its ascospores. The spores are then isolated in the same manner as random spores, but, because of their immaturity, they are not heat shocked until several weeks after isolation, at which time it is often necessary, prior to the heat shock, to add a few drops of water to each tube to make up for evaporation loss.

In the experiments to be described, recombination frequency in a cross is estimated by measuring the frequency at which prototrophs are produced in the progeny ascospores. A recombination event between two auxotrophic markers A and B, in the repulsion or "trans" arrangement will produce an equal number of double auxotrophs (AB) and prototrophs (+ +). Hence, although prototroph frequency is ideally but half of the real recombination frequency, it can be assumed that prototroph frequency is a real index of the frequency of recombination events in the marked region. The advantage of this technique is that relatively rare recombination events can be detected without resort to the laborious isolation of a very large number of individual spores. The fact that very much larger spore samples are screened provides an additional advantage, in that small variations in recombination frequency between crosses of different types are less readily attributable to random fluctuations. The following technique has proved to be the most favourable, in that reasonably consistent prototroph frequencies are obtained for a given cross, and more important, the differentials between crosses are reasonably constant in size and direction.

Mature ascospores are scraped from the walls of the cross tube and suspended in approximately 5 ml. of 0.1% agar. This agar solution

is prepared by dissolving the agar in water and allowing the solution to cool to room temperature while constantly stirring with a magnetic stirrer. The resultant solution is quite homogeneous, with a consistency rather similar to a thin oil. Dilute agar is used in preference to water so that the ascospores will remain in suspension for longer times. Little or no sedimentation has been observed after 30 minutes.

The ascospore suspension is filtered through tightly packed glass wool, to remove ascospore aggregations, after which procedure the volume decreases to about 3 ml.. It was discovered that a convenient rule of thumb spore concentration to use is that which looks just turbid when held against a sheet of white paper after filtration. (The exact concentration is determined by plating a spore sample in complete medium, as described below.) Next, several 0.5 ml. aliquots of the resulting stock ascospore suspension are added to 9.5 ml. amounts of 0.1% agar to effect a x20 dilution. These "dilution" tubes are agitated vigorously in a Vortex mixer to obtain uniform suspensions. Bubbles tend to form during this agitation which can be got rid of by pressing the tube vertically into the centre of the rubber cup of the Vortex mixer, so that the liquid spins round the inside perimeter of the test tube, without agitation.

1.0 ml. aliquots of stock ascospore suspension are each added to flasks containing 100 ml. of molten SFG medium, which have been kept at 60°C. (This SFG medium has been appropriately supplemented for prototroph selection.) The flasks containing the resultant mixture are now shaken by hand to promote mixing, and returned to the 60°C incubator for 40 minutes to heat shock the ascospores. After heat shock, each 100 ml.

mixture is again agitated and poured into 10 Petri plates where the medium is allowed to solidify. These plates are then placed immediately into a 37°C incubator.

One 1.0 ml. aliquot from each of the dilution tubes is individually added to a flask containing 100 ml. of molten complete SFG medium at 60°C. These flasks are treated in the same way as the prototroph flasks, and the resulting plates put into the 37°C incubator.

What has been described is the procedure followed for one cross, and the product of the technique is 10 Petri plates for each 1 ml. aliquot of stock ascospore suspension, and 10 Petri plates for each 1 ml. aliquot of dilution suspension used. However, in every experiment reported here, three crosses were run concurrently and the above manipulation performed on all three at the same time. The three crosses are two homokaryotic strains crossed to the common parent, and their heterokaryon crossed to the same common parent. In this way, the effect of any fluctuation in treatment from day to day is minimised, and the three crosses of any on "set" can be validly inter-compared for any given day.

After 4 days the plates were scored with the naked eye for growing colonies. No appreciable increase in number was observed after 4 days, except on the complete plates where the slight increase was due to a few colonies which had been overlooked on the first count. Consequently the complete plates were re-examined after an additional two days. The reason for incubating the plates at 37°C is that one of the markers (cys-2) is slightly leaky at 25°C (W. N. Ogata, personal communication), but not at 37°C.

Germination estimates were obtained from direct counts on the complete plates at 3 days under a dissection microscope. An area is marked on the bottom of the plate, and all spores in that area are scored for the presence of germination tubes or associated mycelium. Little ambiguity was found as a result of spores in close proximity. Plates were sometimes also observed at 6 days to see if a greater percentage of spores had now germinated, but this was never found to be the case.

Random samples of prototrophs and colonies on complete medium were isolated from the respective plates. This was performed microscopically to avoid inclusion of foreign ascospores or mycelium not associated with the colony being isolated. In order to reduce further this risk, only a small amount of mycelium was removed from the edge of the colony to be isolated. The isolations were performed with a tungsten needle, and the isolated mycelial fragments put into tubes of vegetative medium. The fact that the isolated cultures were rarely mixtures of colonies was evident from the negligible number of tubes seen containing perithecia. The depth of medium in a plate was approximately 2 mm., so little trouble was experienced in isolating colonies which had originated from spores near the bottom of the medium.

Prototrophs were isolated onto minimal-type vegetative medium, while the colonies from the plates containing complete medium were isolated onto fully supplemented vegetative medium. After a week's growth in a 25°C incubator, the cultures were placed in the light to enhance pigment formation, and after 2 more days they were scored for conidial colour.

Samples, from each cross, of colonies growing on complete medium were checked at the start of the research programme to see if the plating technique was selecting for any particular spore genotype. However, no significant divergence from the expected 1:1 distribution of mutant to wild-type was found for any marker.

CHAPTER 3

SYNTHESIS OF SUITABLE STRAINS

The region selected for study was the *cys-2* - *pan-2* region, which spans the centromere on linkage group VI. It is likely that most regions can be expected to show heterogeneity of recombination values, but as the *cys* - *pan* region had previously been shown to be susceptible to variability (Stadler & Towe 1962), it was the final choice. Thus the format of the experiments would be three crosses;

$(\underline{cys\text{-}2},+)_A$ ♀ x $(+\ \underline{pan\text{-}2})$ ♂

$(\underline{cys\text{-}2},+)_B$ ♀ x $(+\ \underline{pan\text{-}2})$ ♂

Heterokaryon $(\underline{cys\text{-}2},+)_A + (\underline{cys\text{-}2},+)_B$ ♀ x $(+\ \underline{pan\text{-}2})$ ♂

(In this symbolism, ♀ stands for protoperithecial parent, and ♂ for conidial.) The two strains A and B are of a similar genotype with respect to the loci *cys-2* and *pan-2*, and the conidial parent is constant in the three crosses. Three crosses of the type set out will be referred to as a "set" of crosses. "A" and "B" will be used throughout to distinguish betwen the homokaryotic protoperithecial parents. If A and B show different *pan* - *cys* recombination frequencies when crossed to the common parent, they could possibly be considered to be genetically dissimilar at recombination-controlling loci. As will be seen below they have to differ at marked loci as well.

Several requirements guided the synthesis of the eventual stocks.

1) The strains of type A and B should be heterokaryon compatible. It is known that compatibility will result only if homozygosity exists at a minimum of three compatibility loci (Garnjobst 1953, Garnjobst & Wilson 1956), and strains can be obtained which are known to be compatible. However, in order to mark these strains and retain compatibility a considerable programme of backcrossing has to be undertaken. In the experiments described here, no attempt was made to introduce compatibility loci; rather it was hoped that by trial and error, compatibility would be attained in some cases. This hope was borne out.

2) A and B type strains should differ in their recombination behaviour when crossed to the common parent. Ideally, perhaps, if A were of Abbott, B of Lindegren and the common parent of Lindegren background, then differences in values could be predicted. No attempt was made to cross markers into definite backgrounds. Nevertheless, recombination heterogeneity was found.

3) Markers should be present in A and B strains which will allow a heterokaryon between them to be forced. The choice of such forcing markers is arbitrary except that they should be auxotrophic and preferably show no leakiness.

4) A and B strains should bear markers which will allow progeny from the heterokaryon cross to be identified with one of the nuclear components of that heterokaryon. These nuclear (and hence meiotic) markers could be the forcing markers themselves, or preferably should be morphological mutants to facilitate scoring of large numbers of progeny. The complementation of these nuclear markers will also provide an additional check on the establishment of heterokaryosis.

5) All three crosses of a set should be fertile and produce an abundant spore progeny.

The strains were built up as follows, the marker arrangements being chosen largely as a result of availability of conveniently marked stocks.

A pan-2,cys-2^{+} strain of mating type a was chosen as the common conidial parent. The actual strain is 467, which also bears the marker ad-1. However, for the purposes of the experiments described below this adenine marker is redundant, and adenine was routinely added to all selective medium.

The B type strains were built up by crossing cys-2,A (Fungal Genetics Stock Centre (FGSC) #125) to arg-6, al-2, a (FGSC #272) and selecting progeny of the genotype cys-2;arg-6,al-2,A. Thus, arg-6 is the forcing marker and al-2 is the morphological nuclear marker.

The A type strains were built up by crossing cys-2,ylo;a (FGSC #489) where ylo is the morphological marker, to three mutants whose feasibility as forcing markers for this nuclear type were to be tested. These latter strains were leu-3,A (FGSC #1124), thi-2,A (FGSC #61) and arom-1,A (FGSC #42). Thus a number of strains of the genotype cys-2, ylo;M,A were selected for each prospective forcing marker, where M represents the forcing marker.

Complementation tests were then performed between all the A strains and all the B strains. It was discovered that none of the cys,ylo;thi or the cys,ylo;arom type A strains were compatible with the cys;arg,al type B strains. However, extensive compatibility was observed between the cys;arg,al strains and the cys,ylo;leu strains. Con-

sequently, cys,ylo;leu was chosen as the A genotype, and leu-3 as the forcing marker.

Fertility on crossing these strains to ad-1,pan-2;a was the next criterion of selection, and five sets were found to give abundant ascospores in this cross. These sets, represented here by their respective heterokaryons were;

(cys;arg,al #59 + cys,ylo;leu #23)

(cys;arg,al #59 + cys,ylo;leu #20)

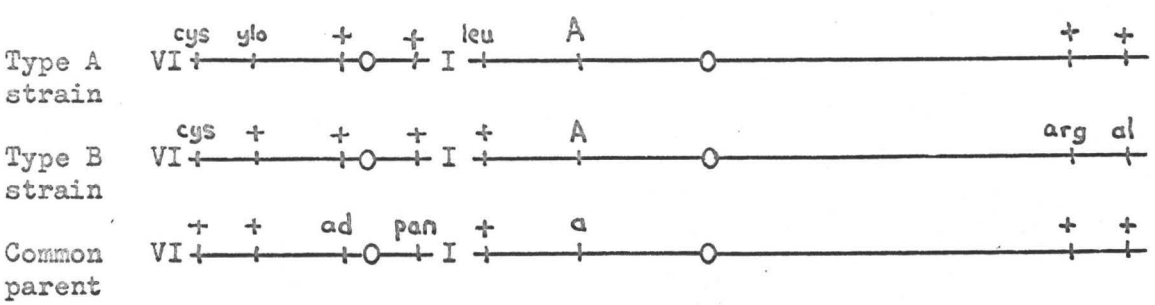
(cys;arg,al #62 + cys,ylo;leu #20)

(cys;arg,al #47 + cys,ylo;leu #23)

(cys;arg,al # 7 + cys,ylo;leu #23)

These sets of three, represented by their respective heterokaryons, will be abbreviated (59+23), (59+20), (62+20), (47+23) and (7+23), where the B-type (cys;arg,al) nucleus is always written first.

The genotypes of A and B-type strains and the common parent can be represented diagrammatically as follows;



approx. 10 map units
(Fincham & Day 1963)

These diagrams are drawn very approximately to scale, and are meant only to show the relative locations of the markers used. The diagrams show

only the marked regions of the marked linkage groups. It is coincidental that both the forcing markers are found on group I. The reported distances between cys and pan in Stadler's and Towe's analysis (Stadler & Towe 1962) varied between about eight units in a parental cross and 17 units in the backcross. However, the fact that variability exists in this region suggested that an even wider spectrum of values might exist.

The three crosses of any individual set can be diagrammatically represented as in Fig.1 with the chromosomes in the paired state. Pan - cys prototrophs from type A crosses may be selected by plating the spores in SFG medium containing adenine and leucine, and prototrophs from type B crosses by plating in medium containing adenine and arginine. Prototrophs from the heterokaryon cross (H-cross) are derived from two component crosses, which have been designated A_H and B_H in Fig. 1. Hence, the selective medium for this cross should contain adenine, leucine and arginine. Consequently, the same selective medium was in fact used for all the three types of crosses, that is, medium containing adenine, leucine and arginine, and any possible differences due to differences in selective medium were thus eliminated.

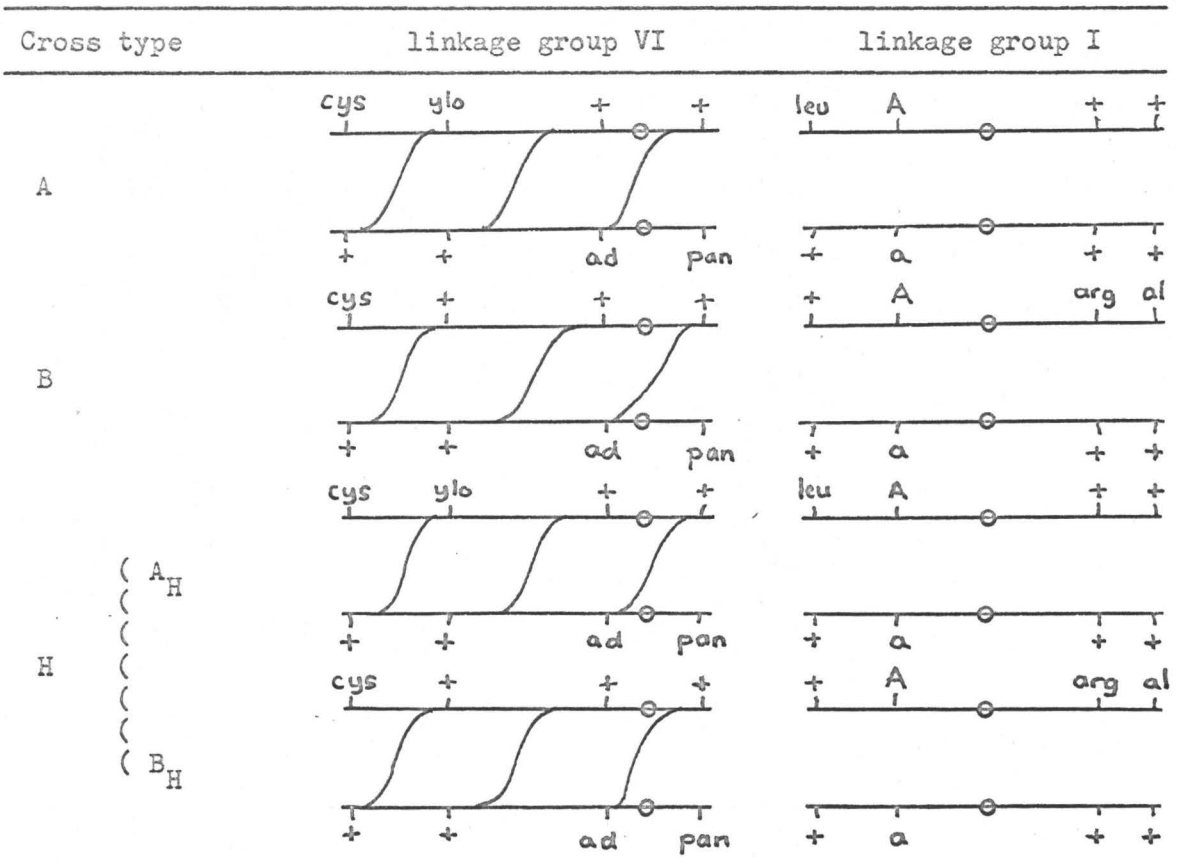


Fig. 1. Marker arrangement in the three types of crosses in a set. Also shown are the recombination events selected for in the experiments.

Inspection of Fig. 1 reveals that pan - cys prototrophs arising from an A or an A_H cross will be of two types with respect to the yellow locus. A certain percentage will be ylo, arising from recombination events between cys and ylo, and a certain percentage will be ylo^+, arising from recombination events between ylo and pan. The relative proportions of these types will depend on the frequency of exchanges in the regions mentioned. Pan - cys prototrophs arising from B or B_H crosses will be of two types with respect to the nuclear marker albino, that is al and al^+.

However, the al marker is on linkage group I, so 50% of these prototrophs should be al, and 50% al^+.

CHAPTER 4

PROTOTROPH FREQUENCIES

Tables 1 to 6 show the experimental values which allow the calculation of prototroph frequency. Tables 1, 3 and 5 have a similar format and each value represents the number of prototrophs in 1.0 ml. of stock ascospore suspension, assayed by plating in ten Petri plates of selective medium. Tables 2, 4 and 6 show the number of viable spores in aliquots of the dilution suspension, and each value represents the number of colonies produced by one 1.0 ml. aliquot taken from one separate dilution tube. Table 7 shows the resulting prototroph frequencies represented as percentages, which are obtained by dividing the values in the last columns of tables 1, 3 and 5 by the values in the last columns of tables 2, 4 and 6, and converting to percentages.

The format of these and many consecutive tables is standard in terms of the two left hand columns showing "set" and "experiment". For example consider the three rows occupied by set 47+23. An "experiment" is defined as the simultaneous analysis of the three crosses of a set, in terms of time of crossing, harvesting and plating of spores and isolation and scoring of colonies. Where experiments have been repeated more than once the Roman capitals are used to designate the repeats. Thus three experiments were performed on set 47+23, each experiment consisting of the simultaneous analysis of the three crosses of that set. Therefore the row represented as 47+23 I (for example) is continuous throughout

TABLE 1

NUMBER OF PROTOTROPHS IN ALIQUOTS OF STOCK ASCOSPORE SUSPENSION IN TYPE-A CROSSES

Set	Experiment*	# prototrophs in 1.0 ml. aliquots of stock ascospore suspension			Mean # prototrophs per ml. stock suspension
		(i)**	(ii)	(iii)	
47+23	I	116	-	-	116
47+23	II	50	-	-	50
47+23	III	211	-	-	211
59+20	I	146	-	-	146
59+20	II	285	-	-	285
59+20	III	162	169	-	165.5
59+20	IV	292	166	253	237
7+23	I	98	-	-	98
62+20	I	129	-	-	129
62+20	II	326	-	-	326
59+23	I	165	122	158	148.3
59+23	II	173	173	-	173
59+23	III	174	189	-	181.5

* The Roman numerals used in this and following tables refer to the repeat number. For example, set 47+23 was repeated three times in experiments I, II and III.

**These numbers refer to the number of aliquots assayed. For example in experiment 59+20 III, two 1.0 ml. aliquots were assayed.

TABLE 2

NUMBER OF VIABLE SPORES IN ALIQUOTS OF DILUTION SUSPENSIONS IN TYPE-A CROSSES

Set	Experiment	# viable spores in 1.0 ml. aliquots taken from the dilution tubes *				Mean # viable spores per 1.0 ml.	Dilution factor	Total # viable spores in 1.0ml. stock ascospore suspension **
		(i)	(ii)	(iii)	(iv)			
47+23	I	701	-	-	-	701	15	10,530
47+23	II	236	-	-	-	236	10	2,360
47+23	III	1,400	-	-	-	1,400	10	14,000
59+20	I	693	-	-	-	693	10	6,930
59+20	II	1,951	-	-	-	1,951	10	19,510
59+20	III	308	331	301	341	320.3	20	6,404
59+20	IV	430	365	429	428	413	20	8,160
7+23	I	609	-	-	-	609	10	6,090
62+20	I	789	-	-	-	789	10	7,890
62+20	II	2,498	-	-	-	2,498	10	24,980
59+23	I	422	375	458	-	418.3	20	8,366
59+23	II	636	651	555	-	614	20	12,280
59+23	III	465	516	480	559	505	20	10,100

* Each figure here represents a count of one 1.0 ml. aliquot taken from one dilution tube. For example in experiment 59+20 III, four dilution tubes were used.

**Obtained by multiplying mean viable spores by the dilution factor.

TABLE 3

NUMBER OF PROTOTROPHS IN ALIQUOTS OF STOCK ASCOSPORE SUSPENSION IN TYPE-B CROSSES

Set	Experiment	# prototrophs in 1.0 ml. aliquots of stock ascospore suspension			Mean # prototrophs per ml. of stock suspension
		(i)	(ii)	(iii)	
47+23	I	262	-	-	262
47+23	II	142	-	-	142
47+23	III	200	-	-	200
59+20	I	125	-	-	125
59+20	II	860	-	-	860
59+20	III *				
59+20	IV *				
7+23	I	88	-	-	88
62+20	I	327	-	-	327
62+20	II	625	-	-	625
59+23	I	348	397	323	356
59+23	II	363	382	-	372.5
59+23	III	511	664	-	587.5

* Results not obtained.

TABLE 4

NUMBER OF VIABLE SPORES IN ALIQUOTS OF DILUTION SUSPENSIONS IN TYPE-B CROSSES

Set	Experiment	# viable spores in 1.0 ml. aliquots taken from dilution tubes				Mean # viable spores per ml.	Dilution factor	Total # viable spores in 1.0 ml. stock ascospore suspension
		(i)	(ii)	(iii)	(iv)			
47+23	I	1,444	-	-	-	1,444	15	21,680
47+23	II	973	-	-	-	973	10	9,730
47+23	III	1,852	-	-	-	1,852	10	18,520
59+20	I	501	-	-	-	501	10	5,010
59+20	II	3,517	-	-	-	3,517	10	35,170
59+20	III *							
59+20	IV *							
7+23	I	482	-	-	-	482	10	4,820
62+20	I	1,061	-	-	-	1,061	10	10,610
62+20	II	3,191	-	-	-	3,191	10	31,910
59+23	I	561	615	615	-	597	20	11,940
59+23	II	537	546	479	-	521	20	10,420
59+23	III	869	953	741	887	862.5	20	17,250

* Results not obtained.

TABLE 5

NUMBER OF PROTOTROPHS IN ALIQUOTS OF STOCK ASCOSPORE SUSPENSION IN TYPE-H CROSSES

Set	Experiment	# prototrophs in 1.0 ml. aliquots of stock ascospore suspension			Mean # prototrophs per ml. stock suspension
		(i)	(ii)	(iii)	
47+23	I	31	-	-	31
47+23	II	178	-	-	178
47+23	III	333	-	-	333
59+20	I	109	-	-	109
59+20	II	290	-	-	290
59+20	III	244	256	-	250
59+20	IV	225	163	204	197.3
7+23	I	74	-	-	74
62+20	I	41	-	-	41
62+20	II	124	-	-	124
59+23	I	54	85	60	66.3
59+23	II	101	73	-	87
59+23	III	216	171	-	193.5

TABLE 6

NUMBER OF VIABLE SPORES IN ALIQUOTS OF DILUTION SUSPENSIONS IN TYPE-H CROSSES

Set	Experiment	# viable spores in 1.0 ml. aliquots taken from dilution tubes				Mean # viable spores per ml.	Dilution factor	Total viable spores in 1.0ml. stock ascospore suspension
		(i)	(ii)	(iii)	(iv)			
47+23	I	251	-	-	-	251	15	3,760
47+23	II	1,076	-	-	-	1,076	10	10,760
47+23	III	2,691	-	-	-	2,691	10	26,910
59+20	I	189	-	-	-	189	10	1,890
59+20	II	1,816	-	-	-	1,816	10	18,160
59+20	III	455	449	427	421	438	20	8,760
59+20	IV	435	435	428	440	434.5	20	8,690
7+23	I	738	-	-	-	738	10	7,380
62+20	I	233	-	-	-	233	10	2,330
62+20	II	1,391	-	-	-	1,391	10	13,910
59+23	I	314	353	352	-	339.7	20	6,792
59+23	II	548	483	526	-	519	20	10,380
59+23	III	860	826	684	741	777.8	20	15,556

TABLE 7

PROTOTROPH PERCENTAGE *

Set	Experiment	A-Crosses		B-Crosses		H-Crosses	
47+23	I	23x ad,pan	1.10	47x ad,pan	1.21	(47+23)x ad,pan	0.83
47+23	II	"	2.12	"	1.46	"	1.65
47+23	III	"	1.51	"	1.08	"	1.24
59+20	I	20x ad,pan	2.14	59x ad,pan	2.49	(59+20)x ad,pan	5.77
59+20	II	"	1.46	"	2.44	"	1.59
59+20	III	"	2.58	"	3.32**	"	2.85
59+20	IV	"	2.90	"	3.32**	"	2.27
7+23	I	23x ad,pan	1.61	7x ad,pan	1.83	(7+23)x ad,pan	1.00
62+20	I	20x ad,pan	1.63	62x ad,pan	3.08	(62+20)x ad,pan	1.76
62+20	II	"	1.30	"	1.96	"	0.89
59+23	I	23x ad,pan	1.77	59x ad,pan	2.98	(59+23)x ad,pan	0.98
59+23	II	"	1.41	"	3.58	"	0.84
59+23	III	"	1.79	"	3.40	"	1.24

* # prototrophs per hundred ascospores

** these values are means of B-cross values in the 59+23 experiments.

all tables of this format. The experiments were performed over two years, so although the three crosses on any given row (an A, B and an H cross) can be validly intercompared, comparisons between rows are less meaningful as they are not performed and analysed simultaneously. In connection with this explanation, the tables concerning B-cross data show no results opposite 59+20 experiments III and IV. These data are absent as the experiments 59+23 I, II and III and 59+20 III and IV were carried out and analysed on consecutive days, consequently it seemed superfluous to repeat the B-cross analysis already carried out one day previously on the same strain (cys;arg,al #59) in 59+23 experiments. Therefore, when values for the experiments 59+20 III and IV are needed, the mean of 59+23 experiments I, II and III are used. This procedure is probably not strictly valid as day to day differences are possible in the analytical technique, however, the cross tubes used would have been from the same batch used in the 59+23 experiments, so any error introduced by the procedure is probably not large.

In Tables 1-6, considerable variation exists between experiments in the number of aliquots assayed; obviously, a prototroph frequency arrived at from a mean of several aliquots of spores is less subject to random fluctuations than one derived from one aliquot .

The dilutions used were x10, x15 and x20, but in any one experiment (ie. horizontal row), the dilution factor is constant.

In this and the following three chapters, the results of the experiments will be presented and discussed in a qualitative manner. The main statistical analysis of the data will be postponed until chapter 8, but some minor statistical confirmations will be presented in this

chapter and in chapters 5, 6 and 7.

Inspection of the prototroph frequencies in Table 7 suggests several tentative general conclusions.

i) There is considerable heterogeneity of data both within the columns and between the columns.

ii) The within-column variation is due to two factors. Firstly, variation due to strain; for example there is no reason why cys,ylo;leu # 23 should give identical prototroph frequencies to cys,ylo;leu # 20 when both are crossed to ad,pan, yet both are found in the A-cross column. A similar argument holds for the B-type crosses, and H-type crosses. Secondly, there is heterogeneity due to random experimental error; for example, in the A-type crosses the cross cys,ylo;leu # 23 x ad,pan shows prototroph percentages of 1.01, 2.12,1.51, 1.61, 1.77, 1.41 and 1.79. These percentages are all estimates of recombination events in the same genetic region, yet a two-fold difference is shown between the extremes. This type of variability seems to be diminished when several aliquots of the suspensions are used in the assay rather than only one, (for example in 59+20 III and IV, and in 59+23 I, II and III) so this is probably the greatest contributing cause of this type of variability. Other contributing causes are no doubt due to slight differences from day to day, and from month to month, in the experimental procedure.

iii) Variability between columns is the main phenomenon under investigation, and two general trends seem to be apparent in the data. Firstly, the B-crosses in general show higher prototroph frequencies than A-crosses. Thus it would appear that the B strains (cys;arg,al) have inherited

characteristics from their common ancestry which cause them to show higher recombination frequencies when crossed to the common parent. (The exception to this general rule is found in the 47+23 set, in which no consistent difference is apparent.) The B strains seem to have inherited these characteristics in different amounts, as witnessed by the absence of a constant A-B differential in set 47+23, and possibly in set 7+23. No trends which can be attributed to differences between the two A-type strains are immediately apparent.

The second trend shown is that in all cases but one, (the abnormally high H-cross value for 59+20 I) the heterokaryon cross values fall either within the range set by the component strains, or more surprisingly, lower than either component strain. The intermediate values fit either the hypotheses of exchange of gene products or lack of exchange of gene products, but the excessively low values would appear to be explicable only in terms of some sort of cross-inhibition of recombination.

However, these conclusions must all be regarded as speculative at this level of analysis. It can be said however, that the 59+23 set shows the trends most markedly and consistently.

It is possible that selective differences in germination behaviour between crosses might give different prototroph frequencies. Germination data are not available for all the crosses performed, but those that were recorded are shown in Table 8, and in order to test the hypothesis a χ^2 test for homogeneity of data was applied to these figures. The resulting χ^2 of 17.6 (12d.f.) gives a p value of between 10 and 20%. Thus the germination values may be considered homogeneous. Thus in the experiments where germination figures are available, differential germination is

TABLE 8

GERMINATION PERCENTAGES

Set	Experiment	A crosses			B crosses			H crosses		
		Sample Size	Germinated Spores	Percentage	Sample Size	Germinated Spores	Percentage	Sample Size	Germinated Spores	Percentage
47+23	I	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-
59+20	I	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-
	III	73	62	84.9	-	-	-	103	84	81.6
	IV	156	131	84.0	-	-	-	189	156	82.5
7+23	I	-	-	-	-	-	-	-	-	-
62+20	I	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-
59+23	I	111	87	78.4	76	67	88.1	127	94	74.0
	II	115	101	87.8	111	92	82.8	111	82	81.2
	III	104	84	80.8	150	126	84.0	103	86	83.4

unlikely to be the cause of the recombination differences. Hence, it seems reasonable to extrapolate to the other experiments and rule out differential germination in these also.

In conclusion to this chapter, the calculated prototroph frequencies permit some broad generalisations to be made, but a deeper understanding of what is going on in the component crosses of the heterokaryon is needed. The next chapter deals with a breakdown of the overall heterokaryon cross frequencies onto their A_H and B_H components by the use of recombination indices.

CHAPTER 5

RECOMBINATION INDICES

In genetic analysis, the frequency of exchange events in a marked region of a chromosome is estimated by dividing total recombinant progeny by total progeny. This is not an absolute measure of the number of recombination events occurring (for example, triple exchanges are usually undetectable), yet if it is consistently used it becomes a pointer or index which can be validly used in comparisons between crosses. However, other indices can be formulated which can be used equally validly as pointers of recombination frequency when comparing crosses.

In the experiments described, it is possible to calculate recombination frequency (via prototroph frequency) in the A and B crosses, but a problem arises in the A_H and B_H crosses where the progeny are mixed and constitute the progeny of the H-cross. However, assumptions can be made which will enable the computation of various recombination indices which can then be used to compare A with A_H and B with B_H frequencies, but not A with B or A_H with B_H frequencies. In computing these indices, use is made of the fact that yellow progeny from an H cross can only have arisen from the A_H component, and albino progeny only from the B_H component. A - B or A_H - B_H comparisons can not be made directly as the yellow marker is in the region under study, and the albino marker is unlinked to that region; thus, whereas prototrophs from

B or B_H crosses can be assumed to show a 1:1 segregation for al:+, this assumption is unjustified for the segregation of ylo:+ in A or A_H crosses. 1:1 segregations of both morphological markers can, however, be expected in the progeny as a whole.

Thus, random samples of prototrophs and of colonies which have grown up on the complete plates (the latter colonies will be abbreviated "random colonies" implying their origin as a random ascospore sample), were isolated from each cross performed, and each colony was grown up in a small slope of vegetative medium as described in chapter 2. The resulting cultures were then scored for conidial pigmentation and the results of this scoring are shown in Tables 9, 10 and 11.

The general segregation of the colour markers can be checked by applying a χ^2 test to the totals of the columns. In Table 9, a comparison of the totals 269 and 218 gives a χ^2 value of 5.34 (1d.f.) which gives a p of between 2% and 5%. Thus, in general, pink prototrophs are more frequent in an A-cross than yellow prototrophs. This suggests that, in general recombination is more frequent in the ylo - pan region than in the cys - ylo region, which might have been predicted from inspection of the linkage map. A comparison of the random colony totals (258 and 211) also in Table 9 reveals a χ^2 of 5.12 (1d.f.) giving a p of between 2% and 5%. Thus a strict 1:1 segregation for yellow is not obtained in A crosses, which is contrary to expectation. Furthermore in some sets (for example 59 + 23), a larger consistent divergence from the 1:1 ratio is manifest, and in other sets (for example 62 + 20) the selection seems to be in favour of the ylo genotype, whereas the overall totals favour ylo^+. Preliminary checks on random colonies had shown 1:1 segregation patterns

for all markers in all the crosses, however in those tests much smaller samples (approximately 10) were used. The cause or causes of these discrepancies are not known, possibly differential germination is responsible.

The segregations in Table 10, however, show good agreement with a 1:1 ratio. The prototroph totals give a χ^2 of 0.04 (1d.f.) which results in a p value of between 80% and 90%, and the random colony totals give a χ^2 of 0.04 (1d.f.) also leading to a p value of between 80% and 90%. Thus in general and in most individual crosses there appears to be no selection for al or al$^+$.

The segregation of pink to yellow plus albino in Table 11 may be checked against a 1:1 ratio. For the prototroph totals a χ^2 of 3.92 is obtained (1d.f.) which gives a p of between 2% and 5%. The random colony totals [illegible] leading to a p of between 10% and 20%, although the differential between 504 and 459 suggests that the segregation of the yellow marker is still in favour of ylo$^+$ even in the A_H cross.

Three types of recombination indices have been computed incorporating the data in Tables 9, 10 and 11. These are explained below.

Index type 1

Yellow prototrophs (YP) are the result of recombination events in an A or A_H cross, and yellow spores (YS) can result only from the same population of A or A_H meioses. Thus, the quotient YP/YS x 100 is a recombination index for A or A_H meioses, and similarly AlP/AlS x 100 is an index for B or B_H meioses. The above A or A_H indices do not estimate recombination over the whole cys - pan region, but rather in the

cys - ylo region, whereas the B or B_H indices measure events over the whole region. (This is why these A index values cannot be compared with the B index values, nor A_H with B_H.) Values for type 1 indices are found in Table 12. They are calculated by combining data on prototroph frequency with data from the colour analysis tables. For example, the B index (2.29) for 47 + 23 I is computed as follows.

$$\frac{\text{Total albino prototrophs}}{\text{Total albino spores from same cross}} \times 100$$

$$= \frac{\text{Mean \# prototrophs in 1.0 ml. stock ascospore suspension} \times \frac{\text{\# albino prototrophs in isolated prototroph sample}}{\text{size of prototroph sample isolated}}}{\text{Mean \# random colonies from 1.0 ml. stock ascospore suspension} \times \frac{\text{\# albino isolates in isolated random colony sample}}{\text{size of random colony sample isolated}}} \times 100$$

$$= \frac{262 \times \frac{28}{43}}{21{,}680 \times \frac{11}{32}} \times 100 = 2.29$$

(This value is not equal to the prototroph frequency for that particular cross (1.21%). This would be expected if the al:al^+ segregation is exactly 1:1 in both the prototroph sample isolated, and the random colony sample isolated i.e. $\frac{262 \times 0.5}{21{,}680 \times 0.5} \times 100 = 1.21\%$.)

The corresponding B_H index is calculated as follows.

$$\frac{31 \times \frac{11}{32}}{3{,}760 \times \frac{16}{54}} \times 100 = 0.94$$

TABLE 9

COLOUR ANALYSIS OF ISOLATED SAMPLES OF PROTOTROPHS AND RANDOM COLONIES IN A-TYPE CROSSES

Set	Experiment	Prototrophs			Random Colonies		
		Sample Size	Pink	Yellow	Sample Size	Pink	Yellow
47+23	I	32	18	14	31	15	16
	II	31	13	18	34	15	19
	III	35	21	14	33	11	22
59+20	I	30	15	15	32	13	19
	II	39	17	22	35	14	21
	III	48	33	15	48	31	17
	IV	50	34	16	49	32	17
7+23	I	20	13	7	20	9	11
62+20	I	32	17	15	24	7	17
	II	32	15	17	29	9	20
59+23	I	44	22	22	42	30	12
	II	47	24	23	45	36	9
	III	47	27	20	47	36	11
TOTALS		487	269	218	469	258	211

TABLE 10

COLOUR ANALYSIS OF ISOLATED SAMPLES OF PROTOTROPHS AND RANDOM COLONIES IN B-TYPE CROSSES

Set	Experiment	Prototrophs			Random Colonies		
		Sample Size	Pink	Albino	Sample Size	Pink	Albino
47+23	I	43	15	28	32	21	11
	II	32	16	16	32	15	17
	III	40	25	15	40	22	18
59+20	I	31	14	17	32	13	19
	II	32	12	20	32	15	17
	III	-	-	-	-	-	-
	IV	-	-	-	-	-	-
7+23	I	20	14	6	20	9	11
69+20	I	32	16	16	24	14	10
	II	32	19	13	32	16	16
59+23	I	44	16	28	47	25	22
	II	48	29	19	48	18	30
	III	48	27	21	45	22	23
TOTALS		402	203	199	384	190	194

TABLE 11

COLOUR ANALYSIS OF ISOLATED SAMPLES OF PROTOTROPHS AND RANDOM COLONIES IN H-TYPE CROSSES

Set	Experiment	Prototrophs				Random Colonies			
		Sample Size	Pink	Yellow	Albino	Sample Size	Pink	Yellow	Albino
47+23	I	32	19	2	11	54	29	9	16
	II	45	21	5	19	50	27	10	13
	III	100	68	3	29	101	43	7	51
59+20	I	47	32	11	4	46	21	12	13
	II	90	35	21	34	89	36	18	35
	III	99	44	20	35	100	57	13	30
	IV	95	42	19	34	100	57	11	32
7+23	I	58	36	15	7	48	16	26	6
62+20	I	40	18	15	7	40	22	10	8
	II	79	49	15	15	78	32	33	13
59+23	I	85	44	15	26	85	47	12	26
	II	95	52	10	33	93	67	7	19
	III	84	46	14	24	79	50	14	15
TOTALS		949	506	165	278	963	504	182	277

409 A nuclei
554 B nuclei

TABLE 12

TYPE-1 INDICES

Set	Experiment	A index	A_H index	Behaviour≠	B index	B_H index	Behaviour≠
47+23	I	0.93	0.31	D	2.29	0.94	D
	II	2.20	0.92	D	1.37	2.67	R
	III	1.41	0.54	D	0.90	0.71	D
59+20	I	1.77	5.17	R	2.30	1.74	D
	II	1.37	1.84	R	2.88	1.53	D
	III	2.28	4.43	R	*(3.08)	3.36	R
	IV	2.68	4.13	R	*(3.08)	2.54	D
7+23	I	1.02	0.48	D	1.00	0.97	D
62+20	I	1.08	2.64	R	3.70	1.54	D
	II	1.00	0.40	D	1.59	1.02	D
59+23	I	3.10	1.22	D	4.05	0.98	D
	II	3.45	1.17	D	2.26	1.43	D
	III	3.27	1.17	D	2.92	1.87	D
					9.23	4.28	

* Means of set 59+23 (See text for explanation)

≠ D = drop in index on entering heterokaryon

R = rise in index on entering heterokaryon

(The figures used in the calculations of these indices are found in Tables 1, 2, 5, 6, 9, 10 and 11.)

The assumption made in computing this type of index is that if the prototroph frequency of the B_H cross is different from that of the B cross, the ratio of $\underline{al}^+$:$\underline{al}$ individuals in the prototroph population or the random colony population does not change. The validity of this assumption will be evaluated in the discussion of index type 2.

Calculations and assumptions analogous to those shown above for the B and B_H indices are used in arriving at the A and A_H indices shown in Table 12.

Index type 2

The assumption that the segregation of the colour markers is unaltered in A_H or B_H crosses is difficult to test directly, but the following argument appears reasonable. The fact that column totals showed a significant difference (at the 5% level of probability) in Table 9 suggests that there is indeed a divergence from a 1:1 segregation for $\underline{ylo}$:$\underline{ylo}^+$ in A crosses. On the other hand, the overall χ^2 tests performed on the column totals in Table 10 showed no significant difference between the overall totals for B crosses. Furthermore, if the 22 possible individual χ^2 tests are performed on the segregation of $\underline{al}$:$\underline{al}^+$ in the crosses in Table 10, only one test reveals a divergence from a 1:1 ratio (at the 5% level). (This is the prototroph sample of 47+23 I.) Consequently, it appears as though deviations from a 1:1 ratio in the segregation of $\underline{al}$:$\underline{al}^+$ are merely random fluctuations. If this is so, and the $\underline{al}$:$\underline{al}^+$ segregation stays 1:1 in the B_H crosses,

then the ylo:ylo$^+$ segregation in the A_H crosses can be computed. If, for example, an H-type cross produces pink, yellow and albino individuals in a hypothetical ratio of 65:10:35, and the above assumptions are made concerning the constancy of a 1:1 segregation for al:al$^+$, then it could be stated that the A_H component is producing a ylo$^+$:ylo segregation of 30:10, and the B_H component is producing an al$^+$:al segregation of 35:35. This ylo$^+$:ylo ratio can be compared to the ylo$^+$:ylo ratio found in the corresponding A cross, using a 2 x 2 contingency table, and if no significant difference is found, it would appear reasonable to conclude that the ylo$^+$:ylo and the al$^+$:al ratios remain constant. This procedure cannot be performed for prototroph samples as the ylo$^+$:ylo ratio might be expected to vary between A and A_H crosses, however, segregation for random colonies in the A crosses and the calculated segregation for random colonies in the A_H crosses have been tabulated in Table 13. A 2 x 2 contingency table using the column totals gives a χ^2 of 0.46 (1d.f.) which gives a p of 30% - 50%. Hence, overall there is little difference between the ratios for ylo$^+$:ylo in the A and A_H crosses. The p values for individual contingency tables are shown in the right-hand column of Table 13. It is seen that out of the eleven χ^2 tests performed, only one gave a p of less than 5%.

This sort of argument, however, cannot be considered as proof that the segregation ratios are not changed in the H crosses: it is possible that both are changed. The argument is based on the principle of Occam's Razor; that is the data are consistent with the absence of a change of segregation, the al$^+$:al remaining 1:1, and ylo$^+$:ylo remaining biased in favour of ylo$^+$. However, the analysis shown in Table 13

TABLE 13

COMPARISON OF ylo$^+$:ylo RATIOS IN RANDOM COLONY SAMPLES OF A AND A_H CROSSES

Set	Experiment	Actual A segregation of ylo$^+$	ylo	Calculated A_H segregation of ylo$^+$	ylo	Probability of A and A_H ratios being identical
47+23	I	15	16	13	9	30-50%
	II	15	19	14	10	20-30%
	III	11	22	*		
59+20	I	13	19	8	12	95-98%
	II	14	21	** 1	18	
	III	31	17	27	13	70-80%
	IV	32	17	25	11	50-70%
7+23	I	9	11	10	26	10-20%
62+20	I	7	17	14	10	2-5%
	II	9	20	19	33	50-70%
59+23	I	30	12	21	12	30-50%
	II	36	9	48	7	30-50%
	III	36	11	35	14	50-70%
TOTALS		258	211	235	175	30-50%

* This ratio cannot be calculated.

** This ratio cannot be used in a 2 x 2 contingency table as one value is < 5.

is a justification for the formulation of index type 2 which may be defined as:

$$\frac{\text{Total prototrophs from an A, } A_H\text{, B or } B_H \text{ cross}}{\text{Total spores from the same cross}} \times 100$$

Values calculated on this basis for the A and B crosses are identical to the prototroph frequencies from the A and B crosses. Indices for the A_H and B_H crosses are calculated below using 47+23 I as an example.

$$A_H \text{ index} = \frac{31}{3,760} \times \frac{\frac{(19-11+2)}{32}}{\frac{(29-16+9)}{54}} \times 100 = 0.63$$

$$B_H \text{ index} = \frac{31}{3,760} \times \frac{\frac{(11x2)}{32}}{\frac{(16x2)}{54}} \times 100 = 0.96$$

The figures used in this calculation are found in Tables 1, 2, 5, 6, 9, 10 and 11. The values for the type 2 indices are found in Table 14. The letters D and R in Tables 12 and 14, signify "Drop" or "Rise" in prototroph frequency and refer to the behaviour of the indices on the relocation of the cross to inside the heterokaryon.

Thus it has been seen that both indices 1 and 2 are based on the assumption that the segregation ratios of the colour markers do not change on entering the heterokaryon. However, index 1 differs from index 2, in that index 1 looks on the segregations found in the prototroph and random colony samples as true reflections of the segregations found in the whole populations and assumes that these do not change.

TABLE 14

TYPE 2 INDICES

Set	Experiment	A index	A_H index	Behaviour	B index	B_H index	Behaviour
47+23	I	1.01	0.63	D	1.21	0.96	D
	II	2.12	0.54	D	1.46	2.69	R
	III	1.51	*		1.08	*	
59+20	I	2.14	11.00	R	2.49	1.74	D
	II	1.46	0.18	D	2.44	1.53	D
	III	2.58	2.09	D	**		
	IV	2.90	1.79	D	**		
7+23	I	1.61	1.01	D	1.83	0.97	D
62+20	I	1.63	1.91	R	3.08	1.54	D
	II	1.30	0.83	D	1.96	1.02	D
59+23	I	1.77	0.98	D	2.98	0.98	D
	II	1.41	0.43	D	3.58	1.43	D
	III	1.79	0.86	D	3.40	1.87	D

* Cannot be computed (see Table 11) as 51 > 43.

** Data not obtained.

Furthermore, if selective techniques seem to favour one genotype on one particular day in an A or B cross (for example the 21:11 ratio of al$^+$:al in the random colony sample in 47+23 I B cross (Table 10)), then the same selection will prevail in segregations in the corresponding A_H or B_H cross. Index type 2 accepts that the segregation of ylo$^+$:ylo in the A or A_H crosses does not conform to a 1:1 ratio, but considers the segregation ratios found in the B or B_H crosses to be random deviations from true 1:1 ratios. Thus both indices assume constancy, but of differing types. Little can be said as to which is a better estimate of recombination frequency; the matter would be clearer if larger samples of colonies had been isolated.

The A and A_H values of the type 1 index measure recombination in the cys - ylo region only, whereas the corresponding values of the type 2 indices measure the frequency of events in the whole cys - pan region. Thus the possibility arises that the cys - pan region can be split into two segments and each examined individually. This possibility is examined further in index type 3.

Index type 3

A yellow prototroph from an A of an A_H cross must have arisen from an exchange in the cys - ylo region, whereas a pink prototroph arising from the same crosses must have arisen from an exchange in the ylo-pan region. The assumption of a constant 1:1 segregation of al$^+$:al, made in the formulation of index type 2, enables the total number of pink progeny in an H cross to be split into an A_H and a B_H component. Thus a third type of index may be formulated which is capable of throwing light on the behaviour of both regions comprising the overall cys - pan region of linkage group VI. This may be stated as:

$$\frac{\text{Total pink (or yellow) prototrophs from an A or } A_H \text{ cross}}{\text{Total spores from the same cross}} \times 100$$

These indices are shown in Table 15, where A(pink) and A_H(pink) indices measure r e combination in the ylo - pan region in A and A_H crosses, and A(yellow) and A_H(yellow) measure recombination in the cys - ylo region in the same crosses. This third type of index does not, of course, measure recombination in B or B_H meioses.

47+23 I can again be used as an example of the type of calculations involved.

$$\text{A(pink) index} = \frac{116 \times \frac{18}{32}}{10,530} \times 100 = 0.62$$

$$A_H\text{(pink) index} = \frac{31 \times \frac{(19-11)}{32}}{3,760 \times \frac{(29-16+9)}{54}} \times 100 = 0.51$$

$$\text{A(yellow) index} = \frac{116 \times \frac{14}{32}}{10,530} \times 100 = 0.48$$

$$A_H\text{(yellow) index} = \frac{31 \times \frac{2}{32}}{3,760 \times \frac{(29-16+9)}{54}} \times 100 = 0.13$$

The figures used in these calculations are found in Tables 1, 2, 5, 6, 9 and 11. (N.B. The prototroph sample size was 32 in both A and H crosses.)

Type 3 A(yellow) and A_H(yellow) indices measure the same recombination events as type 1 A and A_H indices, in fact the numerators are common but the denominators differ in that the type 3 indices use

TABLE 15

TYPE 3 INDICES

Set	Experiment	A (pink) index	A_H (pink) index	Behaviour	A(yellow) index	A_H(yellow) index	Behaviour
47+23	I	0.62	0.51	D	0.48	0.13	D
	II	0.89	0.15	D	1.23	0.38	D
	III	0.90	*		0.60	*	
59+20	I	1.05	7.90	R	1.05	3.10	R
	II	0.64	0.01	D	0.82	1.75	R
	III	1.78	0.65	D	0.81	1.44	R
	IV	1.98	0.53	D	0.93	1.26	R
7+23	I	1.05	0.67	D	0.56	0.35	D
62+20	I	0.87	0.81	D	0.77	1.10	R
	II	0.61	0.58	D	0.69	0.25	D
59+23	I	0.89	0.53	D	0.89	0.44	D
	II	0.72	0.28	D	0.69	0.15	D
	III	1.03	0.53	D	0.77	0.33	D

* Cannot be computed.

total spores and the type 1 indices use total yellow spores. A type 3 A(yellow) or A_H(yellow) index using "yellow spores" as a denominator would of course give indices identical to type 1. However, indices A(pink) and A_H(pink) can be formulated which use "pink spores" as the denominator and can be called type 4 indices (Table 16). This index type 4 should give results similar to A(pink) and A_H(pink) indices of type 3, if the assumptions of constant segregation ratios are valid.

Index type 4

See above paragraph.

The four indices are summarised in Table 17.

The main trends shown by the behaviour of the indices can be discussed qualitatively at this point, and analysed in Chapter 8 using an analysis of variance.

Cys - pan region in B and B_H crosses

The strongest and most consistent trend shown by the index analysis is in the behaviour of this region. Both indices dealing with this region (indices 1 and 2) show consistent drops in the index on entering a heterokaryon (Tables 12 and 14). There is only one case where a strong rise is shown (47*23 II). Thus it can be concluded tentatively, that the B_H meioses are strongly affected by the presence of either cys,ylo;leu #20 or #23, suggesting that the cys,ylo;leu strains have both received a factor in their common ancestry which is capable of influencing all the four cys;arg,al strains in their recombination efficiency. There are no obvious differences in the behaviour of any A-type strain

TABLE 16

TYPE 4 INDICES

Set	Experiment	A (pink) index	A_H (pink) index	Behaviour
47+23	I	1.28	0.86	D
	II	2.01	0.26	D
	III	2.71	*	
59+20	I	2.59	19.75	R
	II	1.59	0.16	D
	III	2.75	0.96	D
	IV	3.02	0.77	D
7+23	I	2.32	2.41	R
62+20	I	2.98	1.38	D
	II	1.97	1.58	D
59+23	I	1.24	0.84	D
	II	0.90	0.32	D
	III	1.35	0.74	D

* Cannot be computed.

TABLE 17

SUMMARY OF INDEX FORMULATIONS AND CORRESPONDING REGIONS EXAMINED BY THEM

Index type	Formulation		Regions examined *
1.	$\frac{\text{Total } \underline{ylo}(\underline{al}) \text{ prototrophs in an A(B) or } A_H(B_H) \text{ cross}}{\text{Total } \underline{ylo}(\underline{al}) \text{ ascospores from same cross}}$	x 100	cys-ylo and cys-pan
2.	$\frac{\text{Total prototrophs from an A, } A_H, \text{B or } B_H \text{ cross}}{\text{Total ascospores from same cross}}$	x 100	cys - pan
3.	$\frac{\text{Total } \underline{ylo} \text{ (pink) prototrophs in an A or } A_H \text{ cross}}{\text{Total ascospores from same cross}}$	x 100	cys-ylo and ylo-pan
4.	$\frac{\text{Total pink prototrophs in an A or } A_H \text{ cross}}{\text{Total pink ascospores from same cross}}$	x 100	ylo - pan

* Where a second region is stated in this column, it refers to the bracketed term in the formulations.

or B-type strain in this respect.

Cys - pan region in A and A_H crosses

The behaviour of this overall region is demonstrated only by the type 2 index (Table 14). Ten experiments show a drop and two a rise in the index. One of the experiments showing a rise is 59 + 20 I, and in all the A_H indices calculated for this experiment (Tables 12, 14, 15 and 16) similar abnormally high values are found. Consequently, the drastic rises shown in this experiment must be regarded with suspicion. Despite the 59 + 20 I value the general tendency of the indices is to drop, in the A_H crosses.

Cys - ylo region in A and A_H crosses

The indices pertinent to this region are types 1 and 3. Examination of Tables 12 and 15 show a perfect agreement in the behaviour of the indices, in every cross performed (A - A_H in Table 12 and A(yellow) and A_H(yellow) in Table 15). Furthermore, a consistent trend is manifest, in that indices concerned with cys,ylo;leu #20 show a rise, whereas those concerned with cys,ylo;leu #23 show a drop. One exception to this generalisation is found (62+20 II). If this is a real trend, it appears that the two different types of behaviour of the indices (drop or rise), must be attributed to differences in the A-type strains, and not to the B-types, as (for example) cys;arg,al #59 produces a rise with cys,ylo;leu #20, but a drop with cys,ylo;leu #23. Thus the different effects seen

in this region must be attributable to a differential reaction to the presence of the B_H component in the heterokaryon.

Ylo - pan region in A and A_H crosses

The behaviour of this region is shown by indices 3 and 4 (Tables 15 and 16, A(pink) and A_H(pink) indices). There is good agreement between these tables, a general drop in index predominating, if experiment 59+20 I is ignored.

Thus, in general, a mutual inhibition of recombination occurs in the heterokaryon, with the possible exception of the cys - ylo region of cys, ylo;leu #20, in which recombination seems to be stimulated. This statement has been made however, without recourse to statistical tests.

The prototroph frequencies in Table 7 showed that H cross frequencies were usually either intermediate between the respective A and B cross frequencies (as might be expected), or lower than either A or B frequencies. The analyses, using various recombination indices, have shown strong evidence that both these phenomena have a common basis, and that whether the H frequency is intermediate or excessively low depends on the nuclear or meiotic ratios in the heterokaryons. The fact that at least one of the component crosses must show an intra-heterokaryon frequency lower than either the A or the B homokaryotic crosses in order to give excessively low H values, suggests that some sort of complementation of inhibiting gene products is occurring.

Even without statistical analysis, it does appear, because of the consistency of the trends, that some sort of interaction is occurring between the components of the heterokaryon, and that this interaction is difficult to visualise in terms of physical heterozygosity arguments.

A problem arises in consideration of the mode of interaction that occurs, and the time in the life cycle at which it occurs. It is known that in the vegetative state, the mycelium of Neurospora is coenocytic, but little is known about the porosity of the septa in the ascogonium and ascogenous hyphae. Furthermore, if the gene products responsible for interaction are at all specific for recombination, one might expect them to be induced only at meiosis, and it is difficult to see how the physical exchange of such products between meioses might occur. In order to see if different meioses can occur inside a perithecium, a perithecial analysis was performed (Table 18), and in fact mixed perithecia were found (perithecia 2, 3, 6, 11, 20 and 25). It is difficult to draw any conclusion about the frequency of mixed perithecia from these data, due to the low germination percentages obtained in most cases. However, it does seem as though they are reasonably frequent. The origin of mixed perithecia is uncertain; they may arise through double fertilisation of a heterokaryotic ascogonium, through the inclusion of two different homokaryotic ascogonia in one perithecial wall, or possibly by other methods (Weijer & Dowding, 1960; Nakamura & Egashira, 1961; Magill, 1966). Consequently, the perithecial analysis is not particularly helpful in throwing light on the mode of exchange of the hypothetical gene products. It shows that two different meioses can occur

TABLE 18

PERITHECIAL ANALYSIS OF A CROSS (59+23) ♀ x *ad pan* ♂

Perithecium #	Size of sample	Wild types (pink)	*ylo*	*al*	Did not germinate
1	50	19	0	6	25 *
2	50	11	6	2	31 *
3	50	23	15	7	5 *
4	50	15	0	23	12
5	50	6	0	17	27
6	50	25	5	11	9
7	50	18	0	21	11
8	50	24	0	17	9
9	50	7	0	3	40 *
10	39	22	0	12	5
11	38	16	1	5	16
12	38	12	0	11	15
13	37	16	0	0	21
14	37	12	0	7	18
15	31	17	0	0	14
16	39	17	0	16	6
17	38	14	0	3	21
18	32	18	0	3	11
19	39	16	0	10	13
20	37	22	1	10	4
21	38	18	0	16	4
22	36	19	0	14	3
23	39	15	18	0	6
24	38	33	1	0	4
25	38	19	3	14	2
26	39	12	0	21	6

* Heavily contaminated with bacteria.

inside one perithecium, but how the insular nature of the young ascus is penetrated (if at all), remains in doubt.

Another possibility is that the effect of one nucleus on another is in some manner permanent or semi-permanent, the effect taking place either in the vegetative stage, or during the synchronous division of the presumptive fusion nuclei in a mixed perithecium. The effect may take place through the production of some long-lived gene product, which can remain in the cytoplasm and hence enter the ascus before it becomes isolated, or through some kind of change, induced in one nucleus by another, that is capable of persisting through mitotic replication. It is conceptually difficult to visualize how a change, other than a type of mutation, can persist through nuclear division: nevertheless, evidence is presented in the next two chapters which necessitates the serious consideration of the suggestion.

CHAPTER 6

RECIPROCAL CROSSES

The experiments described so far have exclusively concerned crosses in which ad-1,pan-2,a (467) was used as the common conidial parent. This chapter describes analogous experiments in which ad,pan was used as the protoperithecial parent in all crosses performed. Of all the sets described in the last two chapters, set 59+23 has shown the most consistent trends, and the behaviour of its component strains reflect the general conclusions tentatively arrived at for the data as a whole. Briefly to summarise the behaviour of set 59+23, it can be said that the prototroph frequencies shown by its H-crosses are lower than the A-crosses, and the A-crosses all show lower frequencies than the B-crosses. In the indices, one trend is consistently manifested in all the three experiments (I,II and III) performed, that is, a drop in all the A and all the B indices on entering the heterokaryon. Consequently, set 59+23 was chosen for the reciprocal crosses reported in the chapter. Six experiments were performed on this set, and they are represented i, ii, iii, iv, v and vi.

The following tables (Tables 19 to 29) are for the most part analogous to the tables described in Chapters 4 and 5, and differences in format are referred to in the footnotes. Tables 19 and 20 show the data which allow the calculation of the prototroph frequencies shown in Table 21.

Inspection of Table 21 shows that again in the reciprocal crosses, B-cross prototroph frequencies are higher that A-cross frequencies. The absolute values of the frequencies themselves seem to be in general lower than the corresponding crosses using ad-pan as the conidial parent, however the reciprocal crosses were performed approximately one year later than the rest of the crosses. The data in Table 21 bear a further resemblance to the original (ad, pan♂) data, in that the prototroph frequencies in the H-cross are on the whole lower than either the A or B heterokaryotic crosses. Thus several conclusions emerge from this table. Firstly, that the original differences between A and B are probably not due to cytoplasmic differences between the strains, but to genotypic differences. Secondly, it seems to be true that the same phenomenon of recombination depression in the H-crosses is occurring in these reciprocal crosses.

At the same time as the reciprocal crosses were being analysed, two H-crosses using ad, pan as the conidial parent were also analysed. These gave prototroph frequencies of 0.99% (performed on the same day as 59+23(i)) and 0.74% (performed on the same day as 59+23 (ii)). The fact that both these are higher than the reciprocal H-cross frequencies obtained on the same day (0.48% and 0.56% respectively) suggests that the ad, pan cytoplasm does provide an environment less favourable to recombination, but not enough data are available for a valid comparison to be made. However, it does appear that the A - B differentials cannot be explained by cytoplasmic differences, as they are still plainly manifest in ad, pan cytoplasm.

TABLE 19

NUMBER OF PROTOTROPHS IN ALIQUOTS OF STOCK ASCOSPORE SUSPENSION IN TYPE A, B AND H RECIPROCAL CROSSES

Set	Experiment	# prototrophs in 1.0 ml. aliquots of stock ascospore suspension ≠ (i)	(ii)	(iii)	Mean # prototrophs per 1.0 ml. stock suspension	Type of Cross *
59+23	i	56	77	-	66.5	A
	ii	75	88	-	81.5	A
	iii	79	70	-	74.5	A
	iv	58	42	-	50	A
	v	31	18	-	24.5	A
	vi	15	14	18	15.7	A
	i	203	223	-	213	B
	ii	213	234	-	223.5	B
	iii	101	38	-	69.5	B
	iv	130	77	-	103.5	B
	v	40	33	-	36.5	B
	vi	61	51	55	55.7	B
	i	15	22	-	18.5	H
	ii	44	54	-	49	H
	iii	39	38	-	38.5	H
	iv	22	22	-	22	H
	v	10	11	-	10.5	H
	vi	48	38	35	40.3	H

* In this table, all three crosses of the set of reciprocal crosses are shown.

≠ The numerals (i) to (iii) here designate aliquots, and have no relation to the experiment numerals.

TABLE 20

NUMBER OF VIABLE SPORES IN ALIQUOTS OF DILUTION SUSPENSIONS IN TYPE A, B AND H RECIPROCAL CROSSES

Set	Experiment	# viable spores in 1.0 ml. aliquots taken from the dilution tubes			Mean # viable spores per 1.0 ml.	Dilution factor	Total viable spores in 1.0ml. stock ascospore suspension	Type of cross
		(i)	(ii)	(iii)				
59+23	i	311	315	-	313	20	6,260	A
	ii	385	428	-	406.5	20	8,130	A
	iii	375	340	-	357.5	20	7,150	A
	iv	451	471	-	461	20	9,220	A
	v	215	243	-	229	20	4,580	A
	vi	83	91	101	91.7	20	1,834	A
	i	457	447	-	452	20	9,040	B
	ii	655	691	-	673	20	13,460	B
	iii	186	185	-	185.5	20	3,710	B
	iv	199	178	-	188.5	20	3,770	B
	v	121	116	-	118.5	20	3,370	B
	vi	70	82	94	82	20	1,640	B
	i	185	204	-	194.5	20	3,890	H
	ii	450	431	-	440.5	20	8,810	H
	iii	289	293	-	291	20	5,820	H
	iv	199	210	-	204.5	20	4,090	H
	v	206	233	-	219.5	20	4,390	H
	vi	270	211	272	251	20	5,020	H

TABLE 21

PROTOTROPH PERCENTAGES IN RECIPROCAL CROSSES

Set	Experiment	ad,pan x 23 (A-CROSSES)	ad,pan x 59 (B-CROSSES)	ad,pan x(59+23) (H-CROSSES)
59+23	i	1.06	2.36	0.48
	ii	1.00	1.66	0.56
	iii	1.04	1.87	0.66
	iv	0.54	2.75	0.54
	v	0.54	1.08	0.24
	vi	0.85	3.39	0.80

TABLE 22

GERMINATION PERCENTAGES IN RECIPROCAL CROSSES

Set	Experiment	A crosses			B crosses			H crosses		
		Sample size	Germinated spores	%	Sample size	Germinated spores	%	Sample size	Germinated spores	%
59+23	i	50	45	90.0	50	47	94.0	50	42	84.0
	ii	55	54	98.2	52	49	94.2	75	71	94.7
	iii	76	70	92.1	70	60	85.7	77	65	84.4
	iv	80	68	85.0	90	86	95.5	76	65	85.5
	v	93	74	79.6	61	56	91.8	76	64	84.2
	vi	55	38	69.1	58	41	70.7	89	80	89.9

TABLE 23

COLOUR ANALYSIS OF ISOLATED SAMPLES OF PROTOTROPHS AND RANDOM COLONIES IN RECIPROCAL CROSSES

Set	Experiment	PROTOTROPHS				RANDOM COLONIES				Type of Cross
		Sample size	Pink	Yellow	Albino	Sample size	Pink	Yellow	Albino	
59×23	i	24	13	11	-	20	8	12	-	A
	ii	104	53	51	-	97	51	46	-	A
	iii	39	25	14	-	26	13	13	-	A
	iv	29	14	15	-	21	13	8	-	A
	v	* -	-	-	-	-	-	-	-	A
	vi	45	24	21	-	45	37	8	-	A
	Total≠	241	129	112	-	209	122	87	-	A
	i	28	11	-	17	21	13	-	8	B
	ii	101	42	-	59	96	43	-	53	B
	iii	52	15	-	37	23	8	-	15	B
	iv	34	12	-	22	35	20	-	15	B
	v	* -	-	-	-	-	-	-	-	B
	vi	47	26	-	21	46	27	-	19	B
	Total≠	262	106	-	156	221	111	-	110	B
	i	38	29	2	7	41	24	1	16	H
	ii	96	53	6	37	86	41	3	42	H
	iii	69	49	6	14	88	50	12	26	H
	iv	41	22	4	15	58	19	14	25	H
	v	* -	-	-	-	-	-	-	-	H
	vi	99	72	10	17	82	60	5	17	H
	Total≠	343	225	28	90	355	194	35	126	H

* Not performed.

≠ Total for that type of cross.

103 A nucl
252 B nucl

TABLE 24

COMPARISON OF ylo^+:ylo RATIOS IN RANDOM COLONY SAMPLES OF A and A_H RECIPROCAL CROSSES

Set	Experiment	Actual A segregation of ylo^+:ylo		Calculated A_H segregation of ylo^+:ylo	
59+23	i	8	12	8	1
	ii	51	46	*	
	iii	13	13	24	12
	iv	13	8	*	
	v	** -	-	-	-
	vi	37	8	43	5
	TOTAL	122	87	75	18

* Cannot be computed as #albinos > #pinks in H cross.

** Not performed.

TABLE 25

TYPE-1 INDICES FOR RECIPROCAL CROSSES

Set	Experiment	A index	A_H index	Behaviour	B index	B_H index	Behaviour
59+23	i	0.81	1.03	R	3.75	0.22	D
	ii	1.04	1.00	D	1.76	0.44	D
	iii	0.75	0.42	D	2.04	0.45	D
	iv	0.74	0.22	D	4.15	0.46	D
	v	* -	-	-	-	-	-
	vi	2.24	1.33	D	3.67	0.67	D

* Not performed.

TABLE 26

TYPE-2 INDICES FOR RECIPROCAL CROSSES

Set	Experiment	A index	A_H index	Behaviour	B index	B_H index	Behaviour
59*23	i	1.06	1.37	R	2.36	0.23	D
	ii	1.00	* -	-	1.66	* -	-
	iii	1.04	0.96	D	1.87	0.45	D
	iv	0.54	* -	-	2.75	* -	-
	v	0.54	* -	-	1.08	* -	-
	vi	0.85	0.90	R	3.39	0.67	D

* Cannot be computed, or data unavailable. 7.62 1.35

TABLE 27

TYPE-3 INDICES FOR RECIPROCAL CROSSES

Set	Experiment	A(pink) index	A_H(pink) index	Behaviour	A(yellow) index	A_H(yellow) index	Behaviour
59*23	i	0.56	1.25	R	0.49	0.11	D
	ii	0.51	* -	-	0.49	* -	-
	iii	0.66	0.82	R	0.37	0.14	D
	iv	0.34	* -	-	0.28	* -	-
	v	* -	* -	-	* -	* -	-
	vi	0.46	0.76	R	0.40	0.14	D

* Cannot be computed, or data unavailable.

TABLE 28

TYPE-4 INDICES FOR RECIPROCAL CROSSES

Set	Experiment	A (pink) index	A_H (pink) index	Behaviour
59+23	i	1.41	1.41	Same
	ii	0.97	* -	-
	iii	1.34	1.23	D
	iv	0.54	* -	-
	v	* -	* -	-
	vi	0.56	0.85	R

* Cannot be computed, or data unavailable.

TABLE 29

PERITHECIAL ANALYSIS OF A CROSS *ad, pan* ♀ x (59+23) ♂

Perithecium	Size of sample	Wild (pink)	Yellow	Albino	Did not Germinate
1	50	25	0	1	24 *
2	50	6	0	7	37 *
3	50	13	0	0	37 *
4	50	9	0	4	37 *
5	50	10	0	2	38 *
6	50	7	0	4	39 *
7	37	8	0	5	14 *
8	30	11	0	15	4
9	38	9	0	15	14
10	35	16	0	0	19 *
11	29	11	0	9	9
12	31	10	0	7	14
13	28	8	0	12	8
14	30	16	0	0	14 *
15	37	16	0	14	7 *
16	30	9	0	12	9
17	39	13	0	22	4
18	39	14	0	15	10
19	31	15	0	1	15
20	31	11	9	0	11
21	31	11	0	11	9
22	31	7	0	16	8
23	39	12	0	21	6
24	30	15	0	8	7
25	39	18	0	10	11
26	39	20	0	14	5

* Heavy bacterial contamination.

It again seems unlikely that the phenomena can be explained in terms of different germination percentages, as these are constantly high and show no apparent differentials which are constant in direction or size (Table 22). Consequently, a colour analysis of isolated prototroph and random colony samples was performed (Table 23), with a view to breaking down the H-cross frequencies in terms of component indices.

Inspection of Table 23 reveals that once again the segregation of $\underline{ylo}^+$:$\underline{ylo}$ is irregular in A crosses. A χ^2 test performed on the random colony column totals (122 and 87) gives a p value of between 1% and 2%. Random colony samples from the B-crosses show close overall agreement with a 1:1 segregation of $\underline{al}^+$:$\underline{al}$ (111:110), the χ^2 leading to a p of 90% - 95%. However, the segregation of $\underline{al}^+$:$\underline{al}$ in the prototroph samples does not appear to conform to a 1:1 ratio. A χ^2 test on the totals 106 and 156 leads to a p value of less than 1%. However, of the $\underline{al}^+$:$\underline{al}$ segregations in the individual prototroph samples, only one (experiment iii) shows a significant deviation from a 1:1 ratio at the 5% level; the deviation is a large one, and is the major contributing factor to the magnitude of the overall χ^2. Hence, the assumption of a 1:1 segregation of $\underline{al}^+$:$\underline{al}$ can be made, although perhaps with less conviction than in the previously analysed data.

The overall segregation of pink:non-pink in the random colony samples of the H-crosses gives a total of 194:35+126, which equals 194:161. If these figures are compared with a 1:1 ratio, the resultant p is just greater than 5%; however, the excess of pink colonies can be assigned to the aberrant $\underline{ylo}^+$:$\underline{ylo}$ segregation of the A_H meioses.

Table 24 compares the ylo$^+$:ylo ratio in A-cross random colonies with that calculated for A_H crosses on the assumption of a 1:1 segregation of al$^+$:al. The ratios of the totals show poor agreement (122:87 compared with 75:18), and individually the only experiment in which a comparison shows a p value of greater than 5%, is experiment vi. However, it should be noticed that two A_H segregations (ii and iv) cannot be computed as the number of albino colonies in those samples is greater than the number of pink colonies (see Table 23, H-crosses). This means that the 3 plus 14 ylo colonies have been missed in the ylo column total. The inclusion of these colonies would no doubt bring the A_H ratio closer to the A ratio, but a discrepancy would probably still exist.

In the last chapter, it was pointed out that the indices are based on two types of assumption. Index type 1 was based on the assumption that segregations are the same (whether normal or abnormal) inside the heterokaryon, as they are in the homokaryotic crosses, and it is difficult to evaluate this assumption quantitatively. (The assumption does not apply to the ylo$^+$:ylo segregation of A and A_H prototrophs which might be expected to vary.) Type 2 indices however, (and types 3 and 4) depend on a constant 1:1 segregation of al$^+$:al in B and B_H progeny, whether prototrophs or random colonies. It has been seen above that this assumption is reasonably well justified by the reciprocal cross data, but its corollary, that of constant abnormal ylo$^+$:ylo segregation in random colony samples, is not upheld by the available figures.

The conclusions drawn from the indices types 1 to 4 shown in Tables 25 to 28 must be evaluated in the light of the above considerations.

Once again the most consistent trends shown by the indices are those shown by the B indices (Tables 25 and 26), where drops occur in every case. This would correspond to an inhibition of recombination (in B_H meioses) over the cys - pan region of linkage group VI.

The behaviour of the cys - ylo region in A and A_H meioses is again shown by index 1 (A and A_H indices) and index 3 (A(yellow) and A_H(yellow) indices). The most common behaviour of these indices is a drop, the only exception being index 1, experiment 59+23 i. This trend is shared with the ad,pan ♂ crosses in which strain cys,ylo;leu #23 is involved.

The behaviour of the cys - pan region in A and A_H crosses is shown by index 2 (A and A_H indices). No consistent trend is shown (Table 26).

The behaviour of the ylo - cys region in A and A_H crosses is shown by index type 3 (A(pink) and A_H(pink) indices) and also by index type 4 (Table 28). The two types of index, measuring events in this region do not concur, although a rise is the most common behaviour. Furthermore, the lack of trend seen in the behaviour of the overall cys - pan region in A and A_H crosses (Table 26) might be attributed to the drop in the cys - ylo region and the possible rise in the ylo - cys region, although this phenomenon is by no means clear.

Thus, it appears as though the depressed H-cross prototroph frequencies can largely be attributed to a consistent lowering of recombination frequency in the cys - pan region in the B_H type meioses, and, more tentatively in the cys - ylo region of the A_H type meioses.

The depressed H-cross frequencies, even in the absence of the index analysis, must be attributed to some form of interaction between the component strains of the heterokaryon. This same conclusion was obtained from the original ad,pan ♀ crosses, but in the reciprocal crosses it is even more difficult to visualise the stage at which the interaction could occur. If mixed perithecia are common, it is conceivable that gene products from both types of meioses might flood their common perithecium and achieve interaction. In order to investigate this possibility a perithecial analysis was done in a cross ad,pan ♀ x heterokaryon (59+23) ♂, and the results are shown in Table 29. Out of 26 perithecia analysed, none showed both yellow and albino ascospores, one showed a segregation for ylo^+ and ylo (perithecium #20), and 25 showed a segregation for al^+ and al. Again, it is possible that the very poor germination obtained in most of these perithecia might lead to a failure to detect small numbers of ylo ascospores in a mixed perithecium. However, germination was equally bad in the former perithecial analysis (Table 18), yet several mixed perithecia were detected. Furthermore, it does not appear that the technique is selecting against the ylo genotype, since perithecium #20 shows a relatively large number of ylo spores in what is presumably a purely ylo^+:ylo segregation. It may be concluded tentatively that if mixed perithecia do occur they are relatively rare, and that most perithecia contain only al^+ x al meioses.

Thus the overall lowering of the H-cross frequency appears to be largely due to the effect of the A-type nuclei on the B-types, since B_H-type meioses seem to predominate in the H-cross and in the homokaryotic B crosses a relatively high prototroph frequency is found. 103:252

It is still possible, of course, that inter-perithecial effects are occurring, although this seems less likely. Alternatively, as suggested in the previous chapter, the interaction may occur at the heterokaryon level, and the effect may be of a permanent or semi-permanent nature. This permanence seems necessary as a postulate since the "male" fertilising nucleus undergoes numerous mitotic divisions prior to the establishment of ascus initials.

In order to test the hypothesis of a permanent effect through interaction, the original A and B nuclei were re-isolated from the heterokaryon, and again crossed to the common parent ad-1,pan-2. The results of these experiments are described in the next chapter.

CHAPTER 7

CONIDIAL ISOLATES

For these experiments, heterokaryon (59+23) was used. The heterokaryon was grown up vegetatively on glucose medium, and a conidial suspension was plated, in an appropriate concentration, on sorbose plates. The plates were examined under a dissection microscope after approximately eighteen hours, at which time sparse colonies approximately 1 mm. - 2 mm. in diameter are visible. Using a fine tungsten needle, it is possible to isolate hyphal tips from such colonies and be reasonably certain that no foreign conidia are included. The cultures resulting from these isolations are either pink (which are presumably still heterokaryotic), or yellow or albino (which are homokaryotic). These cultures are referred to as conidial isolates, implying their origin as single conidia. The nuclear ratio of the heterokaryon can be calculated from a knowledge of the frequency of these three types of vegetative isolate (Prout, et al. 1953), but no attempt was made to do so in this study. Heterokaryotic isolates are designated by P, yellow by Y and albino by A. Conidial isolates of each type were crossed both as protoperithecial and conidial parents to ad-1,pan-2:a 467, and two of each type were selected for further analysis on the basis of their fertility in the crosses. The six isolates analysed in this chapter are P1, P2, Y1, Y3, A2 and A3. The strains heterokaryon (59+23), cys,ylo;leu

#23, and cys;arg,al #59 were run simultaneously in each experiment to act as controls.

The results of these experiments are shown in Tables 30 to 36. In these tables, the letters m to x are used to designate experiments handled simultaneously, in the same way that Roman numerals have been used in previous chapters. Letters m to r refer to crosses using ad,pan as conidial parent, and in s to x ad,pan was used protoperithecially. Tables 30 to 33 show the data which permit the calculation of the prototroph frequencies shown in Table 34. The trends shown in Table 34 are discussed below.

In the A-type crosses where ad,pan is used as the conidial parent (experiments m and n), both the conidial isolates show slightly reduced frequencies when compared with cys,ylo;leu #23. This trend is not manifest in the crosses where ad,pan is the protoperithecial parent (s and t).

In all the B-type crosses performed, the albino conidial isolates show prototroph frequencies consistently lower than the crosses involving the strain cys;arg,al #59. This phenomenon seems to be more marked in the crosses where ad,pan is used as the protoperithecial parent (experiments u and v). If these results are statistically significant, it must be concluded that the passage of these strains through the heterokaryons has conferred upon them some permanent change in their ability to undergo recombination. This sort of phenomenon is usually associated with extranuclear inheritance; however, this appears unlikely in these crosses as the trend in the B crosses is shown most markedly in experiments u and v, where the B-type strains are used as

TABLE 30

NUMBER OF PROTOTROPHS IN ALIQUOTS OF STOCK ASCOSPORE SUSPENSION FROM CROSSES INVOLVING CONIDIAL ISOLATES AS PROTOPERITHECIAL PARENTS

Strain crossed to *ad,pan*	Experiment *	# prototrophs in 1.0 ml. aliquots of stock suspension		Mean # prototrophs per 1.0 ml. stock suspension
		(i)	(ii)	
Cys,ylo;leu,23	m	119	106	112.5
Y1	m	351	310	330.5
Y3	m	283	322	302.5
Cys,ylo;leu,23	n	124	126	125
Y1	n	344	386	365
Y3	n	338	398	368
Cys;arg,al,59	o	423	437	430
A2	o	240	282	261
A3	o	131	137	134
Cys;arg,al,59	p	525	584	554.5
A2	p	340	381	360.5
A3	p	104	102	103
Heterokaryon(59+23)	q	214	193	203.5
P1	q	338	331	334.5
P2	q	200	198	199
Heterokaryon(59+23)	r	236	240	238
P1	r	412	401	406.5
P2	r	404	366	385

* crosses analysed in the same experiment, i.e. on the same day, are designated by an arbitrary letter, in this and in following tables. Also, m and n (for example) are duplicates of the same groups of crosses.

TABLE 31

NUMBER OF VIABLE SPORES IN ALIQUOTS OF DILUTION SUSPENSIONS FROM CROSSES INVOLVING CONIDIAL ISOLATES AS PROTOPERITHECIAL PARENTS

Strain crossed to ad,pan	Experiment	# viable spores in 1.0 ml. aliquots taken from dilution tubes (i)	(ii)	Mean # viable spores per ml.	Dilution factor	Total viable spores in 1.0 ml. stock ascospore suspension
Cys,ylo;leu,23	m	208	193	200.5	40	8,020
Y1	m	1,236	1,256	1,246	20	24,920
Y3	m	1,366	1,363	1,364.5	20	27,290
Cys,ylo;leu,23	n	163	187	175	40	7,000
Y1	n	1,223	1,257	1,240	20	24,800
Y3	n	1,178	1,171	1,174.5	20	23,490
Cys;arg,al,59	o	845	785	815	20	16,300
A2	o	821	810	815.5	20	16,310
A3	o	361	373	367	20	7,340
Cys;arg,al,59	p	1,152	1,218	1,185	20	23,700
A2	p	809	879	844	20	16,880
A3	p	301	295	298	20	5,960
Heterokaryon(59+23)	q	649	702	675.5	20	13,510
P1	q	539	592	565.5	20	11,310
P2	q	834	773	803.5	20	16,070
Heterokaryon(59+23)	r	1,040	1,013	1,026.5	20	20,530
P1	r	698	741	719.5	20	14,390
P2	r	1,534	1,687	1,610.5	20	32,210

TABLE 32

NUMBER OF PROTOTROPHS IN ALIQUOTS OF STOCK ASCOSPORE SUSPENSION FROM CROSSES INVOLVING CONIDIAL ISOLATES AS CONIDIAL PARENTS

Strain crossed to *ad,pan*	Experiment	# prototrophs in 1.0 ml. aliquots of stock suspension		Mean # prototrophs per 1.0 ml. stock suspension
		(i)	(ii)	
Cys,ylo;leu,23	s	295	219	257
Y1	s	130	105	117.5
Y3	s	186	193	189.5
Cys,ylo;leu,23	t	127	185	156
Y1	t	113	143	128
Y3	t	185	247	216
Cys;arg,al,59	u	206	179	192.5
A2	u	54	52	53
A3	u	27	28	27.5
Cys;arg,al,59	v	685	645	665
A2	v	163	151	157
A3	v	115	145	130
Heterokaryon(59+23)	w	129	84	106.5
P1	w	321	334	327.5
P2	w	168	196	182
Heterokaryon(59+23)	x	154	123	138.5
P1	x	268	276	272
P2	x	246	317	281.5

TABLE 33

NUMBER OF VIABLE SPORES IN ALIQUOTS OF DILUTION SUSPENSIONS FROM CROSSES INVOLVING CONIDIAL ISOLATES AS CONIDIAL PARENTS

Strain crossed to *ad,pan*	Experiment	# viable spores in 1.0 ml. aliquots taken from dilution tubes (i)	(ii)	Mean # viable spores per ml.	Dilution factor	Total viable spores in 1.0 ml. stock ascospore suspension
Cys,ylo;leu,23	s	734	657	695.5	20	13,910
Y1	s	441	452	446.5	20	8,930
Y3	s	593	639	616	20	12,320
Cys,ylo;leu,23	t	635	614	624.5	20	12,490
Y1	t	449	478	463.5	20	9,270
Y3	t	833	727	780	20	15,600
Cys;arg,al,59	u	611	629	620	20	12,400
A2	u	511	559	535	20	10,700
A3	u	388	445	416.5	20	8,330
Cys;arg,al,59	v	1,623	1,590	1,616.5	20	32,130
A2	v	902	909	905.5	20	18,110
A3	v	912	936	924	20	18,480
Heterokaryon(59+23)	w	591	634	612.5	20	12,250
P1	w	775	800	787.5	20	15,750
P2	w	732	845	788.5	20	15,770
Heterokaryon(59+23)	x	896	999	947.5	20	18,950
P1	x	870	829	849.5	20	16,990
P2	x	1,481	1,573	1,527	20	30,540

TABLE 34

PROTOTROPH PERCENTAGES IN CROSSES INVOLVING CONIDIAL ISOLATES

Experiment *	Strain crossed to ad,pan		
	cys,ylo;leu 23	Y1	Y3
m	1.40	1.33	1.11
n	1.79	1.49	1.57
s	1.85	1.32	1.54
t	1.25	1.38	1.38
	cys;arg,al 59	A2	A3
o	2.64	1.60	1.83
p	2.34	2.14	1.73
u	1.55	0.49	0.33
v	2.07	0.87	0.70
	Heterokaryon(59+23)	P1	P2
q	1.51	2.96	1.24
r	1.16	2.83	1.20
w	0.87	2.08	1.15
x	0.73	1.60	0.92

* In experiments m, n, o, p, q and r, ad,pan is conidial parent.
In experiments s, t, u, v, w and x, ad,pan is protoperithecial parent (see tables 30 to 33).

TABLE 35

COLOUR ANALYSIS OF CONIDIAL ISOLATES FROM THE THREE HETEROKARYONS (59+23), P1, and P2, IN ACTUAL NUMBERS AND AS PERCENTAGES

Heterokaryon	Total conidial isolates analysed	Pink	Yellow	Albino
59+23	202	72	0	130
P1	184	145	5	34
P2	197	99	12	86
59+23	100%	36%	0%	64%
P1	100%	79%	3%	19%
P2	100%	50%	6%	44%

TABLE 36

GERMINATION PERCENTAGES IN CROSSES INVOLVING CONIDIAL ISOLATES

Strain crossed to *ad,pan*	*ad,pan* conidial parent				*ad,pan* protoperithecial parent			
	Sample size	Spores germinated	%	Experiment	Sample size	Spores germinated	%	Experiment
Cys,ylo;leu 23	117	64	54.70	m	114	96	84.21	s
Y1	59	52	88.13	m	61	52	85.24	s
Y3	52	47	90.38	m	60	50	83.33	s
Cys,ylo;leu 23	87	53	60.91	n	63	58	92.06	t
Y1	79	65	82.27	n	64	56	87.50	t
Y3	81	76	93.82	n	71	59	83.09	t
Cys;arg,al 59	162	115	70.99	o	95	80	84.21	u
A2	69	56	81.15	o	64	56	87.50	u
A3	67	52	77.61	o	42	39	92.85	u
Cys;arg,al 59	112	83	74.11	p	86	74	86.04	v
A2	103	75	72.82	p	120	95	79.17	v
A3	87	55	63.21	p	71	62	87.32	v
Heterokaryon(59+23)	77	69	89.61	q	62	54	87.09	w
P1	78	63	80.76	q	74	63	85.13	w
P2	75	64	85.33	q	95	82	86.31	w
Heterokaryon(59+23)	81	72	88.88	r	76	68	89.47	x
P1	137	105	76.64	r	104	83	79.81	x
P2	69	60	86.95	r	116	90	77.59	x

conidial parents and are presumably freed from their cytoplasmic particles during the passage of the fertilising nucleus down the trichogyne. These points will be pursued in the discussion in Chapter 9.

The H-crosses show an even more surprising trend in that the heterokaryotic conidial isolate P1 shows a prototroph frequency consistently higher than the heterokaryon (59+23) from which it was derived. (P2 shows little difference from (59+23).) Thus we have the situation where a heterokaryotic conidial isolate from a heterokaryon shows behaviour different from its parental heterokaryon. One explanation of this finding is that the nuclear ratio in this conidial isolate is different from that of the original heterokaryon. This is possible as the original conidium from which the isolate grew need not have a nuclear ratio identical to the mycelium from which it is pinched off. In order to test this hypothesis, a large number of conidial isolates were isolated from the three types of heterokaryon (59+23), P1 and P2, and analysed for colour. The results are shown in the top portion of Table 35. A 3 x 3 contingency table composed of the distributions shown in the top part of Table 35 gives a χ^2 value of 94.3 (4d.f.) which leads to a probability of far less than 1%. Thus it appears that if the ratios of different types of conidial isolate are a reflection of nuclear ratios, as is likely, the nuclear ratios are indeed different. The figures in the top of Table 35 can be expressed as percentages of the total conidial isolates analysed, and these are shown in the bottom portion of the table. From these percentages it appears that the main difference between the three heterokaryons is that P1 has far fewer albino nuclei than either 59+23 or P2. Superficially this situation

is the reverse of what might be expected, since the B-type nuclei are, after all, those which on the whole show high recombination frequencies, and a small proportion of these should result in a low recombination frequency for P1. However, the data presented so far have suggested that the low prototroph frequencies in H-crosses are largely due to depressed B_H-cross values. Furthermore, there is evidence that the effect which leads to these depressed B_H values is some permanent effect on the cys,arg,al nuclei. If this argument is sound, the elevated value shown by P1 can be explained in terms of it containing a smaller proportion of cys;arg,al nuclei, that is, a smaller proportion of the agents responsible for depression of H-cross frequencies. Under this argument, the interaction presumably took place in the (59*23) mycelium, so that most or all of the cys;arg,al nuclei in the conidium which would give rise to P1 were in the changed state, and each capable of passing this change on to their progeny. The way in which this change is brought about, and the nature of the change, remain in doubt.

Table 36 shows the germination percentages for the experiments described in Tables 30 to 34. No consistent trends are seen in these germination figures which could account for the prototroph frequency data.

In this chapter, more evidence has been presented that an interaction takes place between two heterokaryon components, furthermore, that the reaction is inhibitory in nature. The main effect seems to be that of the A-type components on the B-type components, and the fact that A2 and A3 are consistently low compared with 59, whereas Y1 and Y3 show no powerful trend in relation to 23, provides an unexpected confirmation of

this finding which was found originally in the indices data. Evidence has also been presented that the effects of the interaction are lasting, as witnessed by the reduced prototroph frequencies in most crosses involving conidial isolates as parents. The increased Pl frequency is interesting in that it can apparently be explained only on the basis of a permanent interaction effect. The fact that the direction in which the crosses are made seems to have little effect on the phenomena observed, is a further indication of the lasting effect of the interaction and of its ability to be transmitted through mitotic division.

CHAPTER 8

STATISTICAL ANALYSIS

This chapter consists of a statistical analysis of the prototroph frequencies and recombination indices presented in previous chapters.

The basic types of comparison to be made are those between A, B and H prototroph frequencies, between A-type and A_H-type indices, between B and B_H indices, and between prototroph frequencies in crosses involving conidial isolates and the prototroph frequencies of the original homokaryotic strains. The values which can be most validly compared are those within any given experiment, for example those within 47+23 II, those within 59+23 i, or those within conidial isolate experiment "m". These comparisons are valid since the experimental procedure is, as nearly as possible, identical within such an experiment. It follows from this statement, that comparisons of means of several experiments are also valid, for example the mean of the type-1 A indices for set 47+23 can be compared with the mean of the type-1 A_H indices for set 47+23. Most of the experiments reported have been performed at least in duplicate, consequently a comparison of means is the most common comparison.

A standard test for the comparison of means in small samples is the student's t-test; however, although the result of any one t-test can be readily interpreted, the interpretation of large numbers of t-tests is complicated, due to the following argument. Consider seven samples taken from populations with identical means. In order to test all possible

pairs of samples for differences among their means, 21 t-tests would have to be performed, for each of which the probability of arriving at the correct conclusion of no significant difference would be 0.95 (assuming the 5% level of significance is being used). Thus the probability of arriving at the correct conclusion for all 21 pairs is $(0.95)^{21}$, and the probability of obtaining at least one incorrect conclusion is $1 - (0.95)^{21}$ which equals $1 - 0.34$ or 66%.

The analysis of variance provides a procedure for the intercomparison of many means under one probability value, and in addition can lead to statistical confirmation of effects which are difficult to confirm using t-tests. The analysis used exclusively in this chapter is a two-way analysis of variance. This analysis is based on the partitioning of the total sum of squares into four components, and using each component to provide an estimate of the variance of the population. The estimates of variance are then compared using an F distribution.

The basic format used in an analysis of variance can be represented as in the following diagram, which shows a 2 x 2 format, the simplest two-way analysis of variance.

	Column 1	Column 2
Row 1	Cell 1,1	Cell 2,1
Row 2	Cell 1,2	Cell 2,2

In the present experiments the columns could represent indices inside and outside the heterokaryon, and the rows could represent various sets. The cells each contain at least two values of the index.

Each individual value (x) in the format will inevitably differ from the overall mean $\bar{x}$ by some value d , where

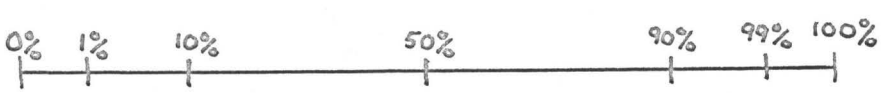
$$d = \bar{x} - x.$$

The total sum of squares can then be represented Σd^2. A large value for the total sum of squares (total SOS) arises when the figures in the table show a wide range of values. In order to pinpoint the basis of this wide range of values, the total SOS is partitioned into "between columns SOS", "between rows SOS", "interaction SOS" and "residual SOS". If a large difference exists between the column means, a large between-column SOS is found, similarly a difference in row means is reflected in a high between-row SOS. A large interaction SOS is expected if, for example, the mean of cell 1,1 is in excess of the mean of cell 2,1 and at the same time the mean of cell 2,2 is in excess of the mean of cell 1,2. A situation such as the latter would undoubtedly show little difference between column means or between row means. Residual SOS is ofter referred to as "error SOS", and a high value for this term reflects a wide range of values due to random fluctuation, or "error" in measurement.

By dividing these four partitions of the total SOS by their respective degrees of freedom, four values are obtained (mean squares) which can be looked upon as different estimates of the variance of population. If no column, row or interaction effects are present, all these estimates of variance must be reflections of the same source of variability, that is, variance due to error. Thus by comparing actual column,

row or interaction mean squares to the residual mean square, some insight can be obtained as to the existence of column, row or interaction effects. The procedure used is to calculate three F values by dividing either the column, row or interaction mean squares by the residual mean square. Tables can then be used to determine the probabilities of finding F values of the order calculated.

The F analysis is only valid if the data being examined conform to normal distributions. However, the prototroph frequency and recombination index values in the present experiments are all expressed as percentages, and cannot extend to less than 0% or to greater than 100%. Consequently, if the mean is close to either theoretical limit, the distribution will tend to skewed, and not normal. This is the case with the data reviewed, where few percentages higher than 5% have resulted from the calculations. Partially to remedy this situation, all the values to be compared in the analysis of variance have been transformed to "arcsin" values. The transformation is to the angle whose sine is the square root of the percentage, and is effected through the use of arcsin tables. The effect of the arcsin transformation is illustrated by the following sketch of the arcsin range.



The values for the sums of squares can be calculated from the following formulae.

If k = # columns,

r = # rows,

rk = # cells,

n = # values per cell (assume constant),

N = total # values = krn,

and x represents one individual value,

$$\text{Total SOS} = \sum_{N} x^2 - \frac{(\Sigma x)^2}{N}, \qquad \text{with } (N-1)\text{d.f.}$$

$$\text{Between columns SOS} = \frac{\sum_{nr}^{k} (\Sigma x)^2}{nr} - \frac{(\Sigma x)^2}{N} \qquad \text{with } (k-1)\text{d.f.}$$

$$\text{Between rows SOS} = \frac{\sum_{kn}^{r} (\Sigma x)^2}{kn} - \frac{(\Sigma x)^2}{N} \qquad \text{with } (r-1)\text{d.f.}$$

$$\text{Interaction SOS} = \frac{\sum_{r}\sum_{k}\sum_{n} (\Sigma x)^2}{n} - \frac{(\Sigma x)^2}{N} - \text{Between cols. SOS} - \text{Between rows SOS}$$

with (r-1)(k-1)d.f.

Residual SOS = Total SOS - Between cols. SOS - Between rows SOS - interaction SOS

with rk(n-1)d.f.

In analyses where n is not constant, the expressions for between columns, between rows, and interaction sums of squares have to be adjusted accordingly. The adjustment can be illustrated most simply by stating the amended parts of the expression in words.

Thus,

$$\text{Between columns SOS} = \sum^{k} \frac{(\text{column total})^2}{(\#\text{ values in that column})} - \frac{(\Sigma x)^2}{N}$$

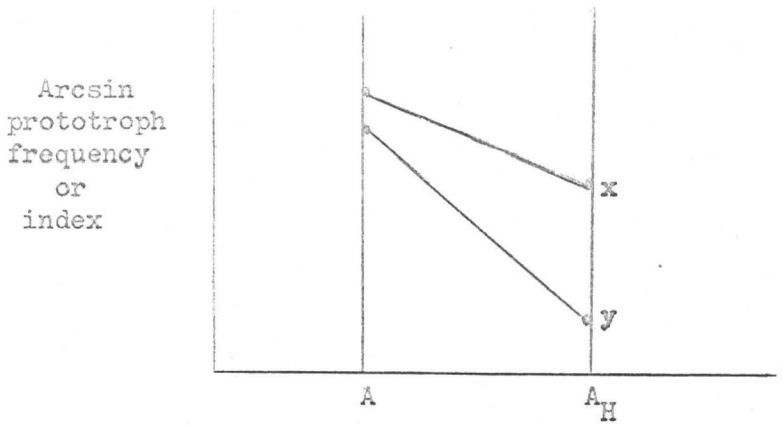
$$\text{Between rows SOS} = \sum^{r} \frac{(\text{row total})^2}{(\#\text{ values in that row})} - \frac{(\Sigma x)^2}{N}$$

$$\text{Interaction SOS} = \sum^{r}\sum^{k} \frac{(\text{cell total})^2}{(\#\text{ values in that cell})} - \frac{(\Sigma x)^2}{N}$$

$$- \text{Between cols. SOS} - \text{Between rows SOS.}$$

It should be pointed out that none of these expressions define the terms used, but merely represent computational forms which allow the values to be calculated readily.

The analyses are presented in the following way in the succeeding pages. The data to be analysed are presented in their arcsin form (Tables 37, 39, 41, 43, 45, 47, 49, 51, 53, 54 & 57), and on the page following each set of data the analysis of variance is shown (Tables 38, 40,42, 44, 46, 48, 50, 52, 55, 56 & 58) together with a chart showing diagrammatically the behaviour of the prototroph frequency means or recombination index means which are being considered (Figures 2 - 12). These diagrams show arcsin values on the y-axis, whose scale is constant throughout. The x-axis consists of an arbitrary yet constant scale which represents diagrammatically two different "treatments", in the present context usually referring to heterokaryotic and homokaryotic states. The following example shows the behaviour of A-type and A_H-type indices for two hypothetical strains x and y.



(The distance between the vertical lines is constant throughout diagrams bearing this format.) The sample diagram shows that both strains x and y show a drop in index on entering the heterokaryon, however, y is obviously influenced to a much greater extent than x. If this difference in behaviour is significant, the difference will show up in the interaction F value. Thus high interaction F values can result through a difference in sign or degree of the slope of the lines joining the recombination estimates. A large separation of the joining lines might be reflected in a high row F, although the importance of such differences in the present experiments is minimal since the differences probably are a result of slight variations in experimental treatment from experiment to experiment. The sample diagram shows a difference between the mean of the A_H values and the mean of the A values. If this difference is significant it will show up in the column F value.

Only one experiment was performed on set 7+23, and it is impossible to estimate the contribution of the recombination values of this set to the overall variance estimates. Consequently, this set has been eliminated from the statistical analysis in this chapter; however, it is represented

on the diagrams (using broken lines), and is shown in the transformed arcsin tables although not included in the totals.

Values for reciprocal crosses have been combined with the ad,pan ♂ crosses in the analyses of variance, and the conidial isolate data have been analysed separately.

The data, therefore, are presented in "units", each unit consisting of a table of arcsin transformations, an analysis of variance table, and a diagrammatic representation of the data. Each unit will be discussed individually.

The high column F in the prototroph frequency data (Tables 37 & 38, Figure 2) shows that differences in the column means are significant. No post-hoc comparison tests have been performed in these analyses, yet it seems probable that most of the effect here is due to the generally high values shown in B-type crosses. The mean for H-crosses is slightly lower than either A or B crosses, and would be even lower were it not for the abnormally high value shown by set 59+20. It is difficult to categorise all the factors which contribute to the significant interaction effect, yet sets 59+20 and 47+23 seem the most obvious exceptions to the general trend. The significant row differences can not be interpreted as reflecting real differences between the sets, and most likely reflect varying experimental treatments.

This unit of data, therefore, shows that in general, A strains differ from B strains in their recombination behaviour, that this difference is probably not due to cytoplasmic differences (as witnessed by the good agreement of the $\frac{1}{59+23}$ (reciprocal cross) means with the general

trends), and that little can be said about what happens to the component strains in the H-crosses.

The unit dealing with type-1 A and A_H indices (Tables 39 & 40, Figure 3) will be considered in conjunction with that dealing with type-3 A(yellow) and A_H(yellow) indices (Tables 47 & 48, Figure 7), since both concern the estimation of recombination in the cys - ylo region of A-type strains. Neither unit shows any significant differences in column means; however, the significant interaction effects shown by both units reveals an interesting possibility when considered in conjunction with the diagrammatic plots (Figures 3 & 7). It is seen that sets 59+20 and 62+20 in Figure 3, and sets 59+20 in Figure 7 show an increased intraheterokaryon index. The 62+20 set in Figure 7 shows no rise in index, yet the possibility must be considered that in the A strain cys,ylo;leu # 20, recombination in the cys - ylo region is stimulated by the presence of the heterokaryotic partners, whereas in the A strain cys,ylo;leu # 23, recombination in the same region is depressed (if only slightly) in the heterokaryons.

The type-1 B and B_H index unit (Tables 41 & 42, Figure 4) shows an extremely high column F value, confirming the suspicion that recombination in the cys - pan region of B-type strains is profoundly influenced by the heterokaryotic presence of both the A-type strains. It is difficult to pinpoint the main sources of the interaction effect in this unit, yet the fact that the interaction effect is there can be taken to mean that some strains are influenced to a greater extent than others.

The behaviour of the type-1 B and B_H indices is confirmed by the

TABLE 37

ARCSIN TRANSFORMATIONS OF PROTOTROPH PERCENTAGES

Set	Experiment	A cross		B cross		H cross		Totals
47+23	I	5.74		6.29		5.23		
	II	8.33		7.04		7.49		
	III	7.04		6.02		6.29		
		21.11	M*=7.04	19.35	M=6.45	19.01	M=6.34	59.47
59+20	I	8.33		9.10		13.94		
	II	7.04		8.91		7.27		
	III	9.28		10.47		9.81		
	IV	9.81		10.47		8.72		
		34.46	M=8.62	38.95	M=9.74	39.74	M=9.94	113.15
7+23**	I	7.27		7.71		5.74		**-
62+20	I	7.27		10.41		7.71		
	II	6.55		8.31		5.41		
		13.82	M=6.91	18.27	M=9.14	13.12	M=6.56	45.21
59+23	I	7.71		9.98		5.68		
	II	6.80		10.94		5.26		
	III	7.71		10.63		6.29		
		22.22	M=7.41	31.55	M=10.52	17.23	M=5.74	71.00
59+23	i	6.02		8.91		3.97		
	ii	5.74		7.49		4.29		
	iii	5.74		7.92		4.66		
	iv	4.21		9.63		4.21		
	v	4.21		6.02		2.81		
	vi	5.29		10.63		5.13		
		31.21	M=5.20	50.60	M=8.43	25.07	M=4.18	106.88
	TOTALS	122.82	M=6.82	158.72	M=8.82	114.17	M=6.34	395.71

* M = Cell mean

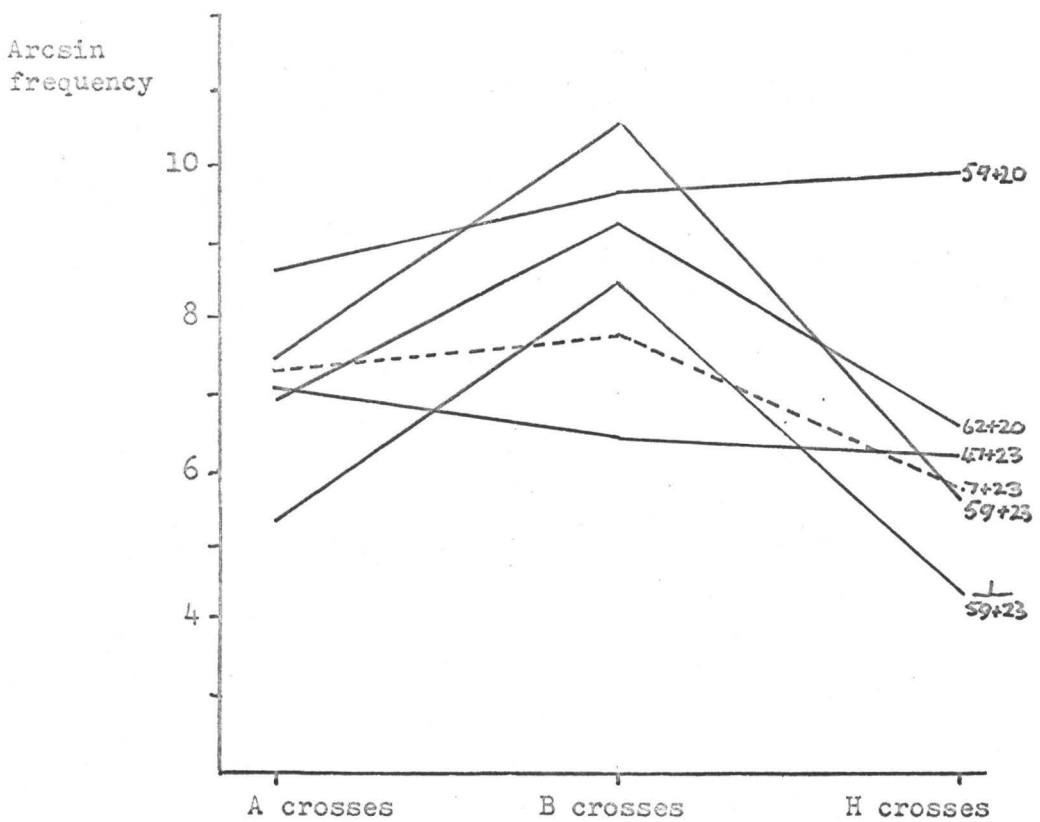
**7+23 not included in analysis of variance in any unit

TABLE 38

ANALYSIS OF VARIANCE OF ARCSIN PROTOTROPHS PERCENTAGES SHOWN IN TABLE 37

	Sum of squares	d.f.	Mean squares	F	Probability
Total	266.5907	53			
Columns	62.0061	2	31.0031	18.91	< 0.005
Rows	95.5247	4	23.8812	14.57	< 0.005
Interaction	45.1138	8	5.6392	3.44	< 0.005
Residual	63.9461	39	1.6396		

Figure 2. Plot of means of arcsin prototroph percentages from table 37.



* $\frac{1}{59+23}$ denotes the reciprocal crosses here and in following figures.

TABLE 39

ARCSIN TRANSFORMATION OF A AND A_H TYPE-1 INDICES

Set	Experiment	A indices		A_H indices		Totals
47+23	I	5.53		3.19		
	II	8.53		5.50		
	III	6.80		4.21		
		20.86	M=6.95	12.90	M=4.30	33.76
59+20	I	7.71		13.18		
	II	6.80		7.71		
	III	8.72		12.11		
	IV	9.44		11.68		
		32.69	M=8.17	44.68	M=11.17	77.37
7+23	I	5.74		3.97		-
62+20	I	6.02		9.28		
	II	5.74		3.63		
		11.76	M=5.88	12.91	M=6.46	24.67
59+23	I	10.14		6.29		
	II	10.78		6.29		
	III	10.47		6.29		
		31.39	M=10.46	18.87	M=6.29	50.26
59+23	i	5.16		5.74		
	ii	5.74		5.74		
	iii	4.97		3.72		
	iv	4.93		2.69		
	v	-		-		
	vi	8.53		6.55		
		29.33	M=5.87	24.44	M=4.89	53.77
	TOTALS	126.03		113.80		239.83

TABLE 40

ANALYSIS OF VARIANCE OF ARCSIN TYPE-1 A AND A_H INDICES SHOWN IN TABLE 39

	Sum of squares	d.f.	Mean squares	F	Probability
Total	230.4952	33			
Columns	4.3992	1	4.3992	1.64	0.1 - 0.25
Rows	108.7870	4	27.1950	10.15	< 0.005
Interaction	52.9781	4	13.2445	4.94	< 0.005
Residual	64.3309	24	2.6805		

Figure 3. Plot of means of arcsin type-1 A and A_H indices shown in table 39.

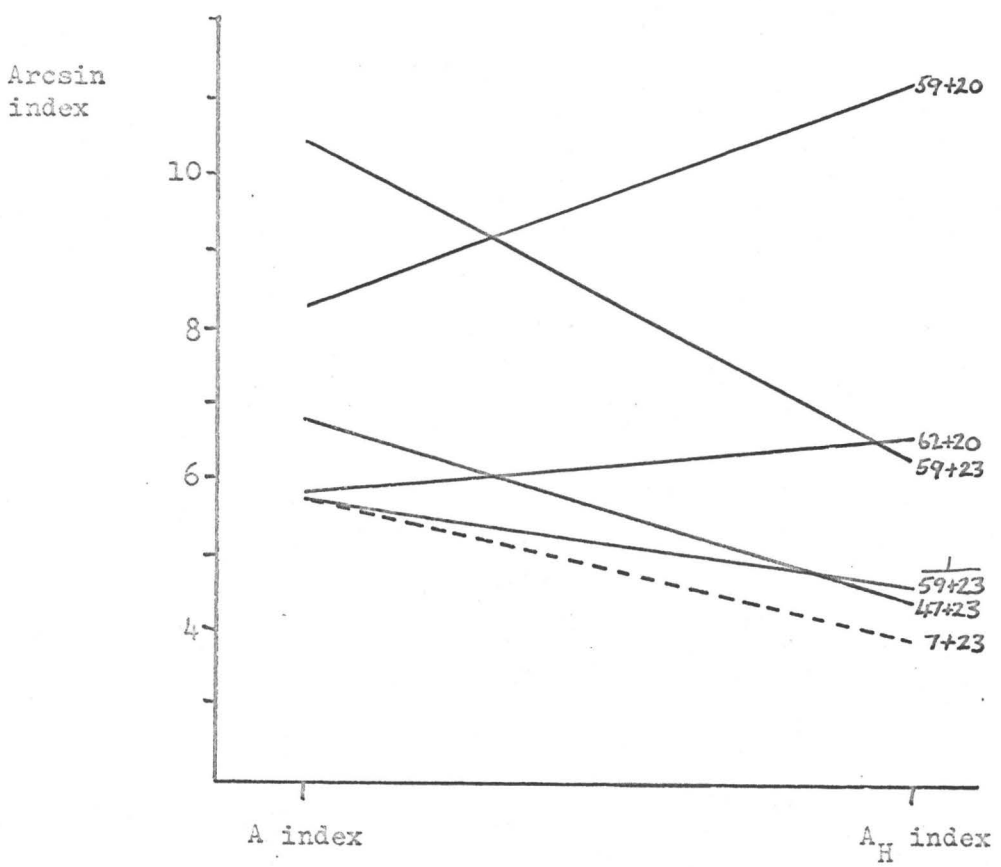


TABLE 41

ARCSIN TRANSFORMATIONS OF B AND B_H TYPE-1 INDICES

Set	Experiment	B indices		B_H indices		Totals
47+23	I	8.72		5.62		
	II	6.80		9.46		
	III	5.44		4.83		
		20.96	M=6.99	19.91	M=6.64	40.87
59+20	I	8.72		7.49		
	II	9.81		7.04		
	III	9.98		10.63		
	IV	9.98		9.10		
		38.49	M=9.62	34.26	M=8.57	72.75
7+23	I	5.74		5.65		-
62+20	I	11.09		7.04		
	II	7.27		5.74		
		18.36	M=9.18	12.78	M=6.39	31.14
59+23	I	11.68		5.68		
	II	8.72		6.80		
	III	9.81		7.92		
		30.21	M=10.07	20.40	M=6.80	50.61
59+23	i	11.24		2.69		
	ii	7.71		3.80		
	iii	8.13		3.85		
	iv	11.83		3.89		
	v	-		-		
	vi	11.09		4.69		
		50.00	M=10.00	18.92	M=3.78	68.92
	TOTALS	158.02		106.27		264.29

TABLE 42

ANALYSIS OF VARIANCE OF ARCSIN TYPE-1 B AND B_H INDICES SHOWN IN TABLE 41

	Sum of squares	d.f.	Mean squares	F	Probability
Total	211.5508	33			
Columns	78.7665	1	78.7665	32.14	<0.005
Rows	29.8917	4	7.4729	3.05	0.01 - 0.05
Interaction	44.0739	4	11.0185	4.05	0.005 - 0.01
Residual	58.8187	24	2.4508		

Figure 4. Plot of means of arcsin type-1 B and B_H indices shown in table 41.

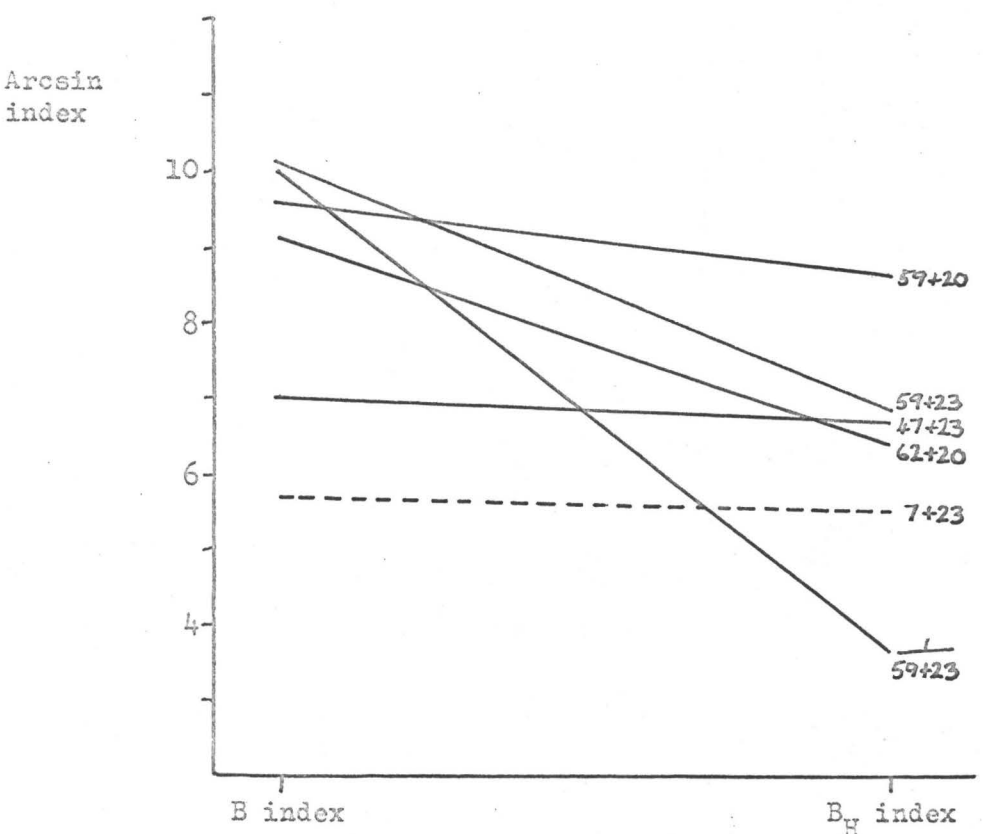


TABLE 43

ARCSIN TRANSFORMATIONS OF A AND A_H TYPE-2 INDICES

Set	Experiment	A indices		A_H indices		Totals
47+23	I	5.74		4.55		
	II	8.33		4.21		
	III	7.04		*-		
		21.11	M=7.04	8.76	M=4.38	29.87
59+20	I	8.33		**-		
	II	7.04		2.43		
	III	9.28		8.33		
	IV	9.81		7.71		
		34.46	M=8.62	18.47	M=6.16	52.93
7+23	I	7.27		5.74		-
62+20	I	7.27		7.92		
	II	6.55		5.23		
		13.82	M=6.91	13.15	M=6.58	26.97
59+23	I	7.71		5.68		
	II	6.80		3.76		
	III	7.71		5.32		
		22.22	M=7.41	14.76	M=4.92	36.98
59+23	i	6.02		6.80		
	ii	5.74		*-		
	iii	5.74		5.62		
	iv	4.21		*-		
	v	4.21		*-		
	vi	5.29		5.44		
		31.21	M=5.20	17.86	M=5.95	49.07
	TOTALS	122.82		73.00		195.82

* Cannot be computed

**This value was omitted from the statistical analysis.

TABLE 44

ANALYSIS OF VARIANCE OF ARCSIN TYPE-2 A AND A_H INDICES SHOWN IN TABLE 43

	Sum of squares	d.f.	Mean squares	F	Probability
Total	88.0681	30			
Columns	11.0141	1	11.0141	5.83	0.025 - 0.05
Rows	19.0248	4	4.7562	2.52	0.05 - 0.1
Interaction	18.3330	4	4.5833	2.42	0.05 - 0.1
Residual	39.6962	21	1.8903		

Figure 5. Plot of means of arcsin type-2 A and A_H indices shown in table 43

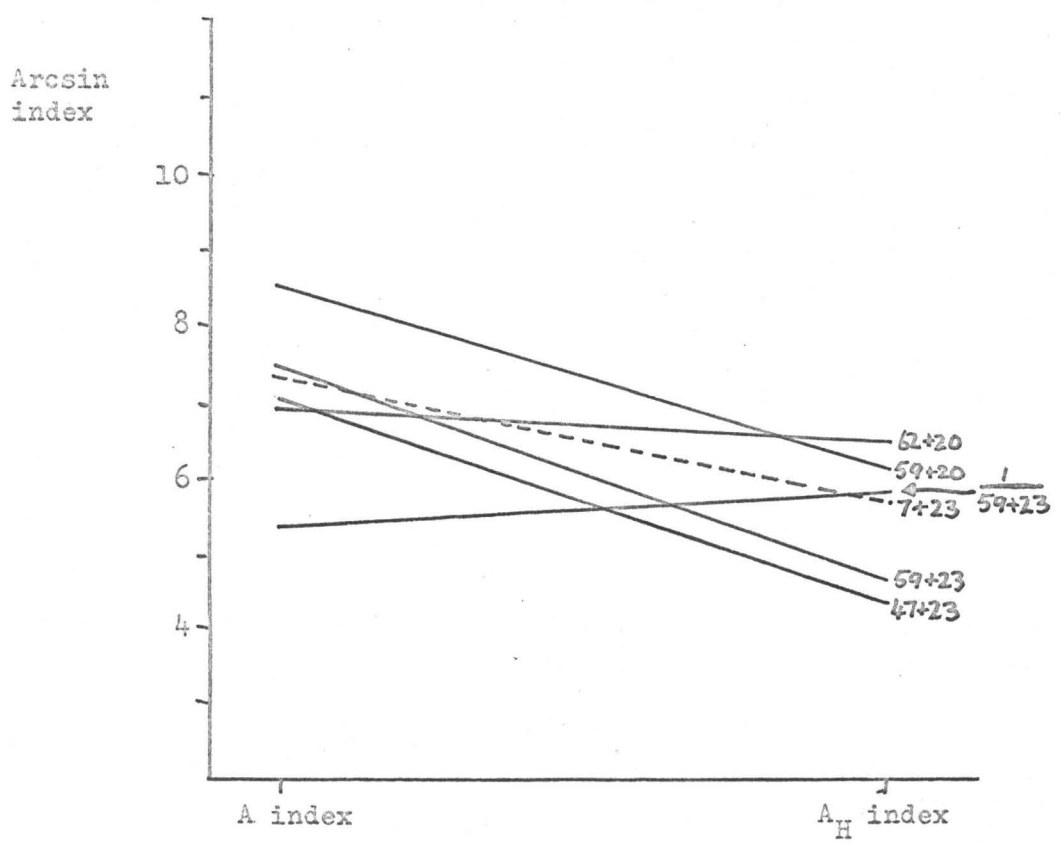


TABLE 45

ARCSIN TRANSFORMATIONS OF B AND B_H TYPE-2 INDICES

Set	Experiment	B indices		B_H indices		Totals
47+23	I	6.29		5.62		
	II	7.04		9.46		
	III	6.02		-		
		19.35	M=6.45	15.08	M=7.54	34.43
59+20	I	9.10		7.49		
	II	8.91		7.04		
	III	-		-		
	IV	-		-		
		18.01	M=9.01	14.53	M=7.27	32.54
7+23	I	7.71		5.65		-
62+20	I	10.14		7.04		
	II	8.13		5.74		
		18.27	M=9.14	12.78	M=6.39	31.05
59+23	I	9.98		5.68		
	II	10.94		6.80		
	III	10.63		7.92		
		31.55	M=10.52	20.40	M=6.80	51.95
59+23	i	8.91		2.75		
	ii	7.49		-		
	iii	7.92		3.85		
	iv	9.63		-		
	v	6.02		-		
	vi	10.63		4.69		
		50.60	M=8.43	11.29	M=3.76	61.89
TOTALS		137.78		74.08		211.86

All blanks are indices which cannot be computed.

TABLE 46

ANALYSIS OF VARIANCE OF ARCSIN TYPE-2 B AND B_H INDICES SHOWN IN TABLE 45

	Sum of squares	d.f.	Mean squares	F	Probability
Total	120.7836	27			
Columns	40.7549	1	40.7549	25.07	<0.005
Rows	15.1972	4	3.7993	2.34	0.05 - 0.1
Interaction	35.5718	4	8.8930	5.47	<0.005
Residual	29.2597	18	1.6255		

Figure 6. Plot of means of arcsin type-2 B and B_H indices shown in table 45.

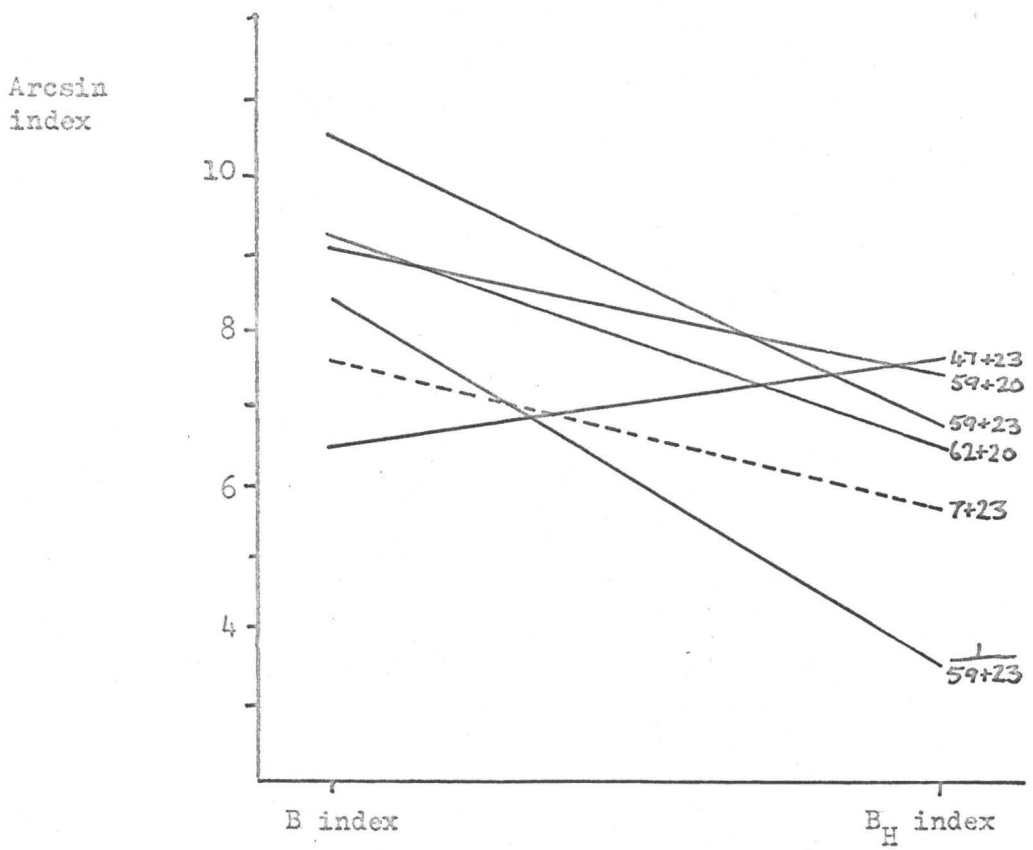


TABLE 47

ARCSIN TRANSFORMATIONS OF A(YELLOW) AND A_H(YELLOW) TYPE-3 INDICES

Set	Experiment	A(yellow) indices		A_H(yellow) indices		Totals
47+23	I	3.97		2.07		
	II	6.29		3.53		
	III	4.44		-		
		14.70	M=4.90	5.60	M=2.80	20.30
59+20	I	6.02		10.14		
	II	5.20		7.71		
	III	5.16		6.80		
	IV	5.53		6.55		
		21.91	M=5.48	31.20	M=7.80	53.11
7+23	I	4.29		3.39		-
62+20	I	5.03		6.02		
	II	4.76		2.87		
		9.79	M=4.90	8.89	M=4.45	18.68
59+23	I	5.41		3.80		
	II	4.76		2.22		
	III	5.03		3.29		
		15.20	M=5.07	9.31	M=3.10	24.51
59+23	i	4.01		1.90		
	ii	4.01		-		
	iii	3.49		2.14		
	iv	3.03		-		
	v	-		-		
	vi	3.63		2.14		
		18.17	M=3.63	6.18	M=2.06	24.35
TOTALS		79.77		61.18		140.95

Blanks = cannot be computed.

TABLE 48

ANALYSIS OF VARIANCE OF ARCSIN TYPE-3 A(YELLOW) AND A_H(YELLOW) INDICES SHOWN IN TABLE 47

	Sum of squares	d.f.	Mean squares	F	Probability
Total	102.1319	30			
Columns	0.7978	1	0.7978	< 1	
Rows	55.6085	4	13.9021	14.74	<0.005
Interaction	25.9120	4	6.4780	6.87	<0.005
Residual	19.8136	21	0.9435		

Figure 7. Plot of means of arcsin type-3 A(yellow) and A_H(yellow) indices shown in table 47.

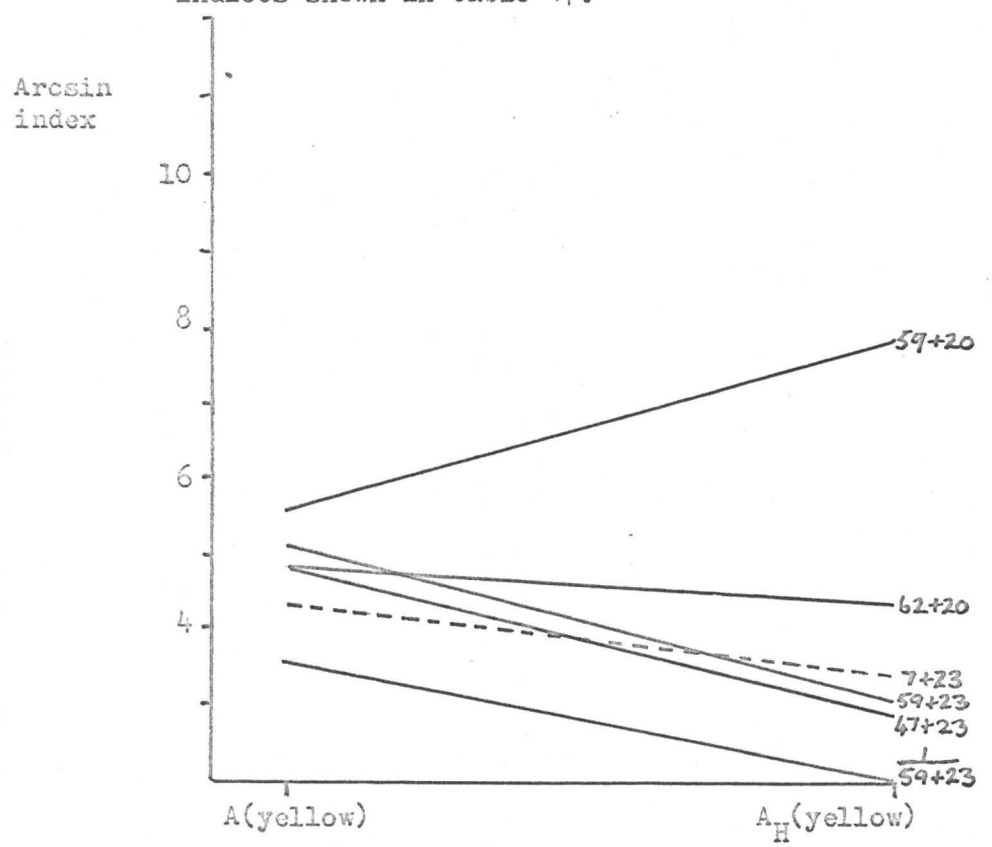


TABLE 49

ARCSIN TRANSFORMATIONS OF A(PINK) AND A_H(PINK) TYPE-3 INDICES

Set	Experiment	A(pink) indices		A_H(pink) indices		Totals
47+23	I	4.52		4.09		
	II	5.41		2.22		
	III	5.44		-		
		15.37	M=5.12	6.31	M=3.16	21.68
59+20	I	6.02		*-		
	II	4.59		0.57		
	III	7.71		4.62		
	IV	8.13		4.17		
		26.45	M=6.61	9.36	M=3.12	35.81
7+23	I	6.02		4.69		-
62+20	I	5.35		5.16		
	II	4.48		4.37		
		9.83	M=4.92	9.53	M=4.77	19.36
59+23	I	5.41		4.17		
	II	4.87		3.03		
	III	5.74		4.17		
		16.02	M=5.34	11.37	M=3.79	27.39
59+23	i	4.29		6.55		
	ii	4.09		-		
	iii	4.66		5.20		
	iv	3.34		-		
	v	-		-		
	vi	3.89		5.00		
		20.27	M=4.07	16.75	M=5.58	37.02
	TOTALS	87.94		53.32		141.26

* Omitted from statistical analysis. All other blanks cannot be computed.

TABLE 50

ANALYSIS OF VARIANCE OF ARCSIN TYPE-3 A(PINK) AND A_H(PINK) INDICES SHOWN IN TABLE 49

	Sum of squares	d.f.	Mean squares	F	Probability
Total	60.0947	29			
Columns	8.4561	1	8.4561	6.92	0.01 - 0.025
Rows	2.0998	4	0.5250	< 1	-
Interaction	25.1148	4	6.2787	5.14	0.005 - 0.01
Residual	24.4240	20	1.2212		

Figure 8. Plot of means of arcsin type-3 A(pink) and A_H(pink) indices shown in table 49.

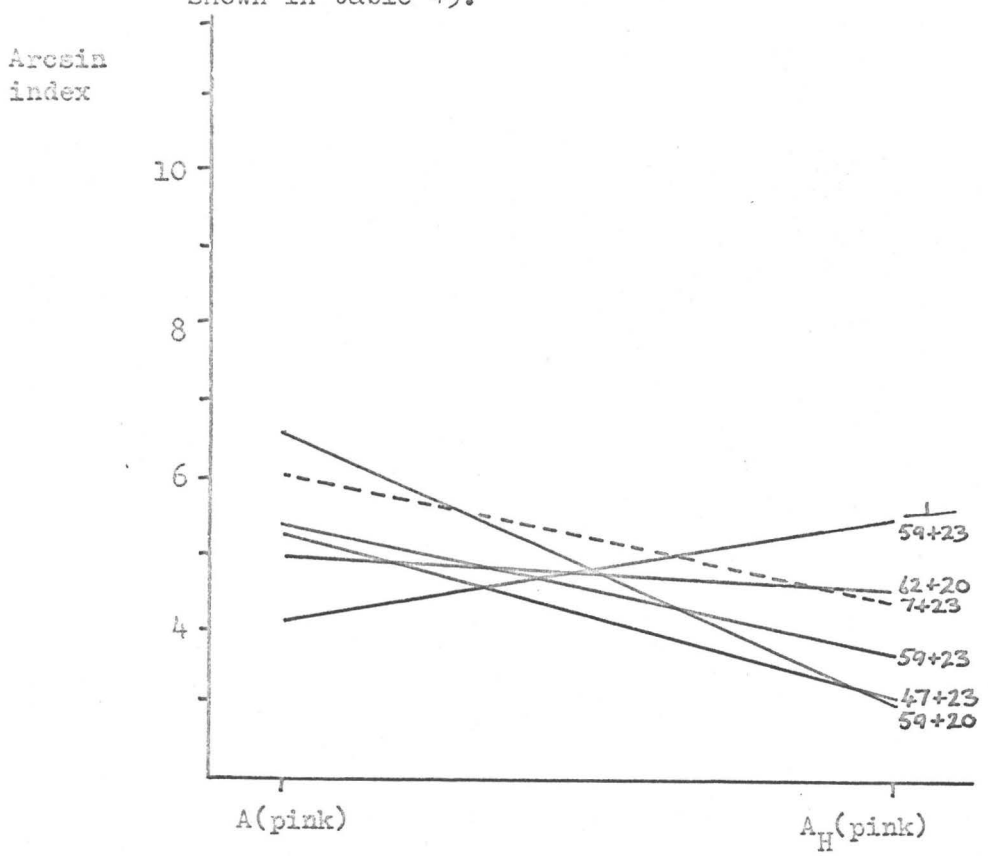


TABLE 51

ARCSIN TRANSFORMATIONS OF A(PINK) AND A_H(PINK) TYPE-4 INDICES

Set	Experiment	A(pink) indices		A_H(pink) indices		Totals
47+23	I	6.55		5.32		
	II	8.13		2.92		
	III	9.46		-		
		24.14	M=8.05	8.24	M=4.12	32.38
59+20	I	9.28		*-		
	II	7.27		2.29		
	III	9.63		5.62		
	IV	9.98		5.03		
		36.16	M=9.04	12.94	M=4.32	49.10
7+23	I	8.72		8.91		-
62+20	I	9.98		6.80		
	II	8.13		7.27		
		18.18	M=9.09	14.07	M=7.04	32.25
59+23	I	6.29		5.26		
	II	5.44		3.24		
	III	6.80		4.93		
		18.53	M=6.18	13.43	M=4.48	31.96
59+23	i	6.80		6.80		
	ii	5.65		-		
	iii	6.55		6.29		
	iv	4.21		-		
	v	-		-		
	vi	4.29		5.29		
		27.50	M=5.50	18.38	M=6.13	45.88
	TOTALS	124.51		67.06		191.57

* Omitted from statistical analysis. Other blanks cannot be computed.

TABLE 52

ANALYSIS OF VARIANCE OF ARCSIN TYPE-4 A(PINK) AND A_H(PINK) INDICES SHOWN IN TABLE 51

	Sum of squares	d.f.	Mean square	F	Probability
Total	119.0950	29			
Columns	34.5500	1	34.5500	23.97	<0.005
Rows	24.1696	4	6.0424	4.19	0.005 - 0.025
Interaction	31.5464	4	7.8866	5.47	<0.005
Residual	28.8290	20	1.4415		

Figure 9. Plot of means of arcsin type-4 A(pink) and A_H(pink) indices shown in table 51.

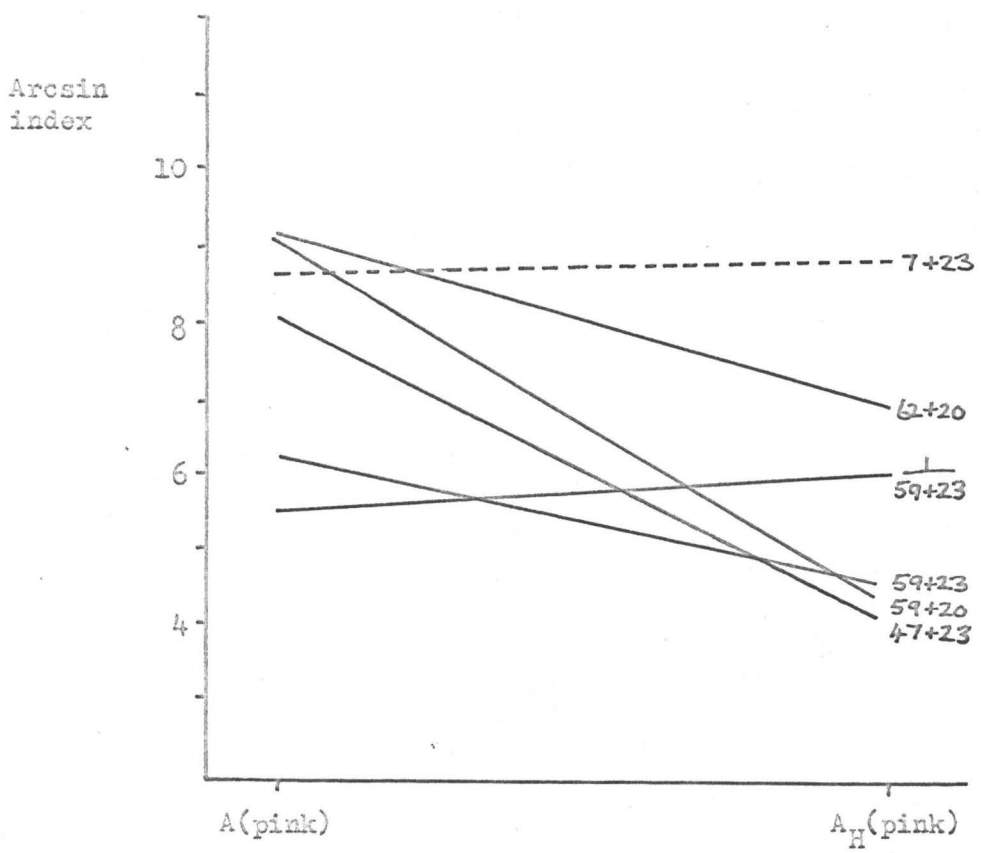


TABLE 53

ARCSIN TRANSFORMATIONS OF PROTOTROPH PERCENTAGES IN CROSSES INVOLVING YELLOW CONIDIAL ISOLATES

Experiment	cys,ylo;leu 23		Y1		Y3		Totals
m	6.80		6.55		6.02		
n	7.71		7.04		7.27		
	14.51	M=7.26	13.59	M=6.80	13.29	M=6.65	41.39
s	7.92		6.55		7.04		
t	6.55		6.80		6.80		
	14.47	M=7.24	13.35	M=6.68	13.84	M=6.92	41.66
Totals	28.98		26.94		27.13		83.05

TABLE 54

ARCSIN TRANSFORMATIONS OF PROTOTROPHS PERCENTAGES IN CROSSES INVOLVING ALBINO CONIDIAL ISOLATES

Experiment	cys;arg,al 59		A2		A3		Totals
o	9.28		7.27		7.71		
p	8.72		8.33		7.49		
	18.00	M=9.00	15.60	M=7.80	15.20	M=7.60	48.80
u	7.27		4.01		3.29		
v	8.53		5.35		4.80		
	15.60	M=7.80	9.36	M=4.68	8.09	M=4.05	33.05
Totals	33.60		24.96		23.29		81.85

TABLE 55

ANALYSIS OF VARIANCE OF ARCSIN PROTOTROPH PERCENTAGES IN CROSSES INVOLVING YELLOW CONIDIAL ISOLATES (SEE TABLE 53)

	Sum of squares	d.f.	Mean square	F	Probability
Total	3.0393	11			
Columns	0.6350	2	0.3175	<1	-
Rows	0.0060	1	0.0060	<1	-
Interaction	0.0845	2	0.0423	<1	-
Residual	2.3138	6	0.3856		

Figure 10. Plot of means of arcsin prototroph percentages in crosses involving yellow conidial isolates (see table 53).

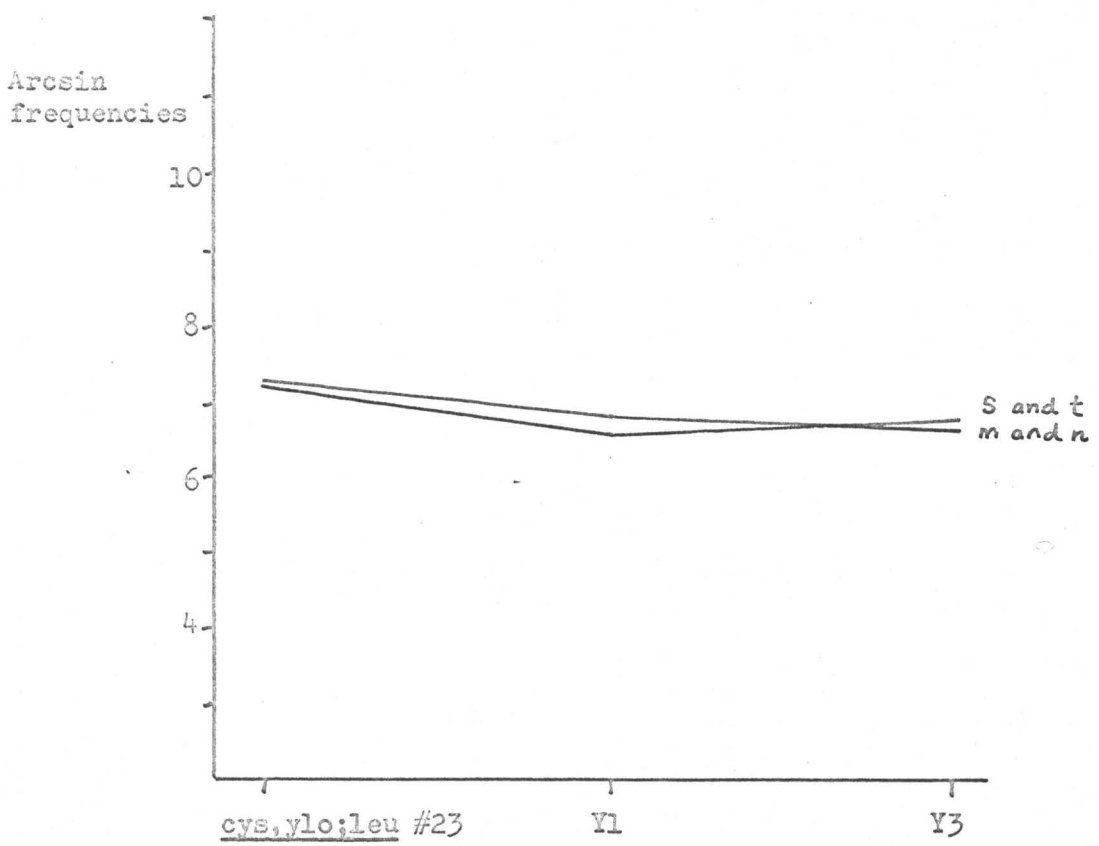


TABLE 56

ANALYSIS OF VARIANCE OF ARCSIN PROTOTROPHS PERCENTAGES IN CROSSES INVOLVING ALBINO CONIDIAL ISOLATES (SEE TABLE 54)

	Sum of squares	d.f.	Mean square	F	Probability
Total	42.4661	11			
Columns	15.3112	2	7.6556	13.74	0.005 - 0.001
Rows	20.6718	1	20.6718	37.11	<0.005
Interaction	3.1407	2	1.5704	2.82	0.1 - 0.25
Residual	3.3424	6	0.5571		

Figure 11. Plot of means of arcsin prototroph percentages in crosses involving albino conidial isolates (see table 54)

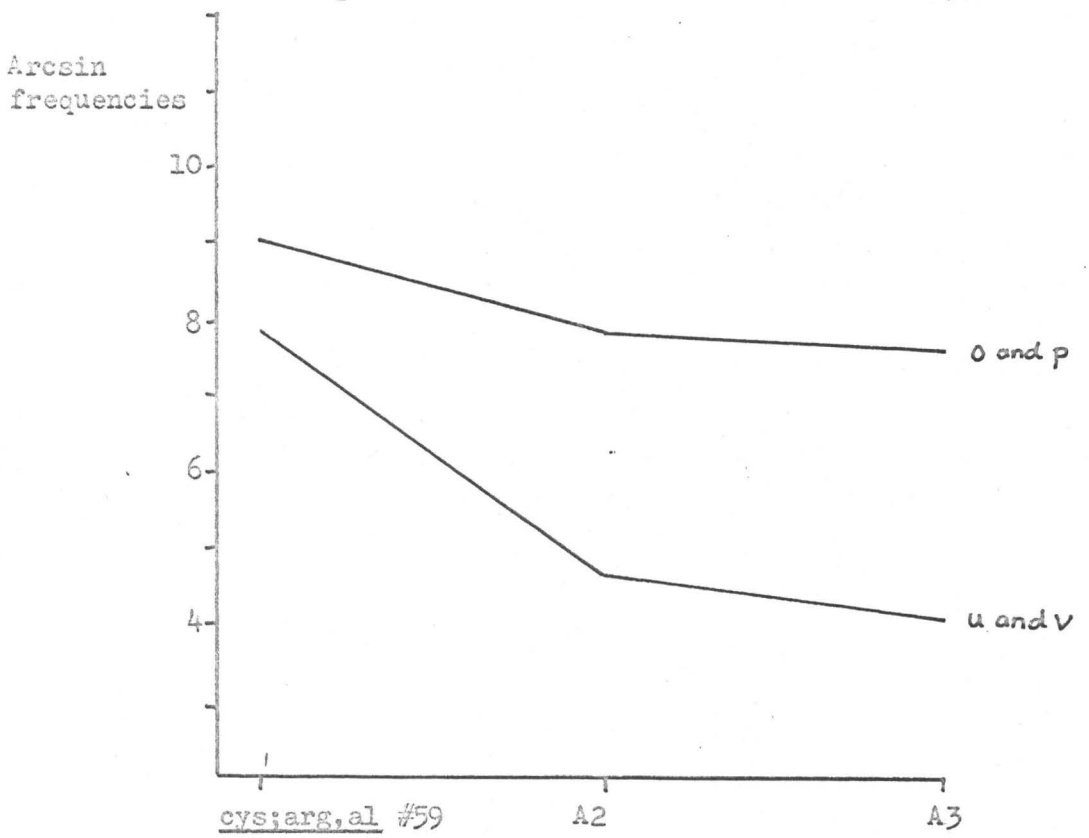


TABLE 57

ARCSIN TRANSFORMATIONS OF PROTOTROPH PERCENTAGES IN CROSSES INVOLVING HETEROKARYOTIC CONIDIAL ISOLATES

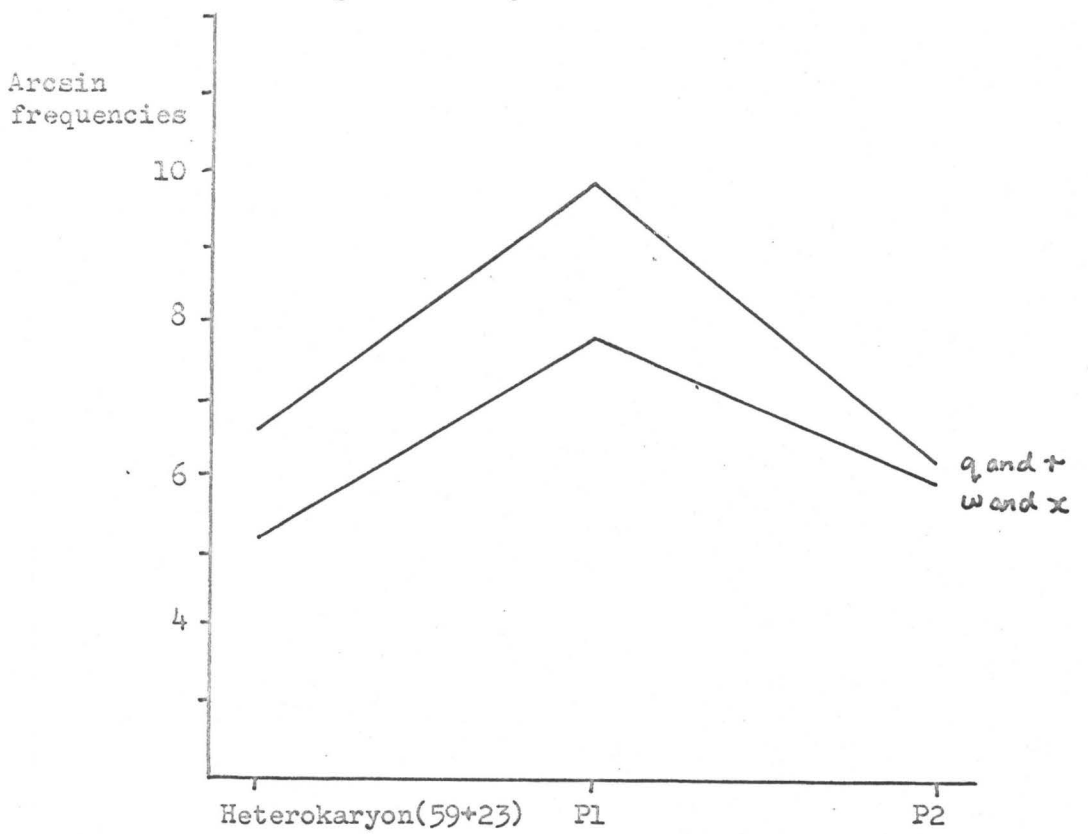
Experiment	Heterokaryon(59+23)		P1		P2		Totals
q	7.04		9.98		6.29		
r	6.29		9.63		6.29		
	13.33	M=6.67	19.61	M=9.81	12.58	M=6.29	45.52
w	5.35		8.33		6.29		
x	4.90		7.27		5.50		
	10.25	M=5.13	15.60	M=7.80	11.79	M=5.90	37.64
Totals	23.58		35.21		24.37		83.16

TABLE 58

ANALYSIS OF VARIANCE OF ARCSIN PROTOTROPH PERCENTAGES IN CROSSES INVOLVING HETEROKARYOTIC CONIDIAL ISOLATES (SEE TABLE 57)

	Sum of squares	d.f.	Mean square	F	Probability
Total	28.9808	11			
Columns	21.1155	2	10.5578	48.08	<0.005
Rows	5.1745	1	5.1745	23.56	<0.005
Interaction	1.3731	2	0.6866	3.13	0.1 - 0.25
Residual	1.3177	6	0.2196		

Figure 12. Plot of means of arcsin prototroph percentages in crosses involving heterokaryotic conidial isolates (see table 57).



data in the type-2 B and B_H indices unit (Tables 45 & 46, Figure 6), where a very large column F value is obtained together with a significant interaction effect. The behaviour of set 47+23 must be considered anomalous in this unit of data, and possibly the main contributor to the interaction effect.

Type-2 A and A_H indices are the only indices which measure recombination over the whole cys - pan region in A-type strains, and the data are analysed in the unit composed of Tables 43 & 44, and Figure 5. No strong effects are observed, and only one F value gives a probability of less than 5% (the column F, Table 44). Thus, the general slight drop in index may be real although this conclusion must be regarded as tentative. The fact that there is no interaction effect suggests that the slight rise shown by the $\frac{1}{59+23}$ line does not reflect a real phenomenon, but is more likely a chance occurrence.

Recombination in ylo - pan region of A-type strains is measured by type-3 A(pink) and A_H(pink) indices, (Tables 49 & 50, Figure 8), and type 4 A(pink) and A_H(pink) indices (Tables 51 & 52, Figure 9). Both units show significant column effects with no differences being apparent between strains cys,ylo;leu # 20 and # 23. Both units also show interaction effects, yet it is not possible to say a priori whether the rises shown by the indices of set $\frac{1}{59+23}$ are largely responsible for these effects. The reciprocal crosses may be genuine exceptions, but the general trend is to a reduction of index inside the heterokaryon.

The statistical analysis presented so far has shown a strong and consistent drop in the B-type indices, and a less strong and less consistent drop in the A-type indices. The two exceptions in the A-type

indices are the possible rise in recombination frequency in the cys - ylo region of strain cys,ylo;leu # 20 and the possible rise in the ylo - pan region of A-type strains in the reciprocal crosses. It should be pointed out that the rise associated with the strain cys,ylo;leu # 20 is probably the reason for the absence of column differences in the unit dealing with the cys - ylo region (Tables 39 & 40, Figure 3). In the same unit, a slight drop for $\frac{1}{59+23}$ is shown, whereas in the cys - pan and the ylo - pan analyses for $\frac{1}{59+23}$, slight increases are found. It seems then, that if the rise in the $\frac{1}{59+23}$ A-type indices reflects a real phenomenon, the action of the B-type strain is mainly on the ylo - pan region.

However, in general, the considerations discussed qualitatively in previous chapters have been statistically verified. Prototroph frequencies in crosses using conidial isolates as parents can also be discussed in unit form.

The unit dealing with yellow conidial isolates (Tables 53 & 55, Figure 10) shows no trend whatsoever, and this result supports the tentative conclusion reached in Chapter 7.

The unit dealing with albino conidial isolates (Tables 54 & 56, Figure 11) shows large column and row effects but no interaction effect. The column effect is mainly due to the low values for the A2 and A3 conidial isolates compared with the original cys;arg,al # 59 strain. Without making post-hoc tests, it is not possible to decide if there is a difference between A2 and A3, but if there is, it must be slight. The fact that no interaction effect exists suggests that the underlying mechanism producing the decreased values is identical, no matter which direction the crosses are made in. The row F value has more meaning in the conidial isolate

crosses, as the whole group of experiments was performed in a relatively short period of time, and repeat experiments show good agreement with the originals. Consequently, the high row F in Table 56 may reflect a true maternal effect on which the inter-strain effect is superimposed.

The unit concerning heterokaryotic conidial isolates (Tables 57 & 58, Figure 12) shows a high column F and a high row F value. The high recombination frequencies for P1 must be held primarily responsible for the high column F, thus confirming earlier observations. Here again the maternal effect may underly the high row F, although the small interaction F value again suggests that this cytoplasmic phenomenon is a background on which the interstrain reaction is superimposed.

In relation to a possible maternal effect, it is interesting to observe that wherever this effect is observed ($59+23$ vs. $\frac{1}{59+23}$ in Figure 2, experiments o and p vs. u and v in Figure 11, and experiments q and r vs. w and x in Figure 12), the ad,pan ♀ crosses show the lower recombination frequencies. Also, it should be pointed out that no such maternal effect exists in the data concerning yellow conidial isolates (see Figure 10).

Statistical confirmation has been presented of the phenomenon of "permanent" alteration of recombination potential in some conidial isolates. Possible mechanisms for these phenomena are discussed in the next chapter.

CHAPTER 9

DISCUSSION

The main trends which emerge from this work are consistent in their occurrence, and have been verified by statistical analysis. The trends may be categorised as follows.

1. B-type strains in general show higher recombination frequencies over a marked region than do A-type strains, when crossed either as protoperithecial or conidial parents to a common parent.
2. When an A and a B strain are combined to form a heterokaryon and the heterokaryon is crossed either as protoperithecial or as conidial parent to the same common parent, recombination frequencies either intermediate between A and B values or lower than either may be obtained.
3. Indices of recombination show that depressed H-cross values and intermediate H-cross values share a common underlying cause, that is, a large and consistent reduction in recombination frequency due to the B component, and, to a lesser degree, the A component. Two possible exceptions to this statement have been shown, both applying to A-type strains. The exceptions are the hint of a rise in frequency in the cys - ylo region of strain cys,ylo;leu #20, and the possible rise in frequency in the ylo - pan region of strain cys,ylo;leu #23 in reciprocal crosses.

4. If A-type conidial isolates from a heterokaryon are crossed to the common parent, recombination frequencies obtained show no difference from those shown in the original A-type homokaryon cross. However, B-type reisolates, crossed as either protoperithecial or conidial parents to the common parent, show prototroph frequencies significantly lower than the original B-type homokaryon cross. One heterokaryotic conidial isolate from a heterokaryon showed a frequency higher than its parental heterokaryon when crossed either as protoperithecial or conidial parent to the common parent. Another heterokaryotic reisolate showed no such difference.
5. Some evidence has been presented that if the common parent is used protoperithecially in crosses, the recombination frequencies obtained are on the whole lower than if it is the conidial parent.

All the trends are based on data concerning prototroph frequency, and the implicit assumption is made that prototrophs arise from recombination events alone. It has been shown, however, (Pittenger, 1954) that prototrophy can result from non-disjunction of the chromosomes at meiosis, giving rise to disomic or pseudowild-type (PWT) ascospores. No selective technique was available in the present work for the detection of PWT colonies; however, several points may be considered which suggest that PWT frequency is not appreciable.

Firstly, in Pittenger's investigation of linkage group VI, the general frequency of PWTs was found to be low. In a cross involving ad-1 (3254) VI L and ribo-t (51602) VI R, 51 wild-type progeny were obtained out of a total of 10,393 ascospores. 23 of the wild-type progeny were tested, but none of them were found to be PWTs. It is pos-

sible, however, that PWT frequency is also a function of the ancestry of strains being used. Barratt (1954) pointed out that a "gene" exists in Abbott stocks which when homozygous results in ascospore abortion, and that a cytological examination of such crosses reveals a highly irregular anaphase disjunction, with many bridges connecting the separating homologues. Such chromosomal behaviour may well account for the production of PWTs, and, although no abortion patterns were observed in the present data, the possibility remains that non-disjunction, too, may be under genetic control, and that PWT frequencies considerably higher than those reported by Pittenger are possible for linkage group VI.

However, inspection of the segregations of ylo$^+$:ylo and al$^+$:al in prototroph samples (Tables 9 and 10), shows that no drastic excess of pink colonies is found in the present data, which might have been expected had non-disjunction been frequent for chromosome VI in A-type crosses, and chromosome I in B-type crosses. It is true that disomics for group I would not affect the frequency of pan - cys prototrophs in B-type crosses. However, if non-disjunction is under genetic control it seems likely that the lack of disomics for linkage group I reflects a general disinclination of B-type strains to undergo non-disjunction. The situation for linkage group VI is complicated by the fact that the ylo$^+$:ylo ratio is also reflective of the frequency of the relative rates of recombination in the two marked parts of the cys - pan region, but Table 9 shows no consistent large excess of ylo$^+$ prototrophs in A-type crosses. The actual excess of ylo$^+$, shown in Table 9, could be attributed to the presence of PWTs, but even if this were the

case, the excess is not large compated with the remainder, and furthermore, it is impossible that the similar ylo^{+} excess shown in the random colony totals could be due to the presence of PWTs.

In order for the large decreases in the B indices to be explained in terms of reduction of PWT frequency in B_H crosses, a strong inter-strains effect on non-disjunction would have to be postulated. In fact, the greater part of the prototroph frequency in B-type homokaryotic crosses would have to be due to pseudo-wild production, and in view of the fact that the cys - pan prototroph frequency in the B crosses in on average about 2.5 - 3.0% (= 5.0 - 6.0% recombination frequency), which is, if anything, smaller than previously reported values for this map distance (Stadler, 1956), the postulate of inter-strain suppression of PWT production seems unlikely.

Thus, the trends manifest in the data can be accepted, with reasonable confidence, as being true reflections of recombination behaviour in the crosses analysed.

The first conclusion that can be drawn from the results is that some form of interaction is taking place between the component strains of the heterokaryon. Even without the index analysis, it is apparent from the colour segregations in H-cross random colony samples that usually at least 50% of meioses in the H crosses are of the B_H type, and if no interaction were occurring an H-cross prototroph frequency intermediate between the appropriate A and B frequencies would be expected. The depressed H-cross frequencies, it seems, can only be explained on the basis of some cross-exchange of inhibitory agents between the component strains. The index analysis confirms this supposition. It therefore appears as though the structural heterozygosity hypotheses can be

ruled out as the major cause of recombination differences between the homokaryotic strains, since it is difficult to visualise how structural heterozygosity in one heterokaryotic meiosis type could influence recombination in another. It was pointed out in Chapter 1 that inversion and translocation heterozygotes in Drosophila show altered recombination frequencies in chromosomes other than those bearing the aberration, suggesting that the function of some recombination-affecting metabolite is altered by the presence of the aberration heterozygosity, possibly via some kind of position effect. This sort of explanation could account for the results of the present work, although there is no evidence for the presence of an aberration in any of the stocks used. However, the results clearly show that it is not the physical dissimilarity of homologous chromosomes in itself which is responsible for the recombination effects observed in these experiments. Rather, if heterozygosity is responsible, it is through its effect on gene function that the control is expressed.

It appears that the agents responsible for the control of recombination frequency in the strains examined, must be capable of passing freely from meiosis to meiosis, or at least from nucleus to nucleus, through the common cytoplasm in the heterokaryon. The origin of these factors or agents responsible for control of recombination may be speculated on, and two general hypotheses can be formulated which cover most of the possibilities.

1. Internuclear interaction. Under this hypothesis the control of recombination is mediated by the gene products of nuclear genes, and the cross-exchange of these gene products result in the heterokaryon interaction effects observed.

2. Intercytoplasmic interaction. Under this hypothesis, differences in recombination frequency reflect differences in cytoplasms, presumably in terms of dissimilar autonomous, self-replicating, cytoplasmic units, whose functional products are capable of affecting nuclear recombination processes in different degrees. In the heteroplasmon, formed simultaneously with the heterokaryon, these extra-nuclear entities can act on either nuclear or meiotic species to effect the observed heterokaryotic interactions. The intercytoplasmic interaction must finally result in an effect on nuclei.

These hypotheses can be more critically evaluated in the light of the evidence presented which suggests that the strain interaction is in some way permanent. This evidence can be summarised as follows.

a) In crosses where a heterokaryon is used as a protoperithecial parent, and if interaction is effected through the exchange of nuclear gene products, the insular nature of the ascus initial cell and the ascus itself suggests that the interaction must have occurred before these meiotic sites were separated off from the rest of the mycelium. If the interaction is intercytoplasmic, however, it is likely that the ascus and ascus initial will themselves contain heteroplasmons.

b) In crosses where the heterokaryon is the conidial parent, interaction must have occurred prior to the passage of the fertilising nucleus down the trichogynes. It is likely that this must be the case whether interaction is inter-nuclear or inter-cytoplasmic, since it has been well established that no cytoplasmic determinants enter with the male nucleus. (One isolated exception has been reported by Muneta & Srb (1959) who described the paternal inheritance of extranuclearly-inherited determinants, but the experiment was not reported in detail, neither has it been confirmed.) Where the heterokaryon is the conidial parent, two types of meioses must be present in the protoperithecial parent, and it is possible that some passage of gene products between meioses might effect interaction, although this possibility appears unlikely.

c) The finding that both the albino conidial isolates tested showed reduced recombination frequency is indicative that they acquired this characteristic from their passage through the heterokaryon. The simplest explanation of this finding is that interaction in the heterokaryon is intercytoplasmic and the heteroplasmon is maintained in the conidial isolates. However, those albino conidial isolates show reduced frequencies also when crossed as conidial parents to the common parent. Hence under either the internuclear or the intercytoplasmic hypotheses, a permanent effect is necessary to explain this phenomenon.

It should be stressed that the intercytoplasmic interaction postulated above has nothing to do with the slight depression of some prototroph frequencies which has been observed when ad,pan is used as a protoperithecial parent. This latter phenomenon appears to be a case of some kind of maternal inheritance in that ad,pan cytoplasm seems to be relatively inefficient as an environment for recombination. Such maternal effects on recombination have not previously been reported in the literature, and the evidence presented in this work concerning the phenomenon is not sufficiently extensive to permit a categorical statement on its occurrence. However, if the effect is real, the underlying cause is not known, and, as stated in Chapter 8, it must be regarded as a background effect on which the A - B interaction is superimposed.

The permanent nature of the interaction seems to be a necessary prerequisite for any model which attempts to explain the data, although little precedent for analogous permanent effects can be obtained in Neurospora. Mitchell (1957) found that by heat-shocking conidia at 60°C and subsequently using the conidia as the fertilising agents in a cross, significant changes in allelic recombination at the pdx locus could be demonstrated. As a fertilising nucleus is believed to undergo several mitotic divisions prior to diploid formation, the effect of the heat-shock must have persisted through the mitotic divisions. Threlkeld (1961) used a random spore analysis to measure recombination frequency between cr and arg-3 on linkage group I in crosses in which the fertilising conidia had been pretreated with ultra-violet light. In two out of the three crosses examined, significant increases in recombination frequency were found compared with untreated crosses. In another simi-

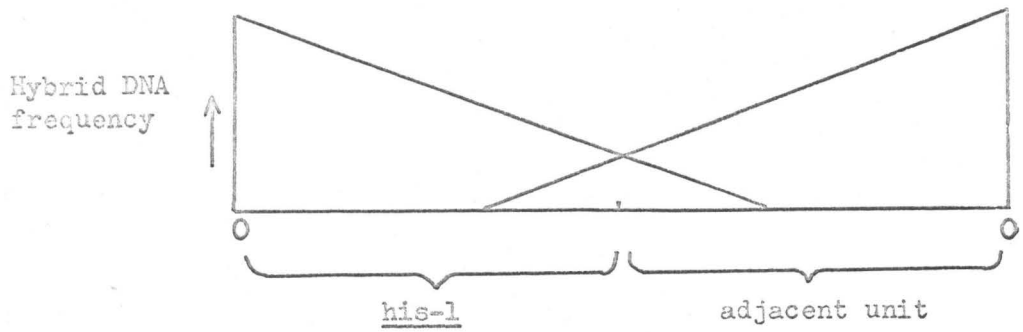
lar experiment, Threlkeld showed that the frequency of production of abnormal segregation patterns in the ascus was also increased by such pre-treatment with UV. It seems that cytoplasmic mutation can be ruled out in the experiments of Mitchell, and of Threlkeld, as presumably no cytoplasmic determinants enter at fertilisation. The only known nuclear change which is capable of self-replication is mutation. Ultraviolet light, and heat, are both known mutagenic agents, but the rate of mutation would have to be exceptionally high if a specific controlling locus is involved in these experiments. Furthermore, if the situations in the present experiments are analogous, it would have to be postulated that the heterokaryotic components are capable of causing mutations in each other's nuclei. It must be concluded that the nature of the permanent effect remains obscure.

There is very little evidence in the present work which can be used in deciding between the hypotheses of nuclear interaction and cytoplasmic interaction. One of the few pieces of data that point to the control being nuclear lies in the elevated values shown by the heterokaryotic conidial isolate P1. It has been seen that this value can be explained in terms of an altered nuclear ratio, and evidence has been presented to support this notion (Table 35). It is also possible to explain the elevated value of P1 by assuming that the conidium from which P1 originated contained mostly "high" type cytoplasmic determinants, as a result of their change segregation from the heteroplasmon, but no corroborating evidence is available to support this idea.

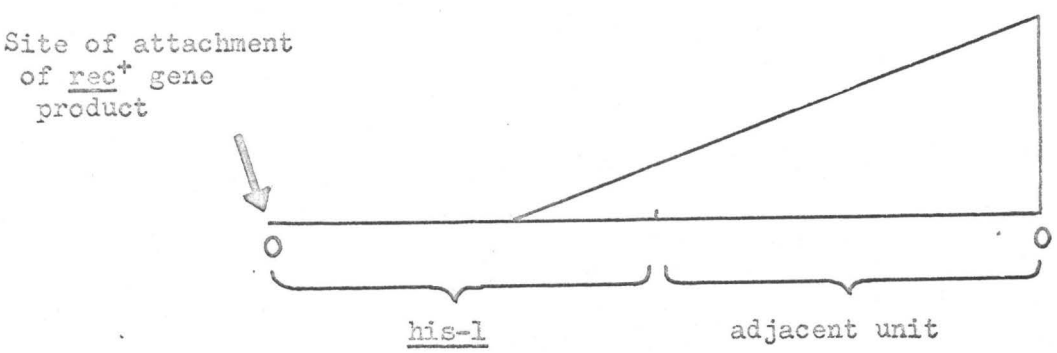
The identification of rec$^+$ and rec$^-$ genes in the last two years has clearly demonstrated that nuclear genes are capable of exerting a control over the recombination process. Thus, precedents do exist for nuclear control but none for cytoplasmic control, and, although a cytoplasmic-type interaction cannot be categorically ruled out as an explanation of the present data, a nuclear exchange seems to be the most plausible explanation. This seems especially true when it is considered that most cytoplasmic mutants which have been identified in Neurospora are of the slow-growing variety associated with abnormal cytochromes.

Whitehouse (1966) has incorporated the rec gene concept into his original model for recombination (Whitehouse & Hastings, 1965), in order to arrive at an attractive operon-like scheme for the recombination process. He visualises rec$^+$ as producing a repressor-like substance which can combine with some kind of operator site and hinder the initiation of the recombination cycle. In a homozygous rec$^-$ situation, the repressor is absent and recombination can proceed efficiently. Whitehouse has re-examined the data of Jessop & Catcheside on the his-1 locus, and has discovered that the presence of the rec$^+$ gene in a cross between his-1 heteroalleles reverses the polarity of recombination in the locus. He considers this evidence that hybrid DNA is entering the his-1 region from two ends, predominantly (say) from the left or operator end, but to a certain extent from the opposite end, from the next operator-like unit which must be assumed to be polarised in the opposite direction from the his-1 unit. The rec$^+$ gene, due to some specificity, only blocks the formation of hybrid DNA originating from the left, leaving the right hand hybridity as the sole, reduced source of recombination between

his-1 alleles. The situation can be represented as in the following sketches.



This diagram shows a rec$^-$ arrangement for his-1 where most hybrid DNA originates from the left-hand operator. The rec$^+$ situation can be represented as follows where the left-hand source of hybrid DNA is eliminated.



The model has led to speculation that the rec$^+$ mechanism is the same as that controlling the rate of transcription of messenger RNA at the site. In order to test this hypothesis, D.E.A. Catcheside (1966) examined the repression of gene activity at the am locus by known exogenous repressor substances in both rec-3$^+$ and rec-3$^-$ cultures. After demonstrating that gene activity was repressed by the same percentage in both cultures, he concluded that the rec-3$^+$ gene is not affecting trans-

cription, although no estimation of the relative levels of gene activity in the cultures was given.

The present data can be satisfactorily interpreted in terms of the recombination gene concept. If the net control of recombination in the cys - pan region is attributable to rec^+/rec^- heterozygosity at (say) three recombination loci (a, b and c), and if the contribution of each is equal and additive, the strains can be represented as follows:

A-type: rec_a^-, rec_b^+, rec_c^+.

B-type: rec_a^+, rec_b^-, rec_c^-.

Common parent: rec_a^-, rec_b^-, rec_c^-.

Heterokaryon: rec_a^+/rec_a^-, rec_b^+/rec_b^-, rec_c^+/rec_c^-.

This situation would give higher frequencies in B-crosses than A-crosses, and the H-cross frequency would be lower than either due to the passage of the rec^+ gene products between nuclei or meioses. The fact that the general phenomenon revealed in the present work is that of reduction in recombination frequencies, points to the role of inhibiting agents of some kind in the interactions observed. The rec^+ gene products appear to fit this role quite well. The possible rise in the cys - ylo region of cys,ylo;leu #20 suggests that, if the phenomenon is real, the action of the rec^+ genes can be modified to some extent, presumably by some factor in the A-type strains, since no qualitative differences in behaviour have been observed in the B-strains. The possible rise shown by A-strain #20 represents one of the few pieces of direct evidence that genes are involved in the present experiments, since A-strain #23 shows no tendency towards a rise in recombination frequency, and both strains

#20 and #23 are derived from isolates from the same cross. (Most known cytoplasmic characters do not show segregation in a cross.)

It has been seen that Whitehouse's operon-like system provides a role for the rec^{+} gene products in the regulation of recombination in (presumably) specific genetic units. Other genes can be visualised which could affect recombination in a manner which is not region-specific. Enzymes undoubtedly exist which perform the various functions which seem to be necessary to effect the exchange of genetic material between homologous chromosomes. Such functions might be breakage of the phosphodiester bonds in the DNA backbone, separation over short distances of the two chains of a DNA double helix, formation of hybrid DNA, excision of mispaired oligonucleotide stretches in the hybrid DNA, and re-synthesis of excised segments of nucleotides (Whitehouse and Hastings 1965). Impairment of these functions would probably affect recombination throughout the whole genome. Evidence exists in bacteria that strains which are sensitive to UV irradiation are also recombination defective (Clark & Margulies 1965: Strauss & Reiter 1965: Howard-Flanders, & Theriot 1966), which provides some correlation between repair of DNA damage and recombination behaviour. In these bacterial experiments, it appears that the enzymes are concerned with excision and re-synthesis of oligonucleotide segments, as this has been shown to be the mode of repair of regions containing thymine dimers (Setlow & Carrier, 1964).

These bacterial strains presumably are recombination-defective due to the inability of the mutant repair enzyme to rejoin DNA breaks to reconstitute a "crossover" strand. However, the rec^{+} genes in Neurospora must act at a different level. The reason for this belief is that

rec$^+$ is dominant to rec$^-$, so rec$^+$ cannot represent a failure to reconstitute a crossover strand as this function would then be fulfilled by rec$^-$, in a heterozygous condition. Neither, of course, can rec$^-$ be considered to provide the faulty enzyme, since the presence of rec$^+$ in a heterozygous situation would then facilitate recombination rather than give the depressed recombination value observed in such situations. Thus, it is difficult to find a role for the gene products of rec$^+$ other than as inhibitory agents blocking some step or steps in the recombination cycle.

What can be said is that rec$^+$ products of a repressor nature would be more likely to show region-specificity in their action, than would the more ubiquitous enzymes which might be postulated from a rec$^+$ analogous in some way to the deficient bacterial mutations. More information is needed on the region-specificity of rec$^+$ genes before their mode of action can be clarified. It is interesting in the present work, however, that H-cross frequencies are regularly lower than either component strain shows when crossed homokaryotically. This suggests that some form of complementation associated with recombination inhibitors has occurred, and implies at least two mutant sites, either at two repressor-like loci affecting different parts of the marked region, or at two loci inhibiting recombination throughout the genome in two different ways, or alleles at one locus producing one recombination-affecting gene product.

Whatever the mode of action of the interaction may be in the present work, the net result is shown in the nucleus at the time of meiosis. Furthermore, it has been seen that a so-called "permanent" effect exists and any hypothesis of interaction must be modified to ac-

count for this phenomenon. It has been seen that the interaction process must occur in the heterokaryon, and that the effect of the interaction appears to be transmitted with a nucleus through mitotic division. This permanent effect resembles the so-called "paramutation" effect described by Brink (1958) in maize, but nothing is known about the mode of interaction which produces paramutation.

Apart from simple mutation of DNA, one of the few other phenomena, for which any sort of precedent exists, that is capable of producing this type of permanent effect is the inheritance of episomic particles associated with the nucleus. In the present context the recombination-affecting loci would have to be borne on an episome or on several episomes, and interaction would be effected by the exchange of episomes between strains. The origin of the episomes could be nuclear or cytoplasmic, but they must be deemed capable of passage through the cytoplasm in either case to become incorporated in either nuclear type in the heterokaryon. A nucleus could then transport the episomes through mitotic divisions either maternally or paternally to the site of meiosis. If there are episomes capable of attachment to the chromosomal complement, they might be found to segregate in an ascus. (Catcheside and co-workers have not reported the segregation of any of their recombination genes in a tetrad analysis, although preliminary reports do suggest that they are mappable.) In the present experiments, the "complementation" of inhibiting agents would suggest that not only are at least two recombination "loci" concerned, but also that a genome is able to incorporate at least two episomes in order that the complementation be realised in any one meiosis.

The above argument must, of course, be regarded as entirely speculative since no case of episomic inheritance has been demonstrated in fungi. The word "episome" has, however, been loosely used, and the above hypothetical particles need not be strictly analogous to bacterial episomes. It is conceivable that the aforementioned data of Mitchell and of Threlkeld, could also reflect a liberation or incorporation of such particles.

In conclusion, it can be stated that the experiments reported here demonstrate that structural heterozygosity *per se* was not responsible for the observed differences in recombination frequency. The control of recombination most likely resides in the action of gene products of various kinds, although the possibility that cytoplasmic factors are involved cannot be ruled out.

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