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TITLE OF THESIS..... Some Effects of Factors for the Control of Recombination in Neurospora

UNIVERSITY..... McMaster

DEGREE FOR WHICH THESIS WAS PRESENTED..... M.Sc.

YEAR THIS DEGREE GRANTED..... Fall 1974

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GENETIC CONTROL OF RECOMBINATION IN NEUROSPORA CRASSA

SOME EFFECTS OF FACTORS FOR THE CONTROL OF RECOMBINATION IN NEUROSPORA

by

ANNE-MARIE LUCIENNE LOMBARD, B.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

September 1974

PREFACE

This thesis describes studies carried out in the Department of Biology, McMaster University, from September 1972 to September 1974. Except where others are specifically mentioned, it consists entirely of my own work. No similar thesis has been submitted at any other university.

I would like to thank my supervisor, Dr. S. P. H. Threlkeld, for his advice and guidance throughout this work.

I extend my thanks to Leslie Györkös, Laura Bentley and Shanta Thomas for their technical assistance, and to the Department of Biology for financial support.

Finally, I would like to thank Miss Judy Street for typing this thesis.

MASTER OF SCIENCE (1974) McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Some effects of factors for the control of recombination in *Neurospora*.

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NUMBER OF PAGES: xi, 93

SCOPE AND CONTENTS:

A factor from *Neurospora sitophila* was shown, prior to this study, to affect recombination in linkage group I.

Hybrid strains of *Neurospora* carrying a *N. sitophila* factor in a *N. crassa* background were used to study the effect of the factor in interallelic crosses and to compare it to its effect in intergenic crosses.

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CHAPTER 1 Introduction

The phenomenon of recombination is one of the most important in genetics. Although it has been known and studied for a long time it is not yet perfectly understood.

The fungi have been very valuable in the study of the mechanism of genetic recombination. A species such as Neurospora crassa, permitting the recovery of all the products of meiosis in a linearly ordered ascus, has been used intensively for this purpose.

Neurospora stocks used in genetic studies have a number of ancestors and quite early (Barrat, 1954) it had been noticed that frequencies of recombination between specific genes could vary between stocks of different origins, as shown in figure 1.

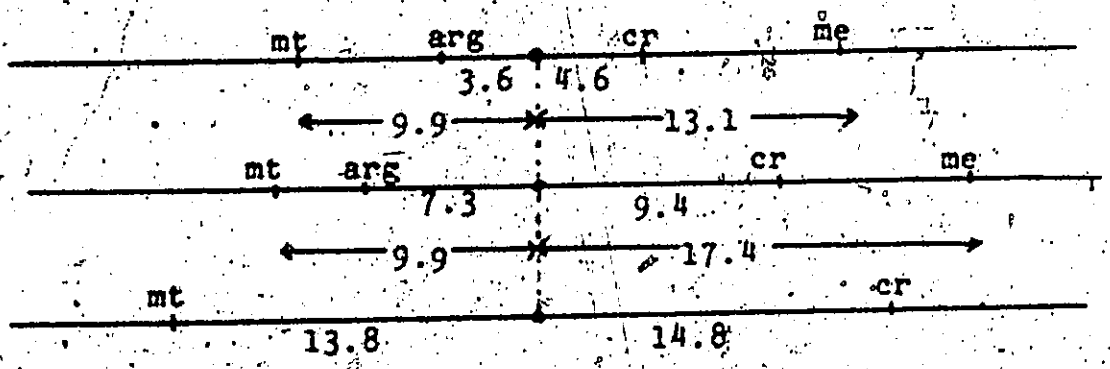

Figure 1: Map of centromere proximal region of linkage group I of Neurospora crassa. Various strains from various sources. (Rifaat, 1969).

This suggests that genetic factors could influence the recombination frequencies.

In fact, a number of genes have been found to affect recombination at specific loci (Jessop and Catcheside, 1965; Catcheside, 1966; Jha, 1969; Angel, Austin and Catcheside, 1970; Smith, 1966). These were called the rec-genes (rec for recombination). The rec genes specifically affect one locus or a short region of chromosome. However it is very possible that further analyses will show that each rec gene affects a number of loci scattered throughout the genome. The dominant alleles, rec^+, decrease the recombination frequencies at the sites they affect.

This suggests that rec^+ produces a substance having a function in the recombination process.

rec^+ could code for an active repressor which binds to a specific site in or near the affected genes, thus preventing or reducing the frequency of access of a recombination enzyme to the affected site. Or it could code for a repressor of a gene specifying an enzyme concerned in recombination and specific to a site (Catcheside, 1970).

Both hypotheses require a recognition site near the affected site, an operator-like locus for the first hypothesis, a promoter-like locus for the second.

Operator is the term introducted by Jacob, Perrin, Sanchez and Monod (1960) for the site at one end of an operon where a repressor molecule binds to the DNA and inhibits transcription.

Promoter is the term introduced by Jacob, Ullman and Monod (1964) for the site at one end of an operon where RNA polymerase binds to the DNA.

Both terms are also appropriate for the corresponding sites when a gene is not part of an operon.

[Operon (Jacob, Perrin, Sanchez and Monod, 1960): group of closely linked genes which appear to affect different steps in a single biosynthetic pathway and which appear to function as an integrated unit.]

A gene which might function as a recognition site has been found: the gene cog, situated near his-3 and hypostatic to rec-w which controls the recombination frequencies in his-3 and between his-3 and ad-3 (Angel, Austin and Catcheside, 1970).

Some results of the effect of cog and rec-w are presented in Table 1.

Table 1: Effect of cog$^+$ and rec-w$^+$ on recombination between his-3 (K874) and ad-3 (K-118). (Angel, Austin and Catcheside, 1970).

	his-3, cog, rec-w	his-3, cog$^+$, rec-w
ad-3, cog, rec-w	x1	x3
ad-3, cog, rec-w$^+$	x1	x1

cog$^+$ is dominant and increases the recombination frequencies. A factor showing characteristics similar to the ones of cog has been found in Schizophyllum commune (Stamberg and Koltin, 1973). It controls recombination between the α and β cistrons of the B incompatibility factor; it is situated between the affected cistrons and the allele increasing the frequency of recombination is dominant to the allele producing low frequency.

These characteristics suggest a recognition (cog) site.

It is a rather long region and is divisible by crossing over, as seen by the heterogeneity in the progeny of crosses heterozygous for this factor. The hypothesis suggested is the presence of several consecutive cog genes with additive effects. A variable number of cog genes in recombinant progeny would produce variable recombination frequencies. This hypothesis is illustrated in figure 2.

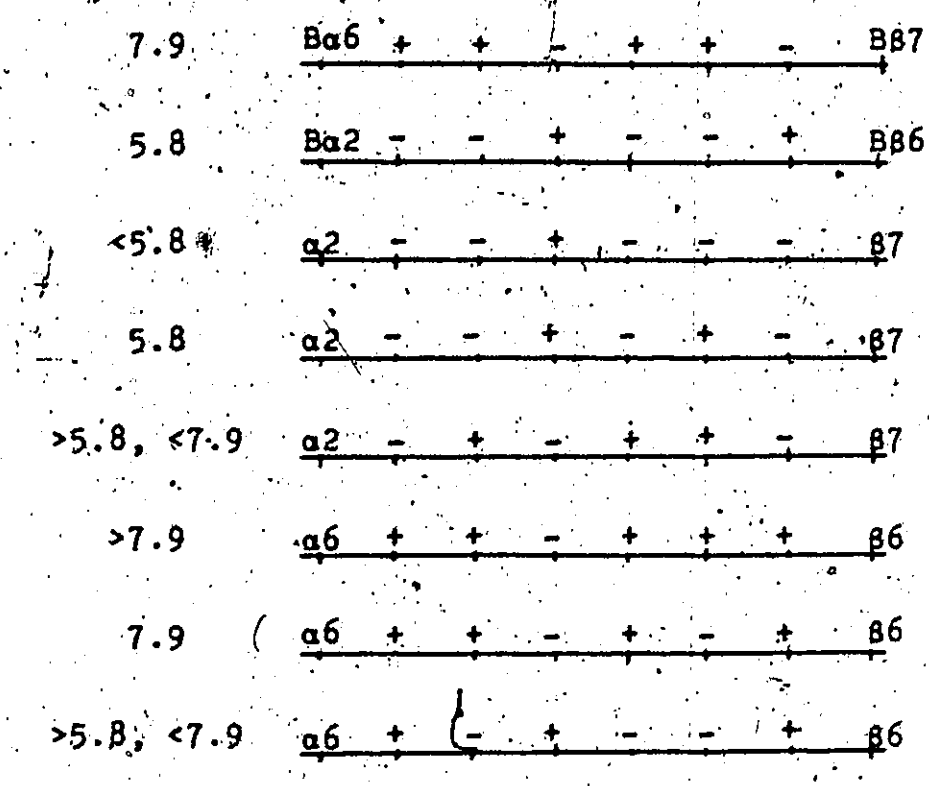

Figure 2: Expected progeny genotypes based on the hypothesis of segregation of a number of recognition sites located between Bα and Bβ. + indicates the dominant, active form of a recognition site and - the recessive, inactive form. (Stamberg and Koltin, 1973).

A mechanism has been proposed to explain the action of rec and cog genes. It is presented in figure 3.

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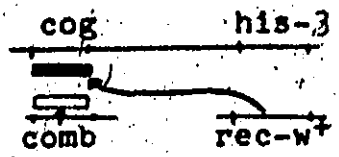

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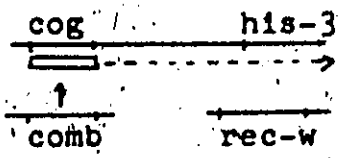
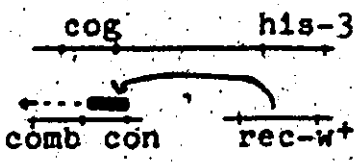
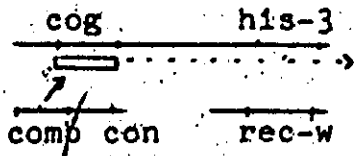
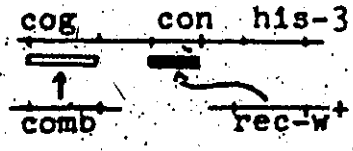


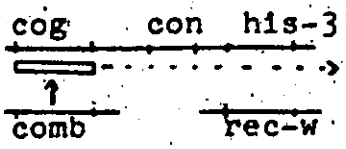
a. Product of rec-w$^+$ interacts with cog to prevent access of recombinase.





b. Product of rec-w$^+$ interacts with an operator site (con) at comb to prevent production of recombinase.





c. Product of rec-w$^+$ interacts with a control site (con) to interfere with the spread of the effect of the recombinase acting primarily at a promoter locus (cog).

Figure 3: Diagrams to illustrate theories of the mechanism of control of recombination by rec-w genes (Angel, Austin and Catcheside, 1970)

cog = recognition site

comb = recombinase

rec = recombination

con = control

If figure 3(a) were the case a very large number of rec genes would occur. A limit in their number implies a limit in the number of cog genes, each of them being present several times in different parts of the genome.

But it is difficult to see how the different cog loci, which are able to distinguish between the products of the different rec^+ genes, would all recognize one single recombinase.

This mechanism also implies that cog and cog^+ respond differently to the presence of $rec\text{-}w^+$ but not to rec-w. In fact just the contrary happens.

If (b) were the case several comb loci responsible each for a recombinase would correspond to each of the rec loci, and the product of each would be specific of a limited number of loci.

cog and cog^+ would be expected to respond differently to a recombinase in the absence of rec^+. This was found to be so.

The number of rec genes in this hypothesis could be limited.

If (c) were the case a larger number of rec genes is required, as well as a larger number of con sites. On the other hand only one recombinase is needed if all cog and cog^+ sites are assumed to react to it with different efficiencies.

It must be noted that a particular rec gene affects all alleles of a specific gene. The specificity of the rec gene is then not due to a selective action of the recombination enzyme based on certain types of allelic differences (Catcheside, 1970).

The rec genes are not linked to the sites they affect and it is probable that their product is diffusible. If a heterokaryon is formed between two strains differing by their recombination frequencies, and the heterokaryon is used in a cross, the prototroph frequencies for each component cross are decreased (Griffiths and Threlkeld, 1966). This can be due to an exchange of gene products between the nuclei in the heterokaryon, resulting in a mutual inhibition of recombination.

A comparison of recombination frequencies between the two species N. crassa and N. sitophilia (Fincham, 1951) suggested that a possible factor (or factors) enhancing recombination near the centromere of linkage group I was present in N. sitophila. See figure 4.

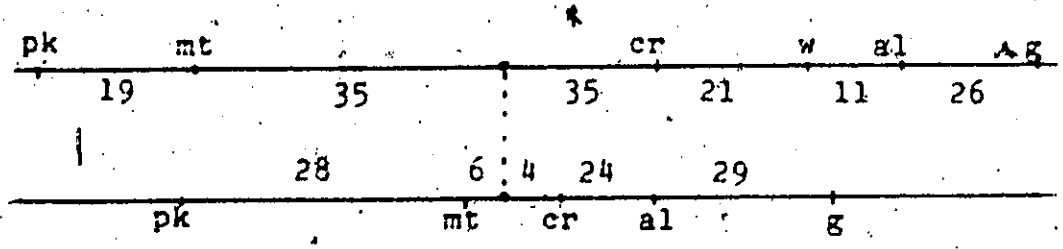


Figure 4: Comparison of N. sitophila and N. crassa maps of the centromere region of linkage group I. (Fincham, 1951)

The centromere itself could be responsible for this effect or it could be due to a factor closely linked to it.

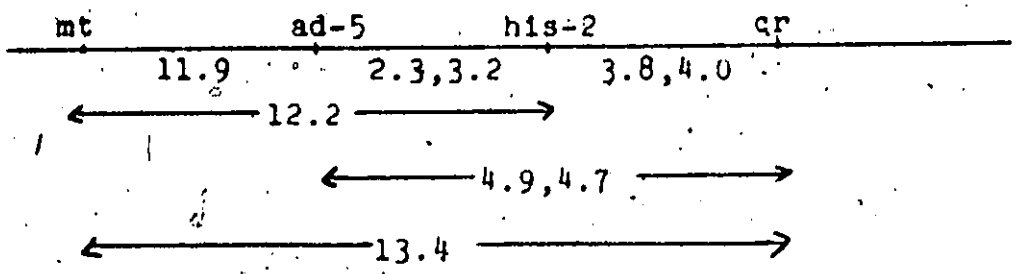
Crosses were made between wild type N. sitophila and his-2 N. crassa (the histidine-2 marker, closely linked to the centromere of N. crassa, was used to follow the origin of the centromere region). Wild type isolates were repeatedly backcrossed to his-2 strains of N. crassa. These isolates were taken only from Tetrads showing first division segregation for his-2, this procedure ensuring that no crossing over separated the marker from its centromere.

Thus strains fertile in crosses with N. crassa but carrying the N. sitophila centromere region were obtained (Newcombe and Threlkeld, 1972).

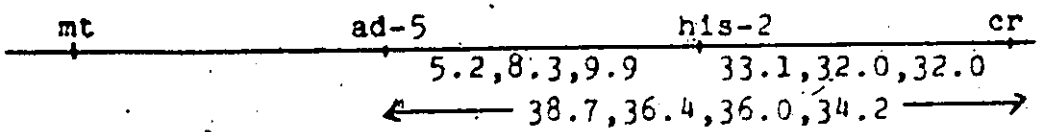
These hybrid strains were used in crosses to N. crassa and it appeared that the N. sitophila factor was dominant: a cross of the type Cs x Cc (Cs = N. sitophila centromere proximal region in N. crassa background, Cc = all N. crassa) gave the high recombination frequencies typical of N. sitophila, and so did Cs x Cs crosses, while a cross Cc x Cc showed the low recombination frequencies of N. crassa. The region mt to cr on linkage group I had definitely increased frequencies of recombination in presence of rec-s, as can be seen in figure 5. The enhancement of recombination

frequencies seemed to affect the centromere - proximal region of linkage group VI, although this increase was not significant (Newcombe, 1969).

Cc x Cc crosses



Cs x Cc crosses



Cs x Cs crosses

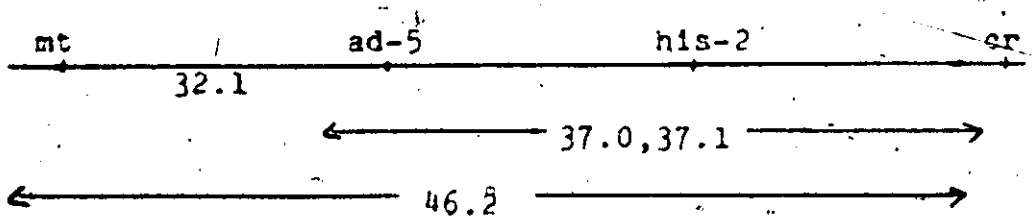


Figure 5: Recombination in Cc x Cc, Cs x Cc and Cs x Cs crosses. (Newcombe, 1969)

The terms Rec-s and rec-c were applied to that part of the genotype responsible for the characteristic recombination frequencies on linkage group I of N. sitophila and N. crassa respectively.

By isolating asci showing second division segregation for the marker his-2, Hargrave and Threlkeld (1973) showed that Rec-s could be separated from its centromere.

Rec-s is situated between his-2 and ad-3 and is composed of two region, Rec-s1 and Rec-s2.
Figure 6 shows the location of Rec-s.

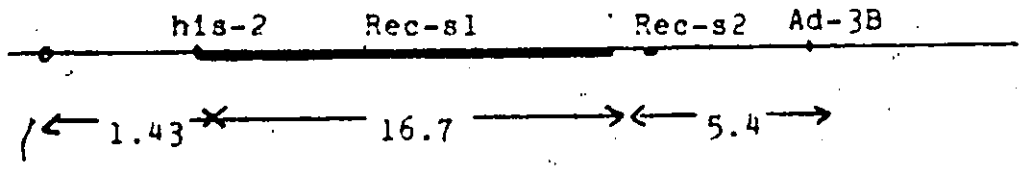


Figure 6: Model for the Rec-s genotype (Hargrave and Threlkeld, 1973). The distances are valid in presence of Rec-s only.

The possible hypothesis of a diffusible gene product from Rec-s was investigated by Ferraro (1971).

A Rec-s strain and a rec-c strain were combined into a heterokaryon which was used as a protoperithecial parent (to avoid the possibility of excluding cytoplasmic determinants of recombination) in a cross with a rec-c strain. No effect of the Rec-s component of the heterokaryon was observed, the frequencies of recombination in rec-c x rec-c (het.) (het. for component of a heterokaryon)

being similar to the ones found in a control rec-c x rec-c cross.

This indicates the lack of any diffusible gene product.

A check for aborted spores did not show them to be in abnormally high numbers, thus eliminating the possibility of a chromosome rearrangement which could be the cause of the observed effects. Also an aberration would produce a low frequency of recombination in heterozygous crosses and a higher one in both homozygous crosses.

So, as opposed to the rec genes, described by Catcheside and his colleagues, Rec-s has been found to affect a rather large segment of linkage group I, from the centromere to cr and possibly also on the other side of the centromere.

It increases interallelic and intergenic frequencies of recombination and is dominant to rec-c which decreases them.

It is closely linked to the affected sites, being situated between his-2 and ad-3. Obviously a mechanism which would explain all rec genes features would not be able to explain how Rec-s affects recombination. In fact it has some characteristics of the cog genes.

Rec-s is a rather long region and it is divisible by crossing over (Hargrave and Threlkeld, 1973). It could well be several genes closely linked to each other and having additive effects.

The present work looked at the effect of Rec-s in interallelic and intergenic crosses on linkage group I and on linkage group VI, in the two regions where Rec-s has been reported to have an influence.

Other rec genes found in Neurospora, as well as the cog gene, affect mostly interallelic crosses. Rec-s has been shown so far to affect a rather long region and one could wonder what its effect on recombination may be between alleles or over very short regions.

When recombination events are studied over short distances it becomes possible to examine the actual recombination process, in addition to its effect, involved in the recombination event. Information on the mechanism of recombination may be obtained in this way (Holliday, 1964).

To study this the gene ad-3 was chosen on linkage group I, in the region most affected by Rec-s.

Although the two mutants chosen are not alleles, strictly speaking, (as is shown below) they are very close to each other and are convenient to use.

Complementation tests have shown that ad-3 mutants can be divided into two groups, ad-3A and ad-3B, each of them a functionally different unit (De Serres, 1956).

However, ad-3A and ad-3B could be separated from each other by a region x containing material of unknown

function: using ad-3 mutants with irreparable recessive mutations, such as deletions, it was found that some ad-3A^{1R} and ad-3B^{1R} (1R for irreparable recessive) would not complement. Therefore they must have some genetic damage in common. The conclusion was that the damage took place in a region situated between ad-3A and ad-3B, called x (De Serres, 1964).

This is illustrated in figure 7.

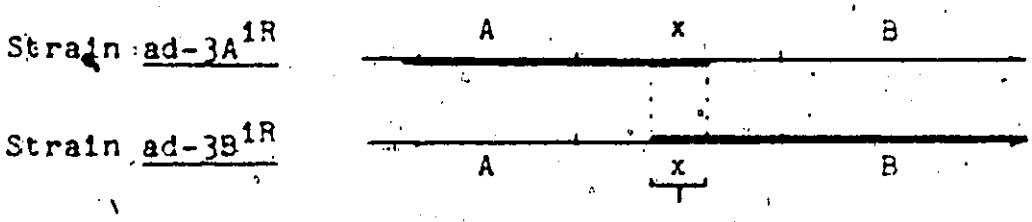


Figure 7: Diagrammatic representation of the extent of genetic damage in the ad-3 region in each component of a dikaryon containing homologous combinations of ad-3A and ad-3B mutations.
(adapted from De Serres, 1964).

——— chromosome

——— deletion

Griffiths (1970), analyzing prototroph frequencies in a series of crosses between ad-3A and ad-3B mutants, provides an estimate of the sizes of the three regions; it is shown in figure 8.

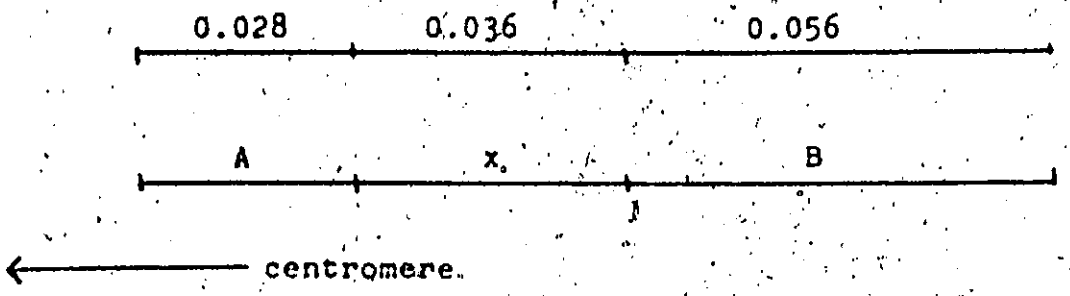


Figure 8: Approximate sizes, in map units, of components of the ad-3 region. (Griffiths, 1970)

Hargrave (1971) shows from her data that one of the two components of Rec-s, Rec-s2, could be situated between ad-3A and ad-3B. It would then occupy the x region of De Serres, as is illustrated in figure 9.

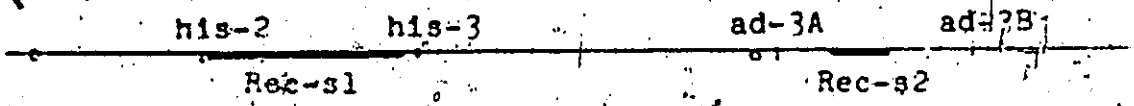


Figure 9: Map of the centromere - ad-3B region showing positions of Rec-s1 and Rec-s2 in relation to other markers. (Hargrave, 1971)

In spite of this the two markers ad-3A and ad-3B are very close to each other and the distance between them is of the order of that found between alleles.

On linkage group VI an interallelic cross was studied using two alleles of the gene pan-2, to ascertain whether the effect reported by Newcombe (1969) is real.

The interest here is two fold: does Rec-s affect recombination frequencies on linkage groups other than linkage group I, at least at the interallelic level, and what type of recombination mechanism might be associated with the activity of Rec-s?

This work also looked at the ability of strains to perform dark repair of DNA damage; ultraviolet sensitivities of strains carrying either Rec-s or rec-c were compared.

It has been shown by previous workers that the mutagenic and lethal effects of ultraviolet light on cells are due to the formation of pyrimidine dimers in DNA (Setlow, Boling and Bollum, 1965). The dimer produces a distortion of the DNA duplex; a specific endonuclease recognizes the dimer or the distortion and cuts the DNA on the 5' side of it.

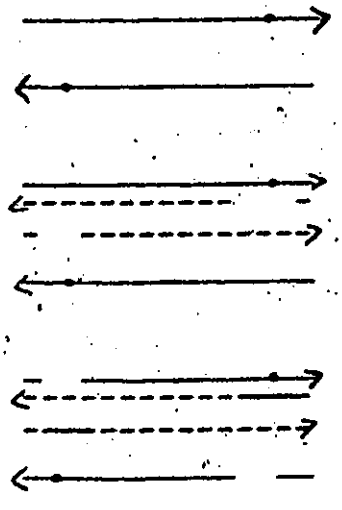
Some mutants of Escherichia coli which are sensitive to ultraviolet radiation lack an endonuclease and cannot excise the dimers (Howard-Flanders, Boyce and Theriot, 1966).

The enzyme DNA polymerase I then excises the nucleotides a few at a time and inserts new ones, using the complementary chain as a template (Kelly, Atkinson, Huberman and Kornberg, 1969). A ligase closes the gene ends.

This mechanism for the dark repair of radiation damage therefore requires a minimum of three different enzymes. A mutation in any of them produces a radiation sensitive mutant. Recombination deficient mutants of E. coli, which exhibit a total lack or a decrease in recombination, show extensive breakdown of DNA after exposure to ultraviolet light (Clark, Chamberlin, Boyce and Howard-Flanders, 1966).

They lack deoxyribonuclease activity; the recombination found in some strains could be due to an alternative pathway not expressed normally (Kushner, Nagaishi and Clark, 1972).

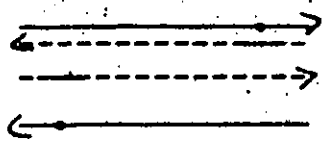
The replication of E. coli containing dimers as the consequence of radiation exposure could take place as seen in figure 10.



dimers are formed in the nucleotide chains after exposure to radiation.

the newly synthetized chains show a gap opposite the dimers

DNA polymerase I requires an intact chain as a template. A recombination event takes place between the sister duplexes to reconstruct an intact chain: a segment of old chain is transferred to the new chain to fill the gap.



The new gap formed in the old chains are filled by DNA synthesis using the complementary chains as a template.

Figure 10: Mechanism of repair of radiation damage in replicating DNA. (Rupp and Howard-Flanders, 1968; Rupp, Wilde, Reno and Howard-Flanders, 1971)

The radiation sensitivity of the recombination deficient mutants is probably due to their inability to carry on this kind of repair.

In fungi the steps for the repair of damage caused by radiation are the same as in bacteria: excision of dimers - single strand degradation widening the gap left by the excision - repair synthesis using the complementary strand as template - rejoining of the phosphodiester links (Holliday, 1967).

Among recombination deficient mutants of Saccharomyces cerevisae there are some which are sensitive to x rays and UV rays, some sensitive to x rays only, but also some which are not more sensitive than the non recombination deficient strains. This shows that there is a link, such as a common enzyme, between recombination and radiation repair but that there is also some steps specific of recombination and not of radiation repair

(Rodarte-Ramon and Mortimer, 1972).

In Ustilago maydis an ultraviolet-sensitive mutation blocks meiosis and decreases mitotic recombination (Holliday, 1967).

Some UV sensitive mutants in N. crassa show no effect on recombination frequency in heterozygous crosses but a block of meiosis in homozygous crosses (Schroeder, 1970).

In Aspergillus nidulans two UV sensitive strains have an increased mitotic recombination but no change in meiotic recombination. Therefore although a link between recombination ability and radiation sensitivity is evident no simple relation is visible in many cases.

Rec-s affecting the meiotic recombination it was decided to see if it had any effect on the ultraviolet sensitivity.

CHAPTER 2: Materials and Methods

Section I. Strains used in this study

The strains used during this study are listed in table 2.

Table 2: Strains used in this study

Stock #	Genotype	Alleles	Linkage Group	M.T.	Obtained from	Chemical Requirements
ORA	wild type			A		
ORa	wild type			a		
2-17-186A	Ad-3A	2-17-186	IR	A	Griffiths A.	Adenine
2-17-186a	Ad-3A	2-17-186	IR	a	Griffiths A.	Adenine
2-17-114A	Ad-3B	2-17-114	IR	A	Griffiths A.	Adenine
2-17-114a	Ad-3B	2-17-114	IR	a	Griffiths A.	Adenine
195-28-7	Ad-3B, Rec-s	2-17-114	IR,IR	A	Hargrave J.B.	Adenine
195-33-5	Ad-3B	2-17-114	IR	A	Hargrave J.B.	Adenine
74-YU-192-1	His-2, Ad-3A, Nic-2	C94,A2, 43002	IR,IR, IR	a	de Serres F.J.	Histidine-Adenine-Nicotinamine
467	Pan-2(5), ad-1	B5,3254	VIR,VIL	a		Adenine, Pantothenic acid
469	Pan-2(3), tryp-2, vio-1	B3,75001; Y30539Y	VIR,VIR, VIL	A		Anthranilic acid, Pantothenic acid
C1198A3-2	Pan-2(3), rec-s	B3	VIR,IR	A	Ferraro M.J.	Pantothenic acid
C1198A3-4	Pan-2(3), Ad-5,rec-s	B3,	VIR,IL, IR	A	Ferraro M.J.	Pantothenic acid adenine
1157	Hist-3	K458	IR	a	Fungal genetics stock center	Histidine

Section II. Media

The media used were those commonly used in Neurospora studies.

They were as follows:

a. 50 x salt solution

Na_3 citrate	125 g
KH_2PO_4 anhydrous	250 g
NH_4NO_3 anhydrous	100 g
$Mg\ SO_4 7H_2O$	10 g
$CaCl_2\ 2H_2O$	5 g
Trace element solution	5 ml
Biotin solution (0.01%)	2.5 ml
Chloroform (preservative)	3 ml

b. Trace element solution

Distilled water	95 ml
Citric acid $1H_2O$	5 g
$Zn\ SO_4\ 7H_2O$	5 g
$Fe\ (NH_4)_2\ (SO_4)_2\ 6H_2O$	1 g
$Cu\ SO_4\ 5H_2O$	0.25 g
$Mn\ SO_4\ 1H_2O$	0.05 g
$H_3\ BO_3$	0.05 g
$Na_2\ Mo\ O_4\ 2H_2O$	0.05 g

Care was taken to add the next chemical only when the previous one had dissolved. The solutions were not heated.

Both solutions were made in quantities large enough to last several months and stored at room temperature.

Chloroform was added to prevent growth in the solutions.

c. Crossing medium

All crosses were made in tubes containing 5 ml of Westergaard and Mitchell's (1947) medium.

50 x salt solution	20 ml
Sucrose	20 g
Difco agar	15 g
Distilled water	up to 1,000 ml

When required the medium was supplemented with 80 mg each of the appropriate chemicals.

d. Vegetative medium

Strains were grown vegetatively on tubes containing 1 ml of Vogel's medium (1956).

50 x salt solution	20 ml
Glucose	20 g
Difco agar	15 g
Distilled water	up to 1,000 ml

80 mg of each chemical was added as necessary.

e. Testing medium

Testing (as well as isolation of prototrophs) was done on Petri plates containing a medium high in

sorbose to induce colonial growth.

The medium contained also fructose and glucose as sources of carbon, to minimise the variations in sorbose toxicity (Brockman and de Serres, 1964).

50 x salt solution	20 ml
Sorbose	10 g
Glucose	0.5 g
Fructose	0.5 g
Difco agar	20 g
Distilled water	up to 1,000 ml

The medium was supplemented with 80 mg of the needed chemicals.

f. All media were autoclaved for 15 mn at a pressure of 16-18 pounds and a temperature of 120°C. The tubes were kept at a angle while the medium was solidifying so as to have a slant, increasing the free surface of the medium.

Section III. Methods

a. Crosses

Crosses were done in 150 x 15 mm test tubes on 5 ml slopes of medium. At first an inoculum from each parent was put in the tube but later on it was found that inoculation of one parent (protoperithecial or female parent) followed 7 days later by the inoculation of the other one (conidial or male parent) yielded a better harvest of spores. Fertilization was effected by pouring

2 or 3 drops of conidial suspension in the protoperithecial parent. All crosses were kept in an incubator set at 25°C.

b. Isolation of random spores.

Three to four weeks after making the cross the mature spores were shot out of the perithecia onto the walls of the tube.

With a wire shaped into a loop the spores were transferred to a drop of sterile water on a block of 4% agar. They were let to dry until they could be picked up easily with a tungsten wire needle, sharpened in molten sodium nitrite, under 35 x magnification. Each spore was transferred to a 1 ml slope of vegetative medium.

The isolates were immediately heatshocked in a 60°C water bath for 45 mn to induce germination and kill any conidia that happened to contaminate the medium. They were grown at 25°C.

c. Isolation of asci

The isolation of asci was started when spores were first noticed to be present on the walls of the cross tubes.

Perithecia were isolated in a drop of sterile water on a block of 4% agar and torn open, using two sharp tungsten wire needles.

The clumps of asci were divided into groups of 4 to 5 asci each and left to dry for a while. Then individual asci were dragged away by their base.

They were left to dry some more before picking the spores up in order under 100 x magnification with a sharp tungsten wire needle and transferred to a 1 ml slope of vegetative medium.

The tubes were stored at 25°C for ten days before heatshocking for 45 mn in a 60°C water bath.

Occasionally a tube would show growth before heatshock took place. This was assumed to be due to contamination by conidia and the tube was discarded.

d. Isolation of prototrophs

Two different methods were used, one in crosses involving a Pan mutation and one in all other crosses.

For all crosses except those involving Pan the two methods gave similar results and therefore the most convenient one was chosen.

For crosses involving Pan there was a significant difference in the data provided by the two methods. The more direct although somewhat lengthier method was thought to be more reliable in this case.

- Crosses not involving a Pan mutation.

About four weeks after fertilization the spores were transferred with a loop to sterile 0.1% agar. The

solution was shaken on a vortex mixer for homogeneization. Spore concentration was estimated by counting the spores in one drop from a sterile Pasteur pipette.

When the solution contained 2,000 to 5,000 spores per ml (depending on crosses) aliquots of 1 ml were transferred each to flasks containing 20 ml of sorbose minimal medium.

A dilution 1/100 of the solution was done and aliquots of 1 ml were transferred each to flasks of 20 ml of sorbose complemented medium.

The flasks were heatshocked 45 mn in a water bath at 60°C, then the suspensions were poured into petri dishes and left to solidify.

After three days the colonies on both types of plates were scored.

- Crosses involving a Pan mutation.

Only the plates of sorbose minimal medium were done. The procedure was the same as stated above.

After one day the percentage of germination was obtained by counting the germinated spores on several fields under the microscope. Two days later the colonies were scored.

- If an isolate of the colonies was desired, each colony was scratched with a wire and the wire in turn touched to a small slope of the appropriate medium.

e. Tests for auxotrophic markers

The cultures were inoculated onto the appropriate sorbose media in Petri plates. The plates were scored 3 days after inoculation.

f. Complementation tests

The complementation tests were carried out in small slopes of glucose medium, minimal with respect to the markers being tested. Two tubes were inoculated with each strain to be tested. One tube was then inoculated with a conidial suspension of one tester and the other tube with a conidial suspension of the other tester. Tubes were scored 4 or 5 days after. The tester strains used were 2-17-186A, 2-17-186a, 2-17-114A and 2-17-114a.

g. Mating type tests

The mating type tests were made in small slopes of appropriatedly supplemented sucrose medium. Each strain to test was inoculated onto two tubes. One was then inoculated with one tester and the other one with the other tester. ORA and ORa were used as testers. The tubes were scored ten days later for presence or absence of perithecia.

h. Irradiation of conidia by ultraviolet rays

The ultraviolet source was a Westinghouse type SB-30 sterilamp. The samples, placed about 50 cm from the

lamp received a dose of 10.5 ugs. $mm^{-2}s^{-1}$. Operations were done in darkness to prevent photoreactivation.

Conidia from the strain to be tested were shaken into a 2 ml of 0.1% agar. After homogenizing the suspension on a vortex mixer, two drops of it were put on a hemacytometer and the conidia counted under magnification x 100. If appropriate the suspension was diluted to obtain a count of about 50,000 conidia/ml.

This was further diluted to obtain 4 suspensions of different concentrations. 0.1 ml of the suspensions were poured each onto Petri plates of supplemented sorbose medium.

The plates were then exposed, with their lids removed, to ultraviolet light for different lengths of time according to the dilution used (times used were 16 mn, 12 mn, 4 mn). The lowest concentration was used as a control and was not exposed to ultraviolet light.

The plates were kept for 3 days in obscurity at 25°C before scoring them.

Section IV. Calculations

The distance between two markers is given by:

$$d = \frac{R \times 100}{N}$$

R = number of recombinant random spores

N = total number of random spores analyzed

If, in some crosses, a differential germination of the markers is observed a correction factor can be used to adjust the poorly germinating parental class:

$$P_1 = \frac{R_1 P_2}{R_2}$$

P_1 = corrected parental type

P_2 = number of spores of the other parental type

R_1 = number of recombinant spores with P_1 marker under consideration

R_2 = number of recombinant spores with P_2 marker under consideration

The distance between a marker and the centromere is:

$$d = \frac{S \times 100}{2N}$$

S = number of asci showing second division segregation for the marker.

N = total number of asci analyzed

When double crossing over were involved a correction was made and the distance was calculated from the formula:

$$d = \frac{(a-2b-c) \times 100}{2N}$$

a = number of asci with single or double crossing over

b = number of asci with two-strand double crossing over

c = number of asci with three-strand double crossing over

N = total number of asci analyzed

The standard error on the distance was calculated from:

$$S.E. = \sqrt{\frac{d\ (100-d)}{N}}$$

N = total number of random spores or asci analyzed

In some cases the standard deviations were calculated, using the formula:

$$S.D. = \sqrt{\frac{\Sigma d^2}{N}}$$

d = deviation from the mean

N = number of measures

When interference was looked at the coefficient of coincidence was calculated.

Coefficient of coincidence = observed frequency of simultaneous crossing over in two linked regions/expected frequency equal to the product of single crossing over frequencies in each region.

The coefficient shows what proportion of the coincidences which would have happened on pure chance really took place.

coefficient > 1	negative interference
coefficient < 1	positive interference
coefficient = 1	no interference

To compare crosses or strains or replicate experiments, a G_H (G test for heterogeneity) was used (Sokal and Rohlf, 1969).

A G test was preferred because it gives more exact probabilities than a X^2 test. Although it requires lengthier calculations it is more efficient and permits certain types of detailed analyses which are impossible with a X^2 test.

Let us suppose we have set up a table (see Table 3) starting from our data.

We can calculate G_H as:

$$G_H = 2(a-b-c+d)$$

a = S

b = X Ln X + Y Ln Y

c = U

d = T Ln T

Degrees of freedom: d.f. = (C-1) (E-1)

C = number of classes

E = number of replicate experiments

To test the goodness of fit to a hypothesis, the following formula of the G test was used:

$$G = 2(\Sigma^a f_i \text{ Ln } f_i - \Sigma^a f_i \text{ Ln } \hat{f}_i)$$

f_i = observed values

$\hat{f}_i$ = expected values

Degrees of freedom: d.f. = C-1

C = number of classes

The critical values for the G tests are looked up in a table of X^2 values (Rohlf and Sokal, 1969).

When comparing crosses or strains the data of several replicate experiments were pooled after it had been found that they were not significantly different.

In some cases where a G test was not possible (for example if one of the measures is 0, Ln 0 being undetermined) a X^2 test was used:

$$X^2 = \Sigma^a \frac{(f_i - \hat{f}_i)^2}{\hat{f}_i}$$

f_i = observed values

$\hat{f}_i$ = expected values

Degrees of freedom = d.f. = C-1

C = number of classes

The critical values were looked up in a table of X^2 values.

Table 3: Heterogeneity Test: G_H-Test

Expt. no.	value of x	value of y	t=x+y	xLnx	yLny	xLnx+yLny	tLnt
1	x_1	y_1	t_1	x_1Lnx_1	y_1Lny_1	S_1	t_1Lnt_1
2	x_2	y_2	t_2	x_2Lnx_2	y_2Lny_2	S_2	t_2Lnt_2
3	x_3	y_3	t_3	x_3Lnx_3	y_3Lny_3	S_3	t_3Lnt_3
4	x_4	y_4	t_4	x_4Lnx_4	y_4Lny_4	S_4	t_4Lnt_4
5	x_5	y_5	t_5	x_5Lnx_5	y_5Lny_5	S_5	t_5Lnt_5
	X	Y	T			S	U
	XLnX	YLnY	TLnT				

Sum of column values

rLnr of sum

CHAPTER 3 Results

Section I Linkage group I

Crosses were set up using markers known to be in the region affected by Rec-s, see figure 11.

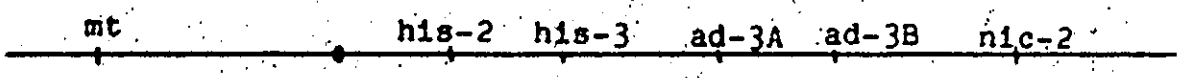


Figure 11: Relative positions of markers used on linkage group I. Distances are not to scale.

Rec-s has been shown to be situated between his-2 and ad-3A (Hargrave and Threlkeld, 1973).

A strain of N. crassa carrying his-2, ad-3A, nic-2, rec-c, a (74-YU-192-1) was crossed to a strain carrying ad-3B, Rec-s, A (195-28-7).

A control cross was done using strain 74-YU-192-1 and a strain having the markers ad-3B, rec-c, A (195-33-5).

Both crosses were studied by taking random spores, asci and prototrophs (for adenine independance). The results of the analysis of random spores from the cross involving Rec-s are presented in table 4.

Table 4: analysis of random spores from cross 28

74-YU-192-1 X 195-28-7

his-2, ad-3A, nic-2, rec-c, a ad-3B, Rec-s, A

Number of spores isolated: 2045

Number of spores germinated: 1595

Percentage of germination: 77.98

Class	Genotype	Number of spores
Parental	his, ad-3A, nic	595
	+, ad-3B, +	613
Recombinant	his, ad, +	189
	+, ad, nic	176
	+, +, nic	19
	+, +, +	3

Of the data presented in table 4, the 3 spores showing wild phenotype were further tested and found to be pseudowild types. They will not be included in calculations of map distances.

A G_H test was done and showed that differences between reciprocal classes were not significant. Therefore it seems that there is no preferential germination of any particular marker and no correction factor had to be used.

In the same way, for each marker the ratio $\frac{m}{m+}$ was not significantly different from 1; for his-2 it was found $G_H = 0.18$ and for nic-2, $G_H = 0.076$; with one degree of freedom it gives $0.9 > P > 05$. The distance between his-2 and nic-2 in presence of Rec-s is 24.06 ± 1.07 map units.

From the same cross, Rec-s x rec-c (cross 28), asci were isolated. The results of their analysis is in Table 5.

Table 5: Analysis of asci from cross 28

Number of asci isolated: 362

Number of asci analyzed: 269

Percentage of germination (analyzed asci only): 76.6

Interval	Number of single crossing overs	Map distance
-o--his	7	1.30±0.69 map units
-o--nic	135	25.09±2.64 map units
his-nic	128	23.79±2.60 map units
ad-3A-ad-3B	12	2.23±0.90 map units

Crossing overs between ad-3A and ad-3B are also included in the his-nic region.

A complementation test was done on the 12 asci containing a pair of *ad*$^+$ spores. The test confirmed that these asci were the result of a single crossing over in the region *ad-3A* - *ad-3B*.

One pair of double mutant spores was present in each of them and there was a normal 4:4 ratio of the two alleles.

None of the asci could have arisen from a conversion at the site of either *ad-3A* or *ad-3B*; such an event would have produced an unequal ratio of the alleles (6:2 or 5:3 ratio) in most cases. (about conversion see chapter 4, section IV)

No double crossing overs were found. The expected number of double crossing overs in the region centromere - *nic-2* is 0.83.

A mating type test was done on some of the asci, the results of which are presented in table 6.

The expected frequency of double crossing over in the interval *mt-his-2* is calculated as 2.208×10^{-3}, which means that out of the 125 asci analyzed 0.276 of them are expected to show such a crossing over. As one was found, the coefficient of coincidence is 3.62. But the sample used here is too small to allow any conclusions to be drawn.

Table 6: Mating type test on asci of cross 28 Number of asci tested: 125

Interval	no C.O.	single C.O.	Double crossing over			Map distances in map units	
			2-strand	3-strand	4-strand	uncorrected	corrected
mt--o-	79	46				18.40 ± 3.46	
mt-his	75	49	1			24.40 ± 3.84	23.60 ± 3.80
-o--his	122	3				1.20 ± 0.97	
his-nic	83	42				16.80 ± 3.34	
mt-nic	23	91	2	5	4	41.20 ± 4.40	37.60 ± 4.33

Crossing overs from doubles are not included in singles.

In the interval mt-nic-2, the expected frequency of double crossing overs is 4.0992×10^{-2}, or 5.124 asci out of 125. In fact there were 11 of them and the coefficient of coincidence is 2.15, indicating negative chromosome interference.

A G test for goodness of fit done on the data for double crossing overs in mt-his-2 and mt-nic-2 gives:

$G = 7.4750$, d.f. = 2, $0.01 > P > 0.005$

indicating a significant difference.

So, although the samples are small, they seem to indicate that a negative chromosome interference is present.

If there is no chromatid interference the ratio of two-strand double crossing overs to three-strand double crossing overs to four-strand double crossing overs will be 1:2:1.

Negative chromatid interference would produce an excess of two-strand double crossing overs while positive chromatid interference would result in an excess of four-strand double crossing overs.

A G test for goodness of fit was done on the data obtained for region mt-nic-2:

$G = 0.7706$, d.f. = 2, $0.9 > P > 0.5$

This test indicates that the data do not depart significantly from the expected ratio of 1 two-strand: 2 three-strand: 1 four-strand double crossing overs.

Table 7: Prototroph frequencies in cross 28

Average number of colonies on complete medium	Average number of colonies on ad-less medium	dilution factor	Average percentage of prototrophs	Average map distance in map units
112	50	1/15	2.99	5.98 ± 0.58
81	35.4	1/20	2.18	4.36 ± 0.51
145.6	49.2	1/10	3.38	6.76 ± 0.66
66.6	16.4	1/10	2.46	4.92 ± 0.84
51.4	14.8	1/10	2.88	5.76 ± 1.03
58	13.2	1/10	2.24	4.48 ± 0.86
57.4	9.8	1/10	1.71	3.42 ± 0.76

Therefore no chromatid interference seems to be present.

Cross 28 was also studied by counting ad$^+$ prototrophs. The distance between ad-3A and ad-3B can thus be calculated, as table 7 shows.

The replicate experiments were compared for heterogeneity by means of a G_H test:

$G_H = 7.64$, d.f. = 6, $0.5 > P > 0.1$

425 of the prototrophs were isolated and tested for the outside markers his-2 and nic-2.

12 of them were in fact pseudowild types, having a wild phenotype similar to other spores found in this study previously which, after testing, were proved to result from a non disjunction of chromosomes rather than from a multiple recombination event.

The 413 remaining had the genotype + nic, as expected if they resulted from a single recombination event between ad-3A and ad-3B. To distinguish ad-3A mutants from ad-3B mutants a complementation test was done on the random spores isolated previously. The results from this test are shown in table 8.

Table 8: Complementation test on random spores from cross 28.

Number of spores tested: 156
(Only the spores showing recombination between his and nic were actually tested. Others were assumed to have the parental allele.)

Class	Genotype	Number of spores
Parental	his, ad-3A, nic	65
	+, ad-3B, +	59
Recombinant	his, ad-3A, +	5
	+, ad-3A, nic	13
	his, ad-3B, +	8
	+, ad-3B, nic	6

From table 8, the distance between his-2 and ad-3A can be calculated as being 13.46 ± 2.73 map units and the distance between ad-3B and nic-2 as 7.05 ± 2.04 map units, both measured in presence of Rec-s.

A control cross homozygous for rec-c, cross 33, was set up and studied in the same way as cross 28. The data from the analysis of random spores is presented in table 9.

Table 9: Analysis of random spores from cross 33

74-YU-192-1 X 195-33-5

his-2, ad-3A, nic-2, rec-c, a ad-3B, rec-c, A

Number of spores isolated: 975

Number of spores germinated: 775

Percentage of germination: 79.57

Class	Genotype	Number of spores
Parental	his, ad-3A, nic	367
	+, ad-3B, +	376
Recombinant	his, ad, +	14
	+, ad, nic	18

In this cross, as in cross 28, there is no significant differences between the sizes of the reciprocal classes and no differential germination of the markers.

The distance between his-2 and nic-2 is 4.13 ± 0.71 map units when Rec-s is absent from a cross.

Asci from cross 33 were isolated and the results of their analysis are in table 10.

Table 10: Analysis of asci from cross 33

Number of asci isolated: 89

Number of asci analyzed: 75

Percentage of germination (analyzed asci only): 88.33

Interval	Number of single crossing overs	Map distance
-o--his	0	
-o--nic	11	7.33 ± 3.01 map units
his-nic	11	7.33 ± 3.01 map units
ad-3A-ad-3B	1	0.67 ± 0.94 map units

Crossing overs in ad-3A - ad-3B are also included in his-nic. No double crossing overs were found and this is expected, in view of what was seen in cross 28.

The distance between ad-3A and ad-3B was measured with more accuracy by counting ad^+ prototrophs, as shown in table 11.

Table 11: Prototroph frequencies in cross 33

Average number of colonies on complete medium	Average number of colonies on ad-less medium	dilution factor	Average percentage of prototrophs	Average map distance in map units
84.2	0.75	1/20	0.0445	0.0880 ± 0.072
93.5	1.25	1/20	0.0668	0.1336 ± 0.084
78.4	6	1/50	0.1520	0.3040 ± 0.088
55.8	1.4	1/50	0.0390	0.0780 ± 0.053
65	2.4	1/50	0.0740	0.1480 ± 0.067
73.25	5.6	1/50	0.1560	0.3120 ± 0.092
57.4	3	1/100	0.0522	0.1044 ± 0.043

A comparison of the replicate experiments by means of a G_H test gives:

$$G_H = 5.121,\ d.f. = 6,\ 0.9 > P > 0.5$$

102 prototrophs isolated and tested for their outside markers were found to be + nic. This can best be explained as the result of a single recombination event between ad-3A and ad-3B.

No pseudowild types were found, in contrast with what was seen in cross 28.

A complementation test was done on the random spores isolated from cross 33 and its results are in table 12.

Table 12: Complementation test on random spores from cross 33

Number of spores tested: 332

(Only spores recombinant for the outside markers were actually tested. Others were assumed to be of parental genotype.)

Class	Genotype	Number of spores
Parental	his, ad-3A, nic	158
	+, ad-3B, +	165
Recombinant	his, ad-3A, +	4
	+, ad-3A, nic	3
	his, ad-3B, +	0
	+, ad-3B, nic	2

From these data the distance his-2 - ad-3A is 0.904 ± 0.52 map units and the distance ad-3B - nic-2 is 1.808 ± 0.73 map units. A cross using the markers ad-3B and his-3, in presence of Rec-s, was done. The results from this cross are presented in table 13.

Table 13: Prototroph frequency from cross 57

195-28-7 x 1157

ad-3B, Rec-s, A his-3, rec-c, a

Average number of colonies on complete medium	Average number of colonies on ad less medium	dilution factor	Average percentage of prototrophs	Average map distance in map units
93	40.71	1/10	4.37	8.74 ± 0.93

This distance between ad-3B and his-3, measured in the presence of Rec-s, can be compared to the similar distance measured in the absence of Rec-s: Cross 114 was set up as a control cross, comporting the same markers as cross 57 but homozygous for rec-c.

The results of the study of cross 114 are in tables 14 and 15.

Table 14: Prototroph frequency in cross 114

2-17-114 x 1157

ad-3B, rec-c, A) his-3, rec-c, a

Average number of colonies on complete medium	Average number of colonies on ad less medium	dilution factor	Average percentage of prototrophs	Average map distance in map units
88	28.8	1/100	0.327	0.0654 ± 0.086

Table 15: Analysis of random spores from cross 114

Number of spores isolated: 608

Number of spores germinated: 467

Percentage of germination: 76.80

Class	Genotype	Number of spores
Parental	+, ad	229
	his, +	235
Recombinant	his, ad	2
	+, +	1

The data from table 15 gives the distance between his-3 and ad-3B, in absence of Rec-s, as being 0.642 ± 0.369 map units.

A comparison of the map distances found can be seen in table 16. The map distances in presence of Rec-s, in absence of Rec-s and the effect on map distances due to Rec-s are illustrated respectively in figures 12, 13 and 14.

From table 16 it is seen that in all the intervals studied there is a significant difference between a cross involving Rec-s and a cross without it.

The maximum effect of Rec-s is found between ad-3A and ad-3B. The genetic distance between these markers is very small in absence of Rec-s. The next higher increases in recombination frequency are between his-2 and ad-3A and between his-3 and ad-3B.

Mention must be made here of the unexpected high number of pseudowild type ascospores found in cross 28 (his-2, ad-3A, nic-2, rec-c, a x ad-3B, Rec-s, A).

Pseudo wild types for linkage group I are relatively rare because of the presence of the mating type, acting as a heterokaryon incompatibility factor.

However 3 out of 1595 random spores and 12 out of 425 prototrophs were recovered in cross 28. Many asci from the same cross contained pseudo wild type spores along

with aborted spores (Most of these asci were not used in calculations for different reasons, one of them being that only asci having at least 5 germinated spores were used.).

In cross 33 (his-2, ad-3A, nic-2, rec-c, a x ad-3B, rec-c, A) no pseudo wild types were found.

A X^2 test (a G test cannot be used, as it is impossible to calculate Lno) indicates that the difference in the occurence of pseudo wild types between the two crosses is significant:

For the random spores $X^2 = 2.6324$, d.f. = 1, $0.5 > P > 0.1$

For the asci $X^2 = 3.7877$, d.f. = 1, $0.05 > P > 0.025$

For the prototrophs $X^2 = 4.0239$, d.f. = 1, $0.05 > P > 0.025$

Rec-s could cause the non disjunction of the chromosomes at meiosis to happen with an increased frequency. However this has yet to be verified for other linkage groups than linkage group I.

Four of the pseudo wild types from cross 28, two from random spores and two from asci, were analyzed in some detail. Conidial isolates were taken from each of them and tested for their biochemical requirements:

PWT 1 gave 12 +++ conidia, 12 + ad + and 1 ++ nic.

PWT 2 yielded 16 +++ and 9 + ad + conidia.

PWT 3 produced 8 +++, 3 his ad nic and 13 ++ nic conidia.

PWT 4 gave 11 +++, 9 + ad + and 3 + ad nic conidia.

The pseudo wild types were allowed to self; all

four of them produced perithecia but these contained very few spores.

The pseudo wild types were crossed to tester strains of each mating type; in each case a pseudo wild type gave perithecia with both testers but spores with only one of them; perithecia from the other cross contained very few ripe spores.

PWT2 and PWT 3 gave spores with tester a, PWT 1 and PWT 4 with tester A.

For all pseudo wild types vegetative growth was slower than for euploid strains.

Summary of results from section 3I.

When Rec-s is present in a cross there is an increase in the recombination frequencies between the centromere and nic-2 on linkage group I. This increase is not uniform along this region.

The cross involving Rec-s produces pseudo wild types with an increased frequency.

Table 16: Comparison of map distances between Rec-s x rec-c crosses and rec-c x rec-c crosses.

Interval	with Rec-s	without Rec-s	Increase	G	d.f.	P
ct--o-	18.40	-		-	-	-
ct-nis-2	23.60	-		-	-	-
ct-nic-2	37.60	-		-	-	-
-:--his-2	1.25	<0.2[1]	x>6.25	-	-	-
-:--nic-2	25.09	7.33	x3.42	105.54	1	<0.001
:is-2-nic-2	21.55	5.73	x3.76	245.9	1	<0.001
:is-2-ad-3A	13.46	0.904	x14.89	33.88	1	<0.001
ad-3A-ad-3B	4.74	0.230	x20.61	824.14	1	<0.001
ad-3B-nic-2	7.05	1.808	x3.90	7.96	1	<0.005
his-3-ad-3B	8.74	0.648	x13.49	213.47	1	<0.001

From Newcombe, 1969

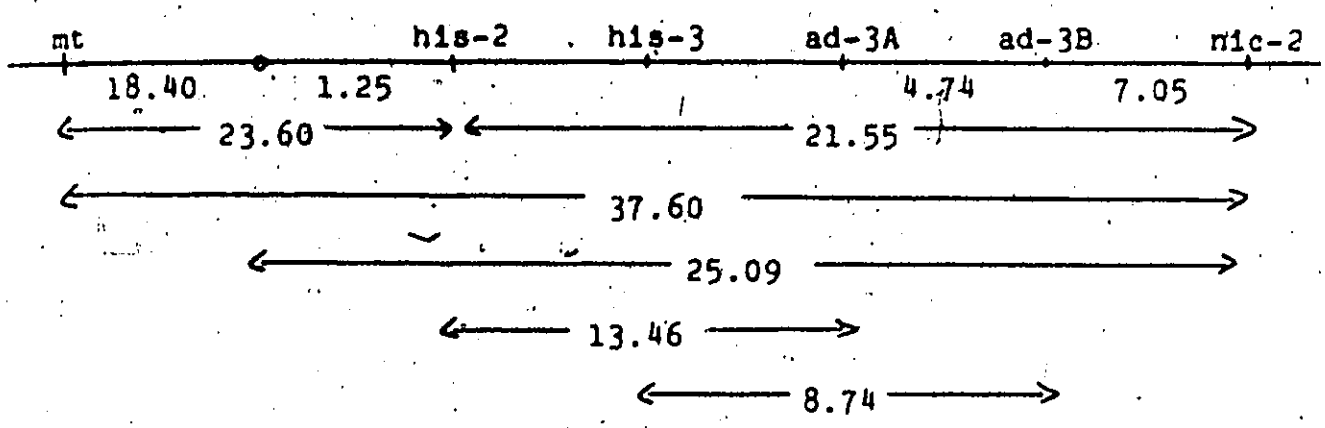


Figure 12: Map distances on linkage group I when Rec-s is present

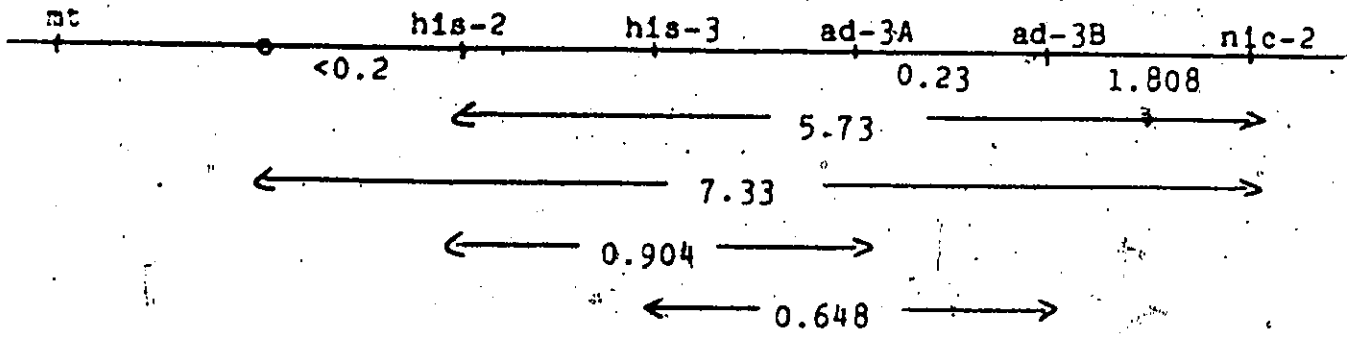


Figure 13: Map distances on linkage group I when Rec-s is absent

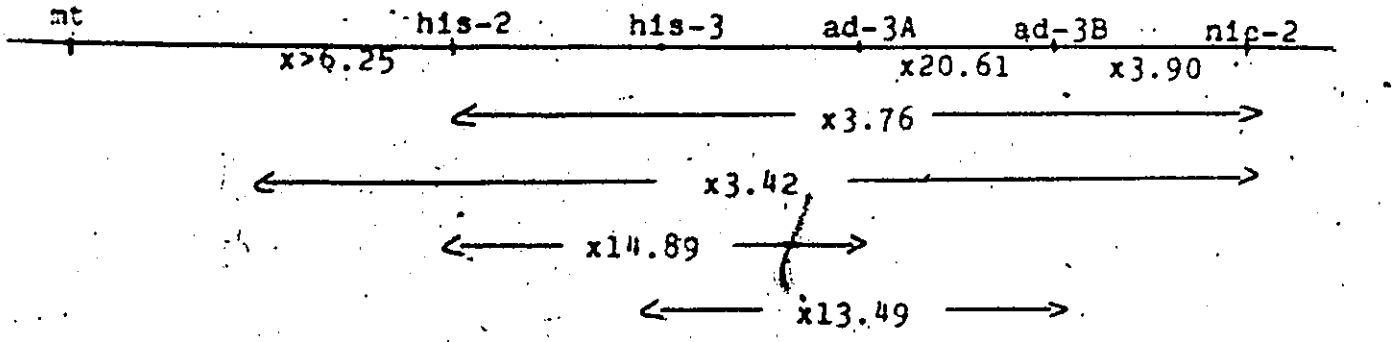


Figure 14: Increases in map distances due to the presence of Rec-s

Distances are not to scale.

Section II. Linkage group VI

Some previous works (Newcombe, 1969) seemed to indicate that there might be an increase in recombination frequency in the centromere proximal region of linkage group VI when Rec-s is present. This possibility was looked at by comparing the recombination frequencies between pan-2(3) and pan-2(5) in the presence and in the absence of Rec-s.

A strain with the markers ad-5, pan-2(3), Rec-s, A (strain C1198A3-4) was crossed to a strain ad-1, pan-2(5), rec-c, a (strain 467). The control cross used a strain tryp-2, ylo-1, pan-2(3), rec-c, A (strain 469) and strain 467.

The results for both crosses are presented in tables 17 and 18.

Table 17: Prototroph frequencies from cross 98

C1198A3-4 x 467

ad-5, pan-2(3), Rec-s, A ad-1, pan-2(5), rec-c, a

Average number of germinated spores per plate	average number of colonies per plate	average percentage of pan prototrophs	average map distance in map units
10,150.50	60.6	0.597	1.194 ± 0.107
3,932.56	14.0	0.356	0.712 ± 0.134
2,949.94	21.7	0.738	1.476 ± 0.221
1,600.00	13.0	0.813	1.626 ± 0.315
293.02	1.2	0.409	0.818 ± 0.525

A heterogeneity G_H test on the values from these replicate experiments gives:

$$G_H = 6.6, \text{ d.f.} = 4, 0.5 > P > 0.1$$

The average distance between pan-2(3) and pan-2(5) in presence of Rec-s, calculated from the data in table 19, is 1.0764 ± 0.078 map units.

Table 18: Prototroph frequencies from cross 69

469 x 467

tryp-2, ylo-1, pan-2(3), rec-c, A ad-1, pan-2(5), rec-c, a

Average number of germinated spores per plate	average number of colonies per plate	average percentage of pan prototrophs	average map distance in map units
1,705.95	7.5	0.439	0.878 ± 0.226
1,284.48	3.0	0.233	0.466 ± 0.190
1,256.38	5.6	0.445	0.890 ± 0.265
1.256.38	4.2	0.334	0.668 ± 0.229
1,471.13	2.8	0.190	0.380 ± 0.160

A G_H test for heterogeneity between the replicate experiments gives: $G_H = 2.46$, d.f. = 4, $0.9 > P > 0.5$

From table 18, the average distance between the pan-2 alleles used is 0.6564 ± 0.096 map units in the absence of Rec-s. A G_H test was done to compare the two sets of data: $G_H = 6.88$, d.f. = 1, $0.01 > P > 0.005$

The difference found between the rec-c x rec-c cross and the rec-c x Rec-s cross is significant. Rec-s appears to have an effect on the recombination frequency between pan-2(3) and pan-2(5) in linkage group VI.

The increase when Rec-s is present is x 0.61.

This is very small and in no way comparable to what has been found for linkage group I.

The pan-2 prototrophs were not tested and there is no way to say if the increase is due to recombination between the pan-2 alleles or if, as was seen in linkage group I, there has been an increase in the frequency of pseudo wild types.

If Rec-s produced a general increase in non disjunction of the chromosomes at meiosis when it is present in a cross, a high frequency of pseudo wild types should be found for all the linkage groups.

Summary of results from section 3II

There is a small but significant increase of pan-2 prototrophs in a cross when Rec-s is present.

Section III. Irradiation with ultraviolet light

Two strains were compared for their percentages of survival after irradiation: strain 195-28-7 (ad-3B, Rec-s, A) and strain 195-33-5 (ad-3B, rec-c, A). The results are presented in tables 19 and 20.

A graph for each replicate experiment is seen in figures 15 and 16. The very variable and sometimes high standard deviations (shown in tables 19 and 20) made a thorough statistical analysis unreliable.

However, by looking at figures 15 and 16, one can suspect that there might not be any significant difference between the behaviors of the two strains. This is even more evident when one looks at table 21 and figure 17.

One of the experiments with strain 195-28-7 (experiment 1) gives unlikely results (112.5% survival is one of the measures). This was thought to be due to a mistake during manipulation. Alternative calculations, excluding this experiment, are provided in table 21 and figure 17.

There is variations between replicate experiments. One reason for this could be either an inhibition or a cross feeding (syntrophy) effect of conidia upon their neighbors when the density of conidia on a plate is high: a conidium would release in the medium compounds which would either inhibit the growth of other conidia, or be used as a substrate by conidia unable to synthetize this particular compound.

In these cases either a negative correlation or a positive correlation would be found between the number of conidia on a plate and the percentage of survival. However the data presented here do not show any such correlation.

Table 19a: Survival to uv irradiation of strain 195-28-7

16 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	standard deviation
200.0	1	3.1	-
154.2	1	2.9	20.02
124.0	1	10.3	33.87
64.2	1	6.6	6.43
82.8	1	12.1	17.77
154.7	1	14.7	20.51

Table 19b: Survival to uv irradiation of strain 195-28-7

12 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	standard deviation
176.0	1/10	27.5	12.58
91.2	1/10	17.7	26.57
39.0	1/10	32.5	24.02
47.2	1/5	23.0	5.49
52.4	1/5	38.5	9.85
104.7	1/5	46.9	11.25

Table 19c: Survival to uv irradiation of strain 195-28-7

4 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	Standard deviation
68.4	1/100	112.5	6.22
84.0	1/50	81.5	6.44
43.6	1/20	72.6	9.90
57.4	1/10	59.7	3.26
56.6	1/10	83.2	1.85
80.3	1/10	72.0	6.24

Table 19d: Survival to uv irradiation of strain 195-28-7

0 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	Standard deviation
6.4	1/1000	100	2.94
20.6	1/250	100	6.41
12.0	1/100	100	5.95
19.2	1/50	100	2.56
13.6	1/50	100	3.72
22.3	1/50	100	1.70

Table 20a: Survival of uv irradiation of strain 195-33-5

16 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	Standard deviation
106.0	1	1.3	-
109.4	1	11.1	5.08
243.0	1	9.0	17.31
251.5	1	15.1	14.55
22.4	1	28.0	10.85

Table 20b: Survival to uv irradiation of strain 195-33-5

12 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	Standard deviation
105.6	1/10	13.6	15.58
80.8	1/5	41.2	8.56
193.8	1/5	37.5	12.83
157.6	1/5	47.2	13.27
7.2	1/5	45.0	2.71

Table 20c: Survival to uv irradiation of strain 195-33-5

4-mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	Standard deviation
79.8	1/50	51.4	9.06
61.2	1/10	62.4	10.89
190.6	1/10	73.9	19.63
122.6	1/10	73.6	15.84
5.6	1/10	70.0	1.85

Table 20d: Survival to uv irradiation of strain 195-33-5

0 mn exposure to radiation

Average number of colonies per plate	dilution factor	Percentage of survival	Standard deviation
31.0	1/250	100	7.13
19.6	1/50	100	5.24
51.6	1/50	100	6.71
33.3	1/50	100	5.56
4.0	1/20	100	2.19

Table 21: Average percentage of survival to ultraviolet radiation of strains 195-28-7 and 195-33-5

Length of exposure to uv	195-28-7 including ept. 1	195-28-7 excluding expt. 1	195-33-5
16 mn	9.95	9.32	10.70
12 mn	31.01	31.72	27.40
4 mn	80.25	73.80	86.14
0 mn	100.00	100.00	100.00

Summary of results from section 3III

The presence of Rec-s does not affect the uv sensitivity of a strain.

Figure 15: Survival to uv irradiation of strain 195-28-7

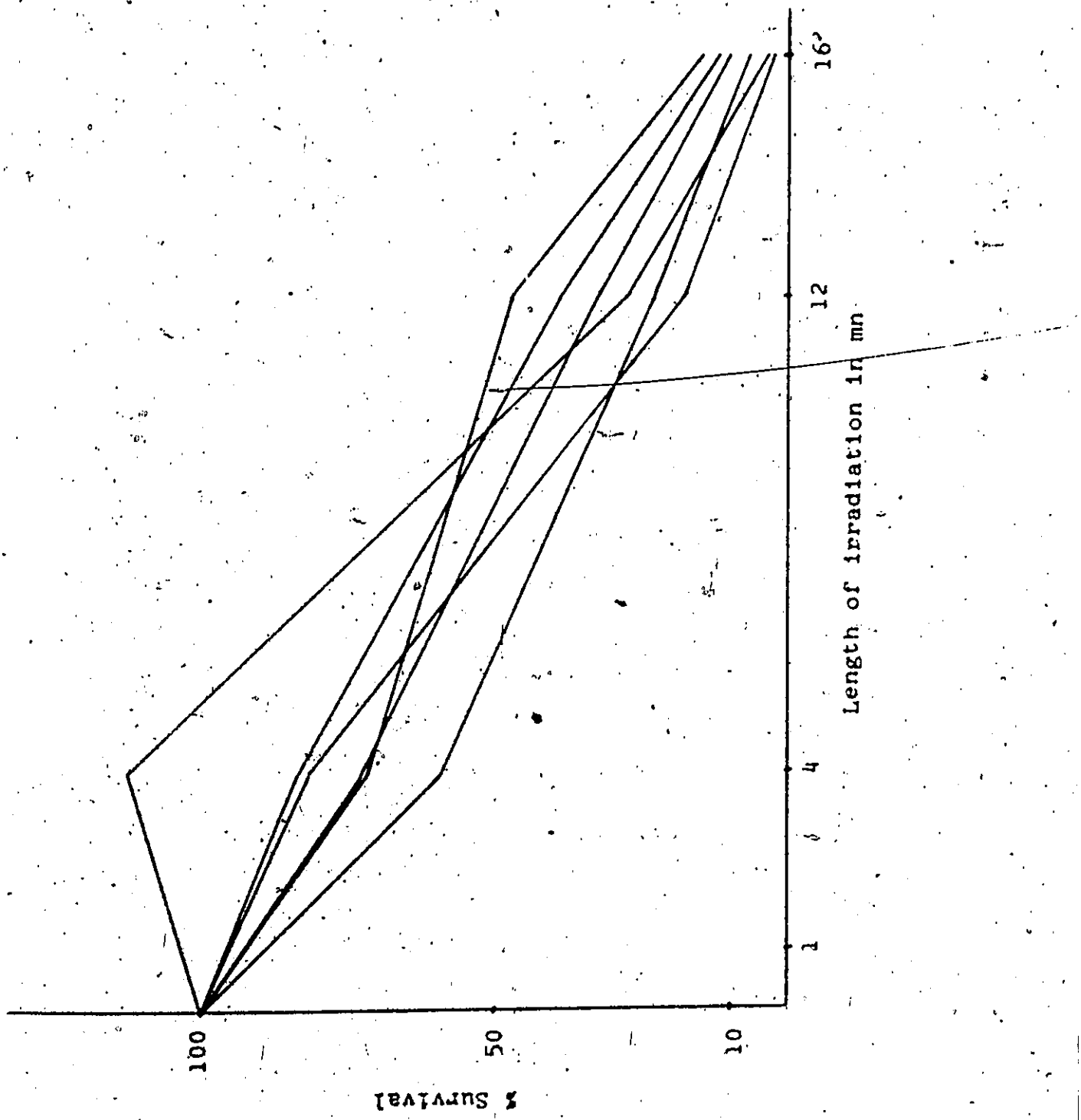


Figure 16: Survival to uv irradiation of strain 195-33-5

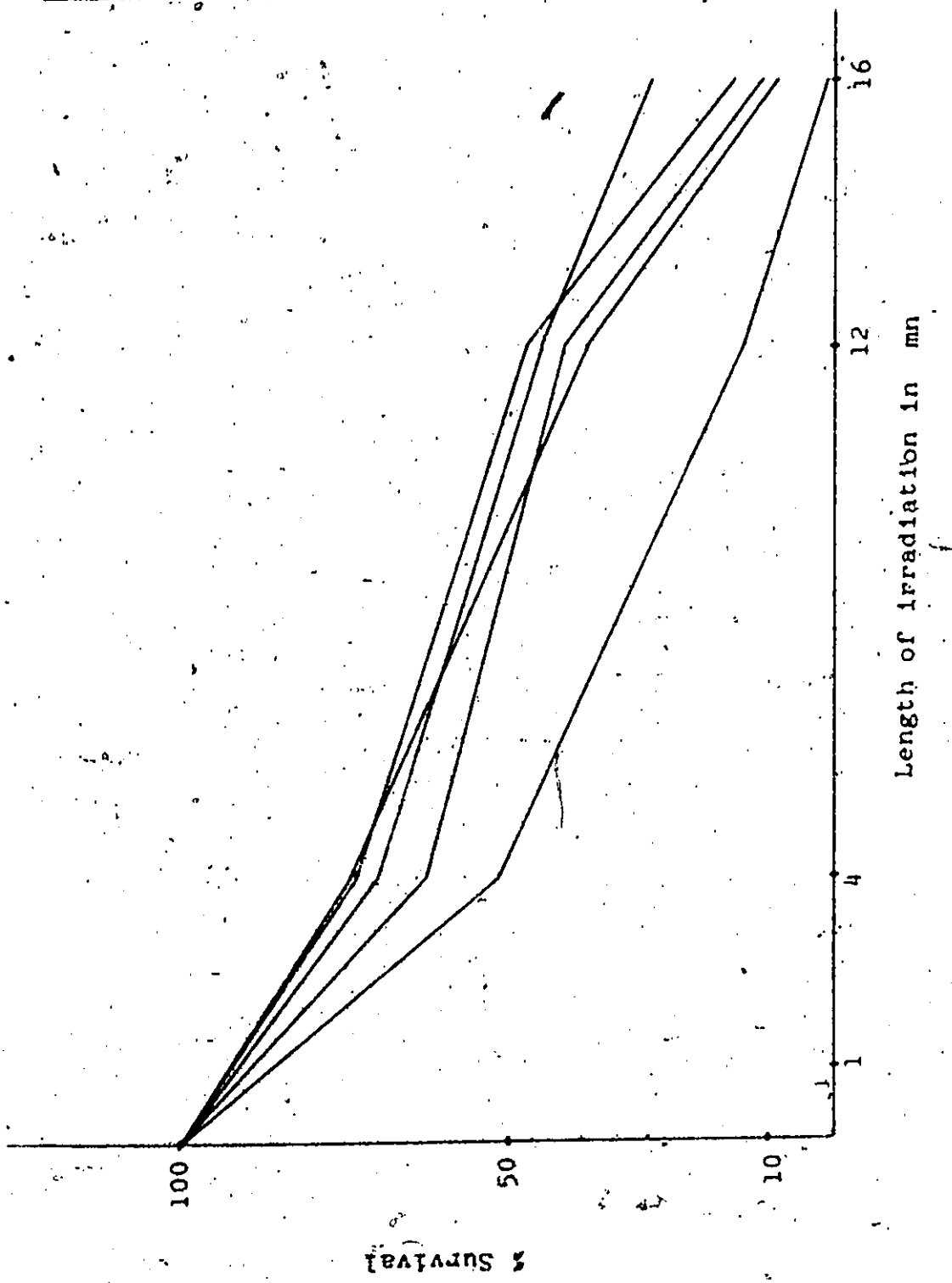
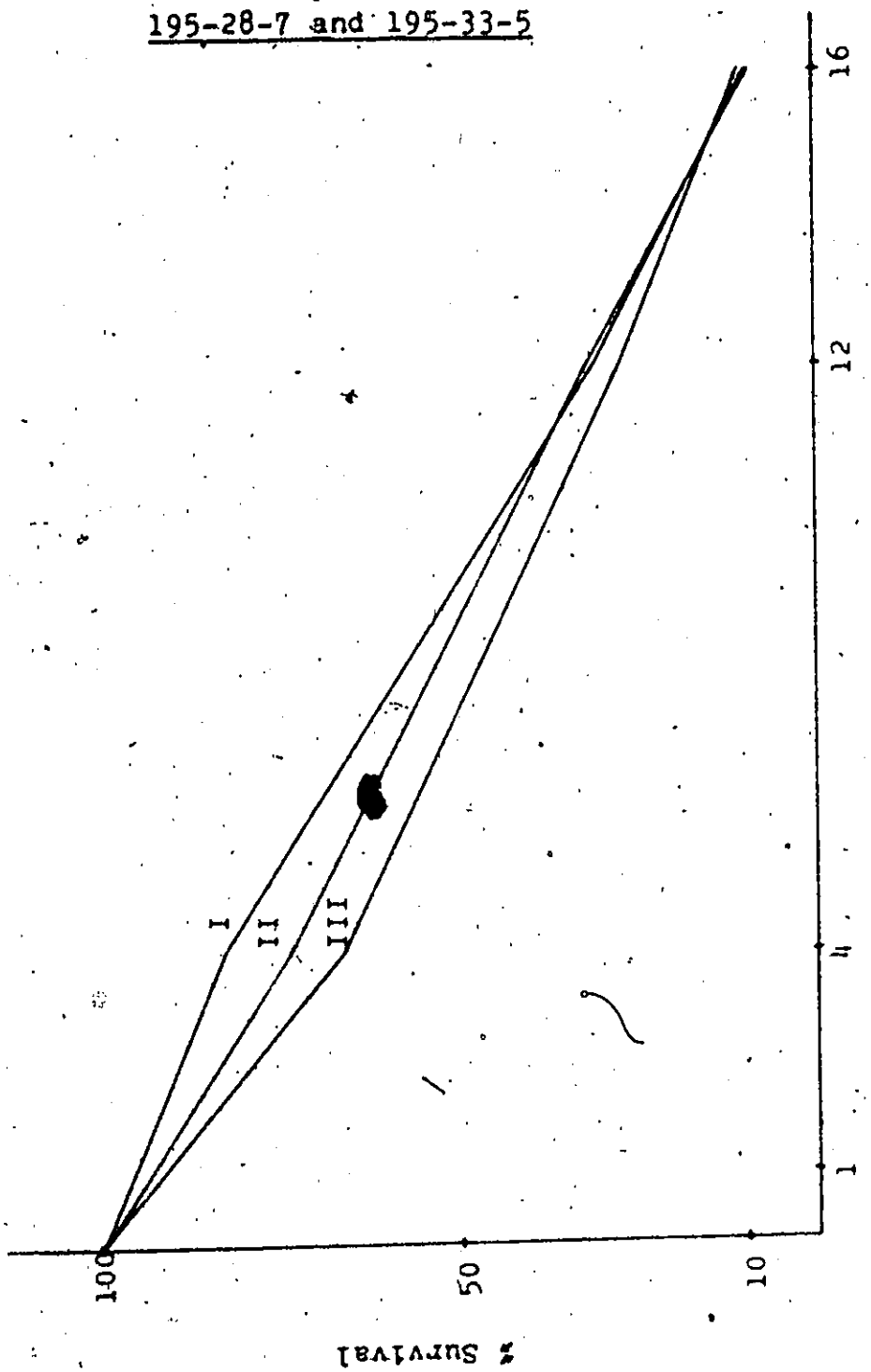


Figure 17: Average survival to uv irradiation of strains 195-28-7 and 195-33-5



I: Strain 195-28-7 including experiment 1

II: Strain 195-28-7 excluding experiment 1

III: Strain 195-33-5

CHAPTER 4 Discussion

Section I: Effect of Rec-s

It has been found, as reported in chapter 3, that Rec-s seems to affect the region between the centromere and nic-2 on linkage group I, and pan-2 on linkage group VI.

On linkage group I the highest increase in recombination frequency, a 20-fold increase, is seen between ad-3A and ad-3B. This distance measured in presence of rec-c happens to be very small (0.230 map units).

The next higher increases in recombination frequencies are between his-2 and ad-3A and between his-3 and ad-3B; both distances, when Rec-s is absent, are small, although not as much as ad-3A - ad-3B (0.904 map units and 0.648 map units respectively.).

Then the effect of Rec-s sharply decreases to give only a 4-fold increase for example in ad-3B - nic-2.

Therefore it could be that the smaller the distance between the markers, the higher is the effect of Rec-s.

But it must also be remembered that Rec-s2 is situated in ad-3A - ad-3B, where the maximum effect of Rec-s is seen, and Rec-s1 is in his-2 - his-3, according to Hargrave (1971).

From her work Hargrave (1971) also concludes that Rec-s2 is more efficient than Rec-s1. The effect in the region centromere-ad-3A would be due to Rec-s1 while Rec-s2 would affect ad-3A - ad-3B and ad-3B - nic-2.

The present study supports these conclusions. On linkage group VI the data presented in chapter 3 are in favor of a significant increase of recombination frequency due to Rec-s in the gene pan-2. However this increase is very small, from 0.6564 map units to 1.0764 map units, only a 0.61 fold increase. This is in no way comparable to what has been found for linkage group I.

Previous studies on linkage group VI reported that Rec-s did not significantly increase recombination frequencies (Ferraro, 1971). The results from different types of crosses are shown in table 22.

Ferraro only looked at recombination between genes, not between alleles.

Table 22: Recombination frequencies on linkage group VI.

(adapted from Ferraro, 1971)

Interval	Type of Cross				G	d.f.	P
	rec-c x rec-c	rec-c x Rec-s	rec-c x rec-c(het.)	Rec-s x rec-c(het.)			
ylo-1--o-	4.8±1.3	6.9±2.5	4.6±1.2	5.2±4.1	1.63	3	0.9>P>0.5
1o--pan-2	3.4±1.1	4.9±2.1	2.3±0.8	8.6±5.2	8.54	4	0.1>P>0.5
pan-2-tryp-2	15.8±2.2	12.3±3.2	16.8±2.0	17.2±7.0	3.25	3	0.5>P>0.1

rec-c(het.): rec-c component of a heterokaryon between a rec-c strain and a Rec-s strain.

If, as seen in the present study for linkage group I, the effect of Rec-s is more marked between alleles or very closely linked genes, the increase on linkage group VI could be so slight as to be unperceived except in interallelic recombination. Indeed even there it is very small as mentioned before.

The presence of another Rec-s like factor, coming from N. sitophila, on linkage group VI is not likely to be the explanation: the Rec-s strains were constructed by selection on linkage group I only and it is highly improbable that a certain factor on linkage group VI would consistantly segregate with Rec-s, after a number of backcrosses to N. crassa.

However a rec- like gene specific for pan-2 could have been introduced by chance from one of the N. crassa strains used in the ancestry of strain C1198A3-4.

Strain 469 which has a different origin from strain C1198A3-4 would not have received this rec gene.

It seems that Rec-s could affect preferentially recombination between very closely linked markers, either in specific genes (ad-3 and pan-2 presently) or in any part of the affected regions.

Other interallelic crosses using markers such as his-2, his-3, ylo-1 and tryp-2 would help answer this question.

De Serres (1960) reports finding one pseudo wild type spore in 16, 339 spores for crosses involving an ad-3B strain of 74A background, and one in 5, 256 with an ad-3B strain of another origin. The ad-3A strain was the same in all the crosses.

The ad-3A strain used in this study being also the same in both Rec-s x rec-c and rec-c x rec-c crosses, any difference between the crosses must be attributable to the ad-3B strains. Both of these, 195-28-7 and 195-33-5, originated from a cross 30JA-5 x C162-461R.

30JA-5 came from a hybrid N. sitophila x N. crassa backcrossed five times to N. crassa FGSC#667. C162-461R has for ancestors ORA1-1, FGSC#667 and 2-17-114, this last having been induced in OR74A.

Therefore the ad-3B strains used here can be considered as being of 74A origin and are expected to yield one pseudo wild type per 16,339 spores.

The Rec-s strain gave 12 pseudo wild types out of 425 prototrophs, or 17,932 spores.

A Poisson distribution was used to calculate the frequency of this event happening and a G test for goodness of fit to test its significance:

$G = 4.9682$, d.f. = 1, $0.05 > P > 0.025$

It seems then that strain 195-28-7 produces more pseudo wild types than is expected and more than

strain 195-33-5. Both strains have the same origin and differ only in the Rec-s region.

Pseudo wild types for linkage group I would be expected to be self fertile and to cross with strains of either mating type, as they presumably contain both alleles of the mating type.

Such pseudo wild type spores were found but it was seen that in fact they are truly fertile with only one mating type. Crosses with the other mating type and selfing produce perithecia containing very few ripe spores (Gross, 1952; Martin, 1959; Newmeyer, 1970). Self fertility is lost after two or three subcultures (Martin, 1959). It has been shown that the disomic nuclei of pseudo wild types break down and conidia of parental types are recovered; some mitotic recombinants can also be found (Michell, Pittenger and Mitchell, 1952). The four pseudo wild types studied in this work (see Chapter 3) behaved as expected.

The very small increase in pan$^+$ spores seen in the pan-2(3) x pan-2(5) cross when Rec-s is present could be due to two phenomena: an increase in recombination in the pan-2 gene; an increase of non disjunction of the homologous chromosomes at meiosis, producing pseudo wild types; or an increase of both types of events, as was found for linkage group I.

However because the effect on linkage group VI was so small it could be, for example, an increase in the frequency of pseudo wild types alone, without increase in recombination frequency.

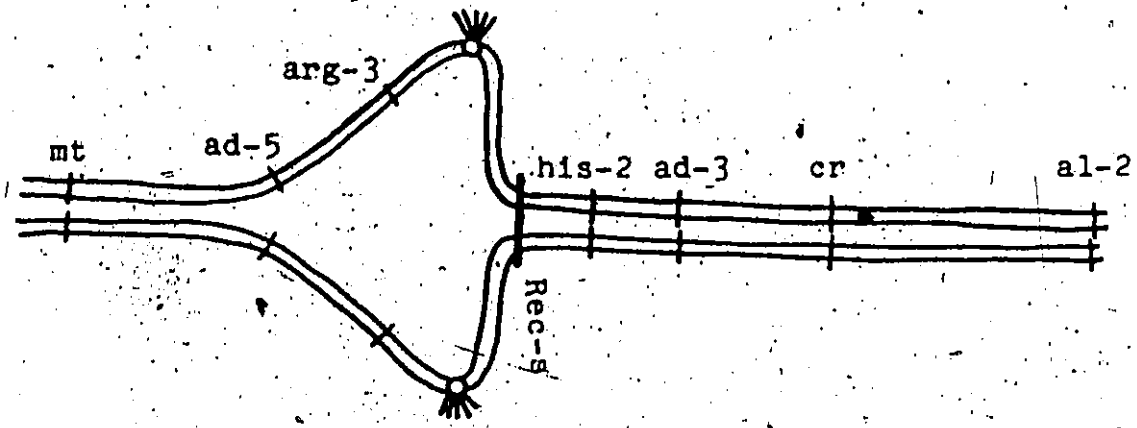
The recombination frequency on linkage group I was increased four to twenty fold, depending on the markers, while on linkage group VI there was only a 0.61 fold increase. Other linkage groups should be tested for pseudo wild type frequency. It might be found that when Rec-s is present in a cross there is a general increase in pseudo wild type frequency, for all linkage groups, but an increase in recombination frequency only in a specific region, on linkage group I.

Section II. Mode of action of Rec-s

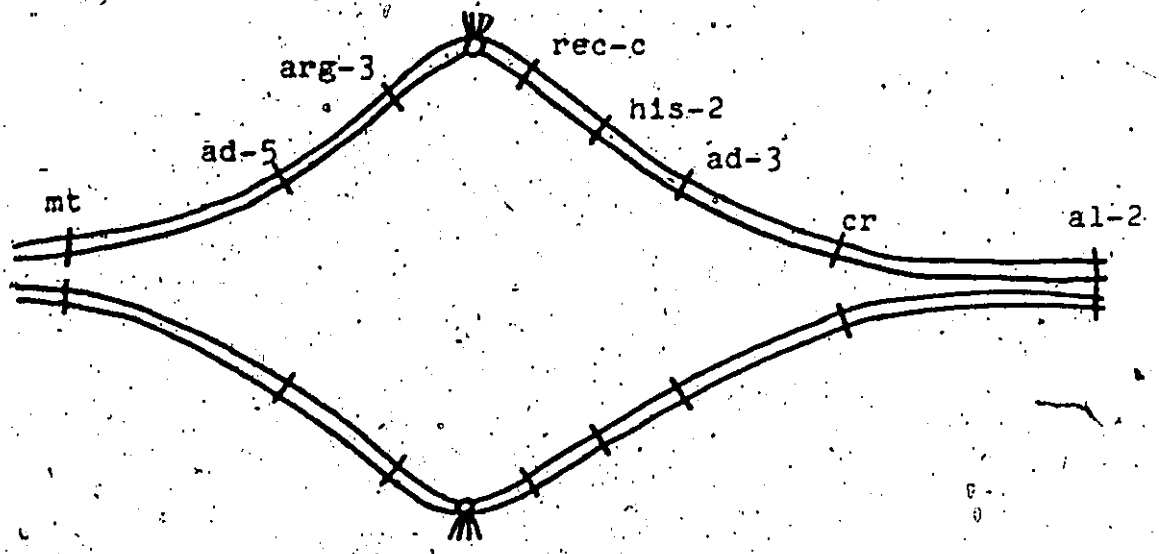
Several models for the mechanism of action of Rec-s have been proposed but none of them is entirely satisfactory to explain all the facts known about this factor.

A "desynaptic model" was proposed by Ferraro (1971). It is shown in figure 18. In this model Rec-s limits the desynapsis between the homologous chromosomes at meiosis so that recombination has more time to take place.

a - Rec-s crosses



b - rec-c x rec-c crosses



(rec-c x rec-c crosses may not have a true "limiting state")

Figure 18: Limiting desynaptic states for Rec-s and rec-c x rec-c crosses.
(Perraro, 1971)

In rec-c x rec-c crosses separation of the homologues starts before recombination has ceased, thus inhibiting recombination near the centromere.

This model could explain the high frequency of pseudo wild types found in presence of Rec-s. Rec-s keeps the homologues together, even after separation and ascension to the poles has started. In some cases this bond would not break in time and the two chromosomes would go together to one pole of the cell. In this way a nucleus with n + 1 chromosomes, disomic for linkage group I, would form and give rise to a pseudo wild type spore pair.

However this model explains the increase in recombination frequencies and in non-disjunction (producing pseudo wild types) on linkage group I only.

The increase seen for linkage group VI, whether it is due to an increase in the number of recombinants or pseudo wild types or of both, is not explained.

This model also requires that his-2 be distal to Rec-s, but Hargrave (1971) found that Rec-s1 is distal to his-2 and Rec-s2 distal to ad-3A.

Another model possible for Rec-s is one similar to the model proposed for cog by Angel, Austin and Catcheside (1970). In this model Rec-s1 and Rec-s2 are binding sites for a recombinase and have more affinity

for it than the corresponding regions in N. crassa, thus producing an increase in recombination frequencies in the surrounding region. The fact that this increase is not uniform can be explained in the following way: As the recombinaae travels on from its original binding site it has a tendency to fall off the chromosome. This could happen mainly while the enzyme is in one of the "spacers" found between genes. Brown, Wensinck and Jordan (1971) have shown that DNA coding for 5S RNA in Xenopus laevis contains spacers, sequences which do not code for any molecule, between each of the 5S RNA genes. These spacers are very heterogeneous in their nucleotide sequences and they differ significantly from the 5S RNA genes for their G-C content.

In Drosophila melanogaster spacer lengths of at least 0.5 µm were found between transcription units (Hamkalo and Miller, 1973). The difference in composition at the level of the spacer sequences could be the cause of the recombinase leaving the chromosome. Therefore the more spacers there are between a certain point on the chromosome and Rec-s, the less molecules of recombinase will be able to reach that point, and the smaller will be the increase in recombination frequency.

In this case the maximum effects of Rec-s1 and Rec-s2 is expected to be felt between the genes on

either side of these two factors; thus Rec-sI would produce a maximum increase in recombination frequency between his-2 and his-3, while the maximum increase for Rec-s2 would be between ad-3A and ad-3B. This last expectation has indeed been found to be true, as is reported in chapter 3.

In these cases there is a minimum number of spacer sequences between the two genes and consequently a maximum number of recombinase molecules able to span the region.

As the number of intercalary genes (and spacers) increases, the effect of Rec-s decreases: while ad-3A - ad-3B shows a twenty-fold increase, there is a fourteen-fold increase in his-2 - ad-3A and in his-3 - ad-3B, both of which have one gene separating the markers considered, and only a four-fold increase in his-2 - nic-2 (with between five and seven intercalary genes).

Or the propensity of the recombinase molecule to fall off the chromosome could simply increase proportionally to the distance travelled by the enzyme. The farther apart two markers are, the less will be the effect of Rec-s.

The data presented here also agree with this explanation. In fact both can work concurrently. However, in this model Rec-s affects only markers in neighbouring

regions, on linkage group I. Its effect on linkage group VI is not explained.

Heterochromatin is known to undergo little or no recombination. (Mather, 1939; Hannah, 1951).

Rec-s1 and Rec-s2 could be regions of euchromatin while the corresponding regions in N. crassa are heterochromatin. An increase in non disjunction of the X and Y chromosomes was seen in Drosophila melanogaster when the X chromosome was deficient for most of the centric heterochromatin (Peacock, 1965). Mention was made before of the high occurrence of pseudo wild types for linkage group I when Rec-s was present in a cross. But again this model would explain the increase in recombination frequencies and in pseudo wild types frequency on linkage group I only. It does not explain the effect found on linkage group VI. In summary, three possible types of models were looked at. None of them satisfactorily explains how Rec-s can affect linkage group VI, although there is no conclusive evidence demonstrating it is Rec-s that affects linkage group VI.

Section III. Ultraviolet sensitivity

No difference was found between a Rec-s strain and a rec-c strain for sensitivity to ultraviolet radiation. Therefore the mechanism by which the recombination

frequency is affected in these strains does not involve the elements of the repair to damaged DNA; rec-c strains would not be deficient in one of the three enzymes needed for repair: endonuclease, polymerase and ligase. In fact this is not surprising, as "rec-c" is the normal constitution of the N. crassa species.

If rec-c synthetized a product which excludes repair mechanism from DNA with mismatched base pairs, a rec-c x rec-c cross would show a decrease in allelic recombination but no change in non allelic recombination. It would also have an increased frequency of asci with unlike sister spores.

The results presented in chapter 3 do not support this hypothesis.

Section IV. Note on conversion

Recombination between alleles can arise from a reciprocal event (crossing over) or from a non reciprocal event (gene conversion). A mechanism accounting for both was presented by Holliday (1964) and it is shown in figure 19.

From this model it is evident that recombination between the outside markers is often linked to a conversion at the sites of the alleles.

This will produce spores with a recombinant composition for the alleles which will belong to all four classes for outside markers composition (two parental and two recombinant classes). In asci it will be seen as tetrads with unequal ratios of mutant to wild type allele, such as 5m:3+, 5+:3m, 6m:2+, 6+:2m. If some of the 425 ad+ prototrophs isolated and tested from cross 28, and some of the 102 from cross 33 arose from conversion of either ad-3 allele to wild type, we would expect the following outside marker compositions to be present: his nic, + +, his +, + nic. The size of the classes would depend on the frequencies of conversion of each allele to wild type; these might be influenced by the molecular nature of the mutations. It will also depend on polarity; an extreme case of this phenomenon has been reported in Ascobobus immersus (Lissouba and Rizet, 1960): in crosses between alleles of series 46, 6m:2+ asci always resulted from conversion of the right hand mutant to wild type.

The only classes of outside marker composition found among the prototrophs from cross 28 were +nic and ++. Even if it is supposed that only ad-3B is converted to wild type in this cross, the composition his + would be expected to be found. Among the prototrophs from cross 33 only the composition + nic was seen.

Among the asci, all the asci containing a pair of ad^+ spores also had a double mutant pair and a 4:4 ratio of the two alleles; they resulted of a crossing over between ad-3A and ad-3B. Therefore we can say that none of the ad^+ spores found resulted of a conversion event. They were produced either by a reciprocal recombination or by non disjunction (pseudo wild types). From the present data it is impossible to say if Rec-s affects non reciprocal recombination and reciprocal recombination equally.

Section V. Note on interference

There is contradictory evidence concerning interference in N. crassa. Positive chromatid interference in the proximal region of an arm has been reported (Lindegren and Lindegren, 1952; Whitehouse, 1958). But Scott-Emuakpor (1965) found negative chromatid interference across the centromere in linkage group I, and Ferraro (1971) positive interference in the same condition with Rec-s present. Negative chromatid interference was reported within an arm and across the centromere (Bole-Gowda, Perkins and Strickland, 1962; Perkins, 1962; Prakash, 1964).

As for chiasma interference De Serres (1971) found differences according to the origin of the strains:

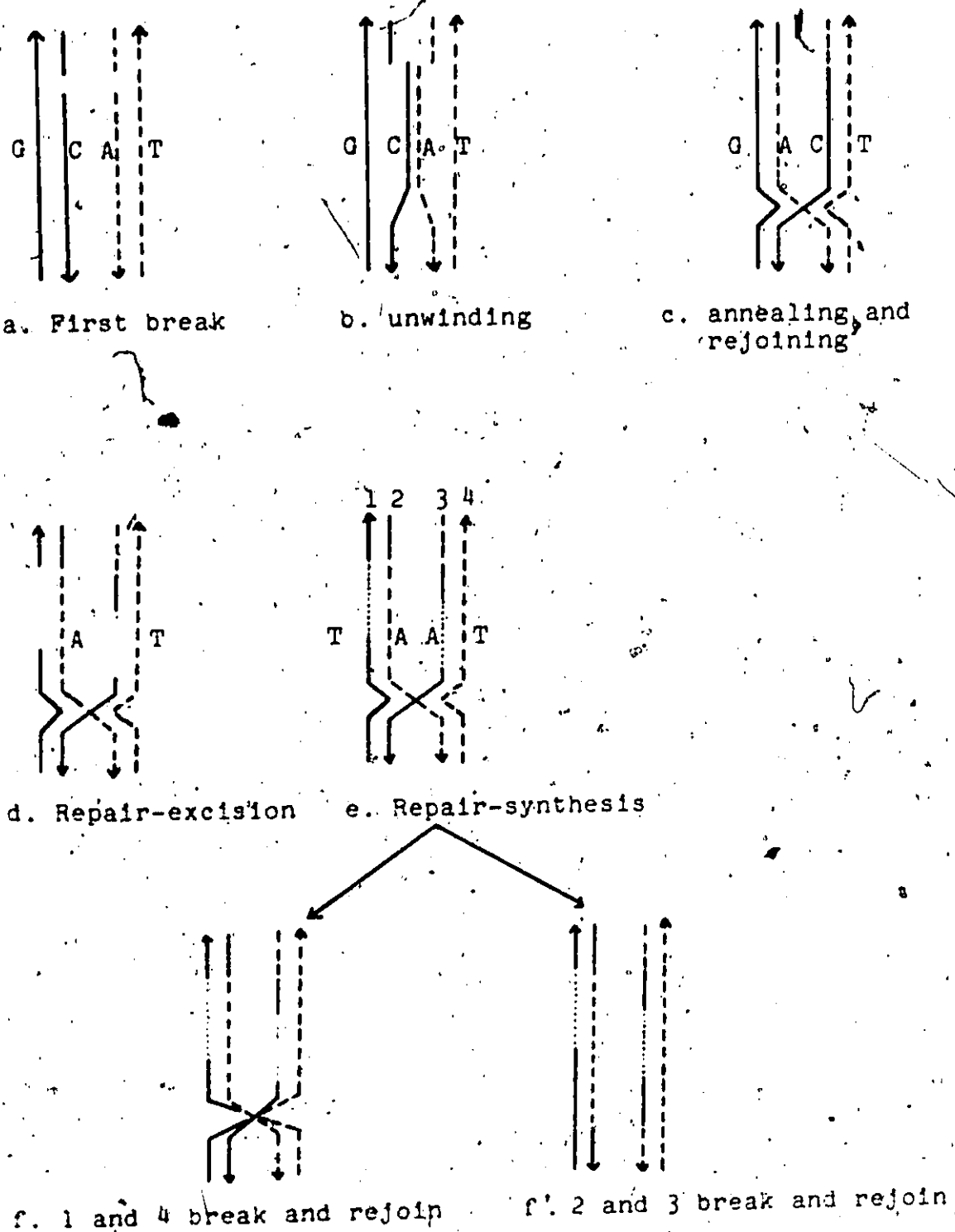


Figure 19: Mechanism of gene conversion in fungi (Holliday, 1964).

some strains have a positive interference, some a negative one and still others do not show any interference, for linkage group I.

Ferraro (1971) found a positive chiasma interference within chromosome arms between short adjacent intervals, and Hargrave (1971) a negative chiasma interference across the centromere.

In the present work a possible negative chromosome interference across the centromere was seen, while no chromatid interference seemed to be present, both when Rec-s was in a cross.

Conclusions

Rec-s increases recombination frequencies on linkage group I between the centromere and nic-2. The maximum effect is seen between ad-3A and ad-3B, where Rec-s2 is probably located.

It also increases the frequency of pseudo wild types for linkage group I.

Rec-s increases the frequency of pan-2 prototrophs on linkage group VI.

There is a negative chiasma interference and no chromatid interference across the centromere on linkage group I in a cross involving Rec-s.

There is no difference between Rec-s and rec-s strains in ultraviolet sensitivity.

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