RECOMBINATION IN NEUROSPORA

THE LOCATION AND EFFECTS OF FACTORS CONTROLLING RECOMBINATION IN LINKAGE GROUP I OF NEUROSPORA

by

JENNY BARBARA HARGRAVE, B.Sc. (Joint Hons.)

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AUTHOR: Jenny Barbara Hargrave, B.Sc. (Joint Hons.) Wales

SUPERVISOR: Dr. S. F. H. Threlkeld

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It was known, prior to this study, that there is a factor, or factors, in the centromere-proximal region of Neurospora sitophila which affect recombination near the centromere whether it is in Neurospora sitophila or transferred to Neurospora crassa. A location of this factor, or factors, was performed using tetrad and prototroph analysis. Further mapping of linkage group I of Neurospora crassa was undertaken when the Neurospora sitophila centromere-proximal region was present.

PREFACE

This thesis describes studies performed in the Department of Biology, McMaster University from September 1970 to December 1971. Except where others are specifically mentioned, it consists entirely of my own work. No similar thesis has been submitted at any other University.

My sincere thanks are due to Dr. S. F. H. Threlkeld, my supervisor, for his helpful advice and encouragement throughout this work. I would like to thank Mr. Michael Ferraro for many useful discussions and the donation of strains, and I am indebted to Mrs. Laura Bentley and Mrs. Shanta Thomas for their technical assistance.

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Section 1:

INTRODUCTION

The phenomenon of recombination has been known since the early days of genetics, but still remains largely unexplained.

Bridges (1916) using XXY females of *Drosophila ampelophila* demonstrated that crossing over occurred at a four-strand stage of homologous pairing. Since then, data have been collected which indicates there is a strong correlation between chiasma formation and recombination. Mather (1938) combined the data of Stern (1931), Creighton and McClintock (1931), and Brink and Cooper (1935) which, by means of genetically and cytologically marked chromosomes - Stern used *Drosophila*, and Creighton and McClintock, and Brink and Cooper used *Zea mays* - demonstrated that both genetically and cytologically detectable crossing over occurred in the same place. John and Lewis (1965) have combined data from *Cricetus aureus*, *Lilium formosanum*, *Allicum fistutosum*, *Calioptamus palaestinensis*, *Lilium maximowiczii*, *Disporum sessile*, *Acrida lata*, *Scilla scilloides* and *Delphinium ajacis*, and have shown that second division segregation frequencies and first division chiasma frequencies are not significantly different.

Cooper (1949) claimed to have observed chiasmata in spermatocytes of Drosophila melanogaster, in which recombination is known not to occur under normal conditions. However, Slizynski (1964), using improved techniques, demonstrated that what Cooper had observed were merely surface associations of the chromosomes.

Thus it seems reasonable to assume that chiasmata and recombinational events are equivalent.

Various models have been proposed to account for recombination. Belling (1931, 1933) proposed that homologues paired when they were unduplicated, and tended to coil around each other. Chromomeres were laid down first, and then the interconnecting fibres. Hence it is possible that the fibres may connect to the chromomeres of non-sister chromatids.

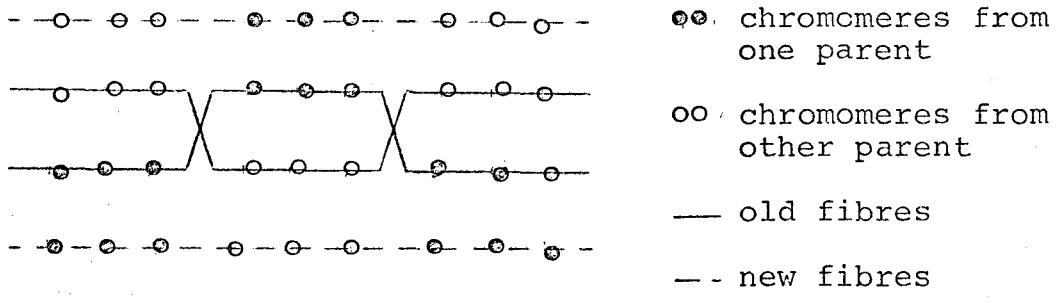


Fig. 1: A double cross over using Belling's model (Modified from Swanson, 1957)

A serious criticism of Belling's model is that only single or two-strand double crossovers will arise.

This difficulty can be overcome if it is postulated that sister-chromatid crossing over occurs, leading to three- or four-strand double crossovers (Swanson, 1957). Evidence from Schwartz (1953) suggests that sister-chromatid exchanges occur at a different time to non-sister chromatid exchanges.

Darlington (1935) proposed that pairing of homologues occurred prior to duplication (as in Belling's model), but were coiled such that the torsion of internal coiling was balanced by the torsion of relational coiling. When homologues were duplicated, the balanced torsion would become unbalanced, leading to breakage of chromatids, and rejoining. If two non-sister chromatids rejoined, a crossover would occur. An objection to this theory is that there is no reason why a break in one chromatid should be accompanied by a break in an identical position in another chromatid (John and Lewis, 1965). However, if it is postulated that breaks only occur in the newly forming chromatids, this difficulty is overcome. But this leads to another objection - that levelled against Belling's model - that only single and two-strand double crossovers will occur.

Essentially, Belling's is a copy-choice model, and the copy-choice theory was expanded and modified

by Lederberg (1955) and Freese (1957). On the basis of this model, it was proposed that replication of the homologues occurred after pairing, and the replicating strands could switch from one homologue to the other causing crossing-over events. Occasionally one homologue would be replicated twice over a particular region, thus giving rise to aberrant genotype ratios.

A serious criticism to all these models so far is that in all cases pairing must occur when homologues are unreplicated. However, it has been demonstrated that replication occurs before pairing, e.g. Taylor (1965) has demonstrated that replication occurs during premeiotic interphase in the grasshopper, and in Lilium longiflorum (Taylor, 1959). Pairing of homologues does not usually occur until leptonema.

Many hypotheses have been proposed to explain recombination on the basis of DNA strands breaking and rejoining (Whitehouse, 1963, 1966, 1967; Hastings and Whitehouse, 1964; Holliday, 1964; Stahl, 1969, later criticized by Holliday and Whitehouse, 1970; and Paszewski, 1970; to name but a few). The general principles involved in these hypotheses are: there is a separation of the DNA strands, which form the double helix, followed by a breakage of two or more of these strands; synthesis of 'new' DNA where the separation has

occurred; removal of the 'old' DNA which has now been replaced by the 'new' DNA; and rejoining of the broken strands. An excision-repair process can then be invoked to explain correction of mis-matched base pairs.

Simchen and Stamberg (1969) have proposed an hypothesis that there are 'coarse' and 'fine' controls of recombination. 'Coarse' controls are those which affect recombination over the whole of the genome of an organism. The magnitude of effect is usually "all-or-none", variations being extremely rare, and usually harmful. 'Coarse' controls have been demonstrated in both prokaryotes, e.g. rec$^-$ (recombination - deficient) strains of Escherichia coli (Clark and Margulies, 1965; Howard-Flanders and Theriot, 1966), and eukaryotes, e.g. asynaptic and desynaptic mutants of barley (reviewed by Riley and Law, 1961) and the c3G mutant of Drosophila melanogaster (Hinton, 1966). 'Fine' controls, on the other hand, affect only small, highly specific regions of the genome. The magnitude of effect of such controls is small, variations being observed as changes in frequency not occurrence, of recombination. So far 'fine' controls have only been demonstrated in eukaryotes, e.g. rec genes in Neurospora crassa (see later in this section for details), and control of frequency in rye (Rees, 1961) and tomato (Griffing and Langridge, 1963).

Schaap (1971) has further extended this hypothesis to include positive and negative correlation of different sites controlled by the same 'fine' control. If two regions are positively correlated, then, as far as recognition sites are concerned there is an unlimited amount of gene products from the 'fine' control to recognize, and each region can thus realize its full potential. (It is suggested that the recognition site is adjacent to the region wherein the frequency of recombination is being controlled, and that it is here that recombination is initiated.) If two regions are negatively correlated, the 'prior' region utilizes what it requires for its recognition site, and the 'subordinate' uses what remains. Thus only the prior region realizes its full recombination potential.

Genotypic control of recombination is known in many organisms. It has been demonstrated that crossing over is under genotypic control in such organisms as rye (Rees and Thompson, 1956; Rees, 1961), wheat (Riley and Chapman, 1958), Hordeum (Gale and Rees, 1970), Drosophila melanogaster (Hinton, 1966) and males of Drosophila ananassae (Hinton, 1970). This last may appear contradictory to an earlier statement to the effect that crossing over does not occur in males of Drosophila melanogaster, but it appears that meiotic crossing over on chromosomes II and III of Drosophila

ananassae is dependent on a dominant enhancer (E) which was mapped on chromosome III R, and on the recessive allele of a dominant suppressor (S) which has been tentatively located on II L.

Further examples of genetic control of recombination come from the recombination genes (rec genes) of Neurospora crassa (Jessop and Catcheside, 1965; Catcheside, 1966; Smith, 1966, 1968; Jha, 1967, 1969; Catcheside and Austin, 1969; 1971; Angel et al., 1970; Smyth, 1971). So far, five distinct rec genes have been located, although seven have been named, see Table 1. It appears that rec-3 and rec-x may be at the same locus (Catcheside and Austin, 1971) and that rec-4 and rec-w may be at the same locus (Jha, 1967; Angel et al., 1970).

Characteristics of rec genes discovered so far are that they appear specific to a particular site, or sites, and that they are not contiguous with the site(s) they affect. For example, rec-1 affects histidine-1 (hist-1), and the order on linkage group V is suggested to be: amination-1, histidine-1, inositol, adenine-7, rec-1, aspartate (Catcheside, 1966). (The uncertainty is in the relative positions of adenine-7 and rec-1). Rec^{+} is dominant to rec, and markedly reduces recombination frequency.

TABLE 1

Recombination genes and the sites at which effective

site(s) at which act. / gene	hist-1	hist-2	hist-3	hist-5	am-1	nit-2	pyr-3 -leu-2
rec-1	+						
rec-2				+			+
rec-3					+		
rec-4			+				
rec-6			on one allele only K504				
rec-w			+				
rec-x		+					
rec-z [1].						+	

[1]. Catcheside (1970)

Rec-1 was found to affect recombination at the hist-1 locus (Jessop and Catcheside, 1965). The presence of rec-1$^+$ gives a decrease in recombination frequency of 15-25 times that when rec-1 is homozygous. An alteration in polarity of recombination within hist-1 has also been attributed to rec-1$^+$ (Jessop and Catcheside, 1965; Whitehouse, 1966; Thomas and Catcheside, 1969). When rec-1 is homozygous, recombination within hist-1 exhibits proximal polarity: when rec-1$^+$ is present hist-1 exhibits distal polarity. In his operation model for recombination, Whitehouse (1966) suggests that an operator model might be diagrammatically represented as follows:

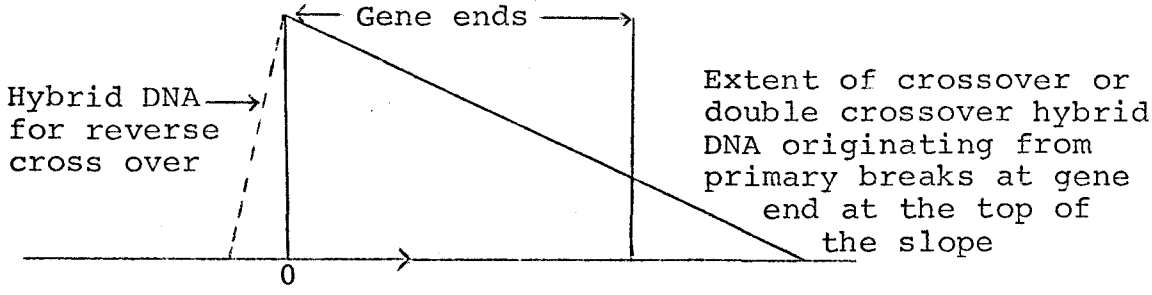


Fig. 2: Diagram to illustrate operator model of crossing over (Whitehouse, 1966)

The situation as regards hist-1 might be explained on the operator model as given in Fig. 3.

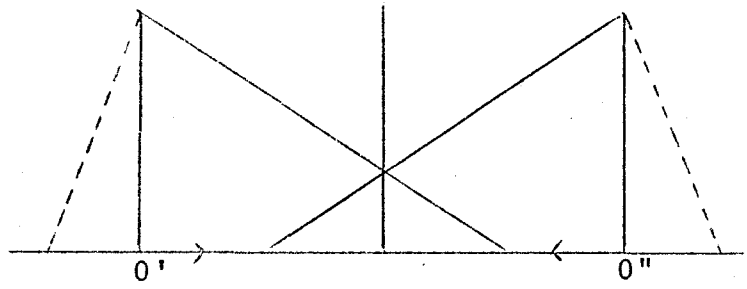


Fig. 3: A possible mechanism to account for the alteration in polarity of hist-1.

It was suggested (Whitehouse, 1966) that if rec-1$^+$ repressed hist-1, then when rec-1$^+$ was present, all polarity would come from the adjacent gene.

The possibility that rec genes are regulatory genes which control transcription at the sites they affect was considered (Catcheside, 1966; Whitehouse, 1966). However, Catcheside (1968) demonstrated that rec-3 (which controls recombination within amination-1 has no effect on the repressibility of the NADP-specific glutamate dehydrogenase specified by amination-1.

A mechanism for the mode of action of rec genes was proposed by Angel et al., (1970). Tightly linked to histidine-3 (hist-3) is a site which affects recombination within hist-3 and without it, since the distance hist-3 - ad-3 (adenine-3) is also affected. This site was denoted recognition (cog), and rec-w is epistatic to cog. They interact in the following way, cog$^+$ being dominant and

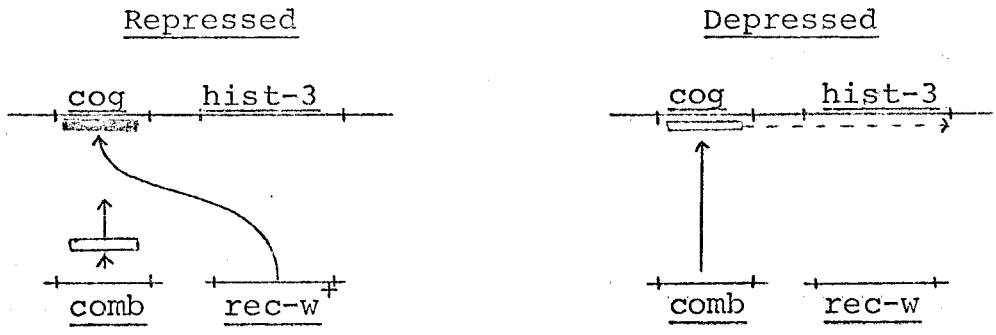
causing an increase in recombination frequency.

	cog$^+$ present	*cog*$^+$ absent
rec-w$^+$ present	low recombination	low recombination
rec-w$^+$ absent	high recombination	low recombination

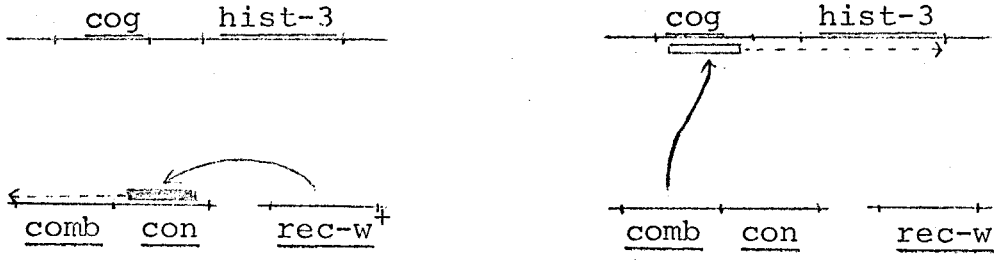
Angel *et al*. (1970) proposed three models as to how this system might work (Fig. 4).

One would expect if 4(a) were the case that *cog* and *cog*$^+$ would respond differently to the presence of *rec-w*$^+$, but this was not observed. If 4(b) were the case, one would expect variations at the *cog* locus to react differently to any given recombinase produced at the *rec-w* locus when *rec-w*$^+$ is absent. This is what was observed. This would also be expected if 4(c) were the case. However, in this case, one would expect to find large numbers of *rec* genes, whereas in the case of 4(b) one would expect to find only limited numbers of *rec* genes. In both these cases, other loci under the control of *rec-w* have to be identified, hence variants would have to be found.

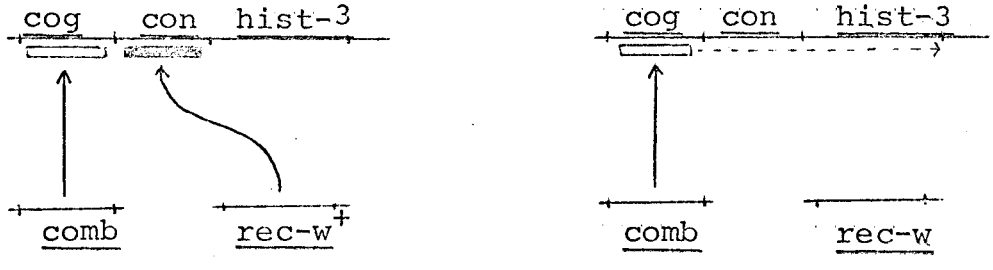
Frost (1961) and Rifaat (1969) obtained evidence that recombination in the centromere-proximal region of linkage group I of *Neurospora crassa* was under genetic control. Strains with different backgrounds exhibited different recombination frequencies. Frost (1961) obtained



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



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



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

Fig. 4: Diagrams to illustrate theories of the mechanism of control of recombination by rec-w genes. (Angel et al. 1970).

cog = recognition
comb = recombinase
rec = recombination
con = control

mt - centromere distances varying from 2.31 to 16.27 m.u.'s (map units). Rifaat (1969) compared his results of map distances in the centromere-proximal region with those of other workers, and found regular intervals of increasing map distances around the centromere, varying only between increases of 3.4 and 5.4 for the markers he observed, see Fig. 5.

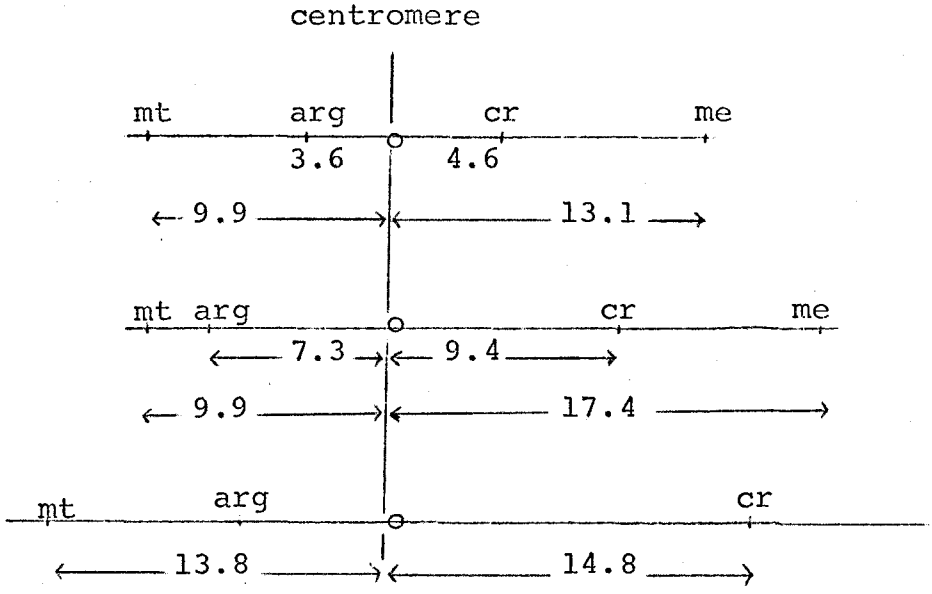


Fig. 5: Maps of centromere-proximal region of linkage group I of Neurospora crassa. Various strains from various sources (Rifaat, 1969).

Rifaat deduced from his work that there are at least two genes, or groups of genes controlling recombination frequency in both arms of linkage group I near the centromere. However, the effect is not as striking as that found by Lincham (1951) and later workers

in the centromere-proximal region of linkage group I.

The work described presently is a continuation of studies commenced by Fincham (1951). He introduced mutants from linkage group I of Neurospora crassa into linkage group I of Neurospora sitophila by means of a back-crossing programme, and compared recombination frequencies in the region pink (pk) to ginger (g). This included pk, mt (mating type), centromere, cr (crisp), w (weak - requires adenine or hypoxanthine), al (albino) and g. He compared the two maps he obtained from N. crassa and N. sitophila.

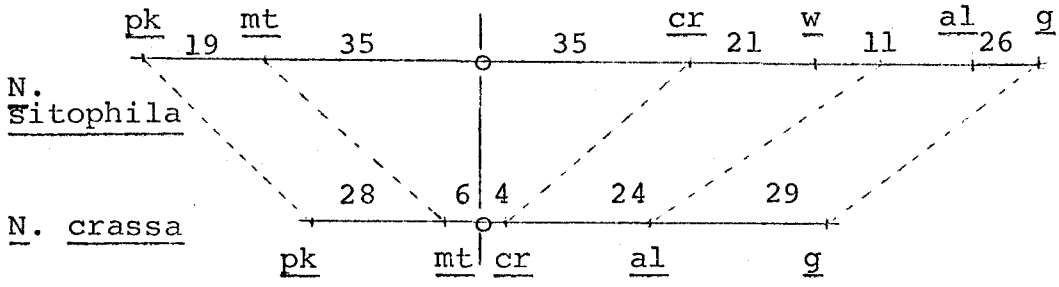


Fig. 6: Comparison of N. sitophila and N. crassa maps of the centromere region of linkage group I. (The pk - mt distances are not significantly different.) (Modified from Fincham, 1951)

Fincham concluded from his work that the most likely cause of the difference in recombination frequencies in this region was an effect of the centromere.

Fincham's data were rather heterogeneous, although this could probably be explained on the basis that his

strains were structurally heterozygous - they were from 3rd, 4th, or 5th backcross generations. Scott-Emuakpor (1965) decided to attempt to confirm Fincham's results and conclusions, and also to extend his observations to other linkage groups. He added two other markers to those Fincham had used, one more proximal than mt, namely arg-3 (arginine-3), and one more distal to cr, me (methionine). His strains were backcrossed more than eight times to give less structural heterozygosity in them. The results he obtained were very similar to those obtained by Fincham, although his map distances were slightly increased right of centromere. An interesting point to arise from his work was that the arg-3 centromere distances were practically identical in both N. crassa and N. sitophila - 6.2 m.u.'s in N. crassa, and 5.7 m.u.'s in N. sitophila. The morphological mutant rg (ragged) has been shown by Threlkeld (1961) to show no second division segregation in either N. crassa or N. sitophila. So it appears that the region immediately proximal to the centromere is insensitive to the factor(s) affecting the other two regions - i.e. left and right of the centromere - or that the centromere itself may be causing interference. This latter suggestion seems possible since work on Drosophila by Beadle (1932) and Graubard (1932) using a homozygous translocation involving chromosomes III and IV

(Beadle) or by means of an inversion (Graubard) indicated it was the position of the genes relative to the centromere which determined this crossing over frequency.

Newcombe (1969) transferred the centromere-proximal region of linkage group I of Neurospora sitophila into Neurospora crassa and examined recombination frequencies in the region mt to cr when the sitophila region was present. He found significant increases in that region over that normally found in N. crassa strains, and very similar to those obtained by Threlkeld (1961) and Scott-Emuakpor (1965).

Ferraro (1971) coined the term rec-s to denote the factor(s) associated with the production of high frequency recombination since it is a recombinational factor derived from Neurospora sitophila. Similarly rec-c denotes the factor(s) associated with recombination derived from Neurospora crassa.

Ferraro extended Newcombe's work, and demonstrated that when rec-s is present, mt - ad-5 (adenine-5), centromere - hist-2 (histidine-2), and hist-2 - cr are significantly increased, but that ad-5 - centromere was not. There was also some indication from the work of Newcombe and Ferraro that when crosses were homozygous for rec-s, there was a significant increase in the mt - ad-5 distance over rec-s x rec-c crosses. No similar

effect was observed right of centromere.

Threlkeld (1961), Scott-Emuakpor (1965) and Ferraro (1971) examined the effect of rec-s on linkage groups II, VI and VII. No significant effect has been found in the centromere-proximal region of any of these linkage groups. However, whether there is an effect on any other linkage group has yet to be determined.

It has this far been ascertained that rec-s is dominant, or partially dominant, to rec-c, and that it is in the centromere-proximal region of linkage group I. Ferraro (1971) demonstrated by means of heterokaryons that if rec-s is a gene (or a group of genes), its product is non-diffusible. This led him to favour the idea that the mode of action of rec-s is structural.

The present work was undertaken to locate more precisely the position of rec-s, and to attempt to elucidate its mode of action.

Section 2:

MATERIALS AND METHODS

(i) General:

Throughout the experimental work performed in this study, aseptic techniques were observed. Workbenches were regularly wiped down with a solution of "Javex" diluted 1:6 parts with tap water. This minimized contamination by bacteria, other fungi, and possibly mites.

When experiments involving procedures which required having tubes or plates of Neurospora exposed for long periods of time were necessary, the work was performed in a small, positive pressure room, which had been previously sterilized for at least 15 minutes with ultra-violet light at bactericidal wavelengths.

Unless otherwise stated, the incubation temperature was 25°C. Stocks and other strains were stored at -18°C until required. This had the dual purpose of halting growth and killing any mites present. Tubes and plates of media were stored at 7°C in polythene bags until required.

(ii) Notation used:

Strains obtained from other workers have retained

the notation used by those workers. Strains derived during the course of this work are labelled as follows:

a) Spores from Asci: Each spore was labelled with the cross number, a dash, the ascus number, a dash, and the spore number. Hence spore 1-11-8 is the eighth spore from the eleventh ascus isolated in cross number 1.

b) Randomly Isolated Spores: Each spore was labelled with C followed by the cross number, a dash, the number of the spore isolated followed by R. Hence C162-461R is the 461st spore randomly isolated from cross 162.

Other nomenclature used is that of Barratt et al. (1954) except in the cases of ad-3 mutants when a distinction is made between ad-3A and ad-3B mutants, and the mating type locus, where mt is used instead of sex.

(iii) Strains used

Number	Genotype	Phenotype	Allele #'s	Comments
ORa-1-3	a	wild type	-	Oak Ridge strain, origin unknown
ORA-1-1	A	wild type	-	Conidial isolate from FGSC #987 after initial work found probably contained a chromosome aberration, so not used in later work.
ORA-5-1	A	wild type	-	Conidial isolate from FGSC #987
PPr8	A, cr	crisp morphology	B123	Obtained from a cross of P369 X P109. A, hist-2, cr x a, ad-5 obtained from S. F. H. Threlkeld
PPr14	A, cr	crisp morphology	B123	As PPr8
2-17-114A	A, ad-3B	requires adenine		Induced in OR74-A by Griffiths using nitrous acid, and back-crossed once to ORa and used as conidial parent.
2-17-114a	a, ad-3B	requires adenine		As 2-17-114A

Number	Genotype	Phenotype	Allele #'s	Comments
C443 R_9	a, hist-2	requires histidine	Y175M611	3rd backcross generation to ORA-1-1 of FGSC #667. Obtained from M. J. Ferraro.
C162 9R	A, hist-2	requires histidine	Y175M611	Obtained from a cross of C443 R_9 X 2-17-114A.
C162 461R	a, hist-2, ad-3B	requires histidine and adenine	Y175M611	As C162 461R
202-1	a, ad-5, hist-2, cr	requires histidine and adenine, and crisp morphology	Y152M40 Y152M14, B123	FGSC #246 backcrossed twice to ORA-1-1 by Newcombe (1969)
C1220 R_{227}	a, hist-3	requires histidine	Y175M614	Oak Ridge background. Obtained from M. J. Ferraro (1971)
C1220 R_{229}	A, hist-3, ad-3A	requires histidine and adenine	Y175M614	As C1220 R_{227}

Number	Genotype	Phenotype	Allele #'s	Comments
C1219 A_{43-7}	A, *ad-3A*, *cr*, *al-2*	albino, crisp morphology, requires adenine	Y175M614, B123, 15300	Oak Ridge background. Obtained from M. J. Ferraro (1971)
C1219 A_{50-8}	A, *ad-3A*, *cr*, *al-2*	As C1219 A_{43-7}	As C1219 A_{43-7}	As C1219 A_{43-7}
C1038 R_{24}	A, *hist-3*	requires histidine	Y175M614	Oak Ridge background. Obtained from M. J. Ferraro (1971)
30JA-5	A, *rec-s*	wild type with high frequency recombination	-	*Neurospora sitophila* centromere region in *Neurospora crassa*, strain derived from a backcrossing programme to FGSC #'s 667 and 668 (Newcombe, 1969)
180-4-7	a, *rec-s*?	wild type with high frequency recombination	-	Obtained from a cross between C1220 R_{229} and 30JA-5

(iv) Media used:

All media used were sterilized by autoclaving at 15 lbs in^{-2} pressure above atomospheric pressure, or 121°C for 15 minutes, except in the case of the sorbose-glucose-fructose medium used for prototroph analysis, which was steamed for 20 minutes immediately after dispensing into tubes, and a further 10 minutes prior to use. Medium containing sorbose tends to burn on autoclaving, and where it was necessary to observe germinating spores, it was found that this could be more easily done in paler coloured medium than in darker coloured medium. The above method ensured the medium was pale enough.

All media used were solid, agar being used as the solidifying agent in 1.5% or 2% concentrations in tubes and in plates respectively.

The following quantities were used per litre of medium:

a) Reproductive Medium

4X salt solution (see below)	250 ml
Sucrose	20 gm
Agar	15 gm or 20 gm
Distilled H_2O	750 ml

4X salt solution (modified from Westergaard and Mitchell, 1947)

KNO_3	4	gm
KH_2PO_4	4	gm
$MgSO_4.7H_2O$	2.0	gm
$CaCl_2.6H_2O$	0.4	gm
NaCl	0.4	gm
Biotin	16	µ g
T.E. solution (see below)	1.0	ml
Distilled water	up to 1000	ml

Trace element (T.E.) solution for 4X salt solution

$NaB_4O_7.10H_2O$	0.01	gm
$CuSO_4.5H_2O$	0.1	gm
$FePO_4.2H_2O$	0.02	gm
$ZnSO_4.7H_2O$	2.0	gm
$Na_2MoO_4.2H_2O$	0.02	gm
Distilled water	up to 250	ml

b) Glucose Medium

50X salt solution (see below)	20	ml
Glucose	20	gm
Agar	15	gm
Distilled water	1	litre

c) Sorbose-glucose-fructose medium

50X salt solution (see below)	20	ml
Sorbose	10	gm
Glucose	0.5	gm
Fructose	0.5	gm

Agar	20	gm
Distilled water	up to 1	litre

50X salt solution (modified from Vogel, 1956)

$Na_3C_6H_5O_7.2H_2O$	124	gm (instead of 150 gm Na_3 citrate 5 1/2 H_2O)
KH_2PO_4 anhydrous	250	gm
NH_4NO_3 anhydrous	100	gm
$MgSO_4.7H_2O$	10	gm
$CaCl_2.2H_2O$	5	gm
T.E. solution (see below)	5	ml
Biotin solution (see below)	2.5	ml
Distilled water	up to 1000	ml
Chloroform (as a preservative)	2	ml

Trace element (T.E.) solution for 50X salt solution

Distilled water	95	ml
Citric acid.H_2O	5.00	gm
$ZnSO_4.7H_2O$	5.00	gm
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1.00	gm
$CuSO_4.5H_2O$	0.25	gm
$MnSO_4.H_2O$	0.05	gm
H_3BO_3 anhydrous	0.05	gm
$Na_2MoO_4.2H_2O$	0.05	gm
Chloroform (as a preservative)	1	ml

Biotin solution

5.0 mg biotin in 50 ml of distilled water

Biochemicals were added to the media where necessary in the following concentrations:

histidine monohydrochloride	50 ppm
adenine	50 ppm

(v) Crossing of Strains

Crosses were set up in large tubes (16X150 mm) containing 5 ml of suitably supplemented reproductive medium, except in the case of crosses used for prototroph analysis which were, for convenience, set up in small tubes (10X75 mm) containing 1 ml of medium.

The following techniques were employed. Quantities for the small tubes which differ from the large tubes are given in parentheses.

The strain to act as the protoperithecial parent was inoculated onto the medium, and incubated for 7 days at 25°C. After this time the conidial parent was added, either in the form of a suspension in sterile distilled water; or dry, and the distilled water added later. The quantity of suspension, or water, used was about 1 ml (4-5 drops from a pasteur pipette). After addition of the liquid, the tube was shaken briefly on a vortex mixer, and the cross was incubated at 25°C until analyzed.

(vi) Isolation of Random Spores

Random spores were isolated in the following manner. A block of sterile 4% agar, about the size of a small postage stamp, was placed on a glass slide, and a drop of sterile distilled water placed on the agar. A sterile loopful of distilled water was touched to the side of the crossing tube where spores had been shed. The surface tension of the water draws sufficient spores into the water film. The spores, still in the film, were then placed in the drop of water on the agar and spread over the agar surface by means of the loop, and allowed to dry until tacky. They were then isolated, under 100X magnification of a dissecting microscope, by touching each one with a tungsten needle sharpened in molten sodium nitrite, and transferring them one at a time to individual small tubes containing 1 ml of appropriately supplemented glucose medium.

Spores were heat-shocked for 45 minutes at 60°C within 18 hr of isolation. A second heat-shock was given 7-10 days after the first heat-shock where germination failed, and a third heat-shock given 7-10 days after the second. After heat-shocking, the spores were incubated for at least 5 days before testing.

(vii) Tetrad Analysis

Tetrad analysis is used to examine all 4 products of meiosis. Neurospora crassa has ordered tetrads, in

the form of 8-spored linear asci, each product of meiosis being represented twice. An ordered tetrad is one in which it is possible to distinguish the position of each meiotic division, and hence how each meiotic product is formed.

To dissect asci, the following procedure was employed. Crosses were left to ripen until about 3 days after spores first appeared on the walls of the tubes. It was found that this was the time when perithecia contained many ripe, intact asci. A perithecium was removed from the cross, and placed in a drop of sterile 10% sucrose solution. The sucrose solution was used rather than water since it was found that asci tended to break open and randomize in the water: when sucrose was used, they usually remained intact until dissected.

Under 35X magnification of a dissecting microscope, any mycelium adhering to the walls of the perithecium was removed with sharpened tungsten needles, and discarded. The perithecium was broken open using the same needles. The rosette of asci isolated from the perithecium was divided into manageable sized portions of about 12 asci, and each portion transferred to drops of 10% sucrose on individual small 4% agar blocks. The clumps of asci were allowed to dry until they become tacky enough, so that when the unattached end of an

ascus was touched with a needle, it would adhere to it, and the entire ascus could be dragged across the agar to a separate position. The asci were then allowed to dry further, before spores were removed in order using extremely sharp needles and 100X magnification. The spores were transferred to individual tubes arranged in rows of 8 in a rack. Note was made of any pale-coloured or aborted spores present.

Racks of asci were stored in polythene bags and maintained at room temperature for 13-15 days to allow the spores to complete maturing, after which time the spores were heat-shocked at 60°C for 45 minutes. Frequent checks were made during the 13-15 days to ensure that there was no growth due to either pre-germination of spores or conidial or mycelial contamination. If such growth was observed, a subculture was made, as much of the aerial growth as possible removed from the original tube, and the spore heat-shocked immediately.

Once heat-shocked, the spores were treated in the same manner as the randomly isolated spores with respect to incubation, further heat-shocking, and testing.

(viii) Testing of Strains

a) Biochemically

Testing for biochemical requirements was performed on sorbose-glucose-fructose plates which had been suitably

supplemented. A wire twist with a sample of the strain to be tested on it was used to inoculate a series of plates. Care was taken to avoid transferring medium from one plate to the next. It was found that 25-30 was the optimum number of strains which could be inoculated on one plate. When complete, the sets of plates were incubated for 2 days, or 3-5 days in the case of crisp strains, at 25°C before scoring.

b) for Mating Type

Mating type tests were performed on plates of unsupplemented reproductive medium inoculated 4-7 days before required with a 'fluffy' mating type tested. 'Fluffies' were used as they are aconidial and so produce no cloud of conidia when the plates are opened for testing. This also means they are difficult to use as the 'conidial' parent. Tests were performed by inoculating the 'fluffy' plate with the strains of unknown mating-types in separate positions with a circular motion. Each strain was tested on 'fluffies' of both mating types to act as a double check. The plates were incubated at 25°C until good production of perithecia was observed. Twenty to twenty-five different strains could be tested per pair of plates using this method.

c) for Female Sterility

Since some problems were encountered with female sterility in some strains, tests were performed in some

cases to determine which strains were female sterile, e.g. on asci from cross 195. The method of Mylyk (1971) was originally employed, using a mixed suspension of female sterile testers as the conidial parent. However some results obtained were inconclusive, and it seemed likely that heterokaryons were being formed between the two different female sterile strains of the same mating type, and these thus being female fertile. This method was modified in the way outlined below.

The strains to be tested were inoculated onto 1 ml of appropriately supplemented reproductive medium, and incubated at 25°C for 8 days. After this time a conidial suspension of a known female sterile strain with the appropriate mating type, either 1-3-a or 18-1-A was added to each tube, the tube briefly vortexed, and incubated for a further 7 days, after which time tubes were scored for perithecial production. After a still further 3 weeks the tubes were scored as to whether they had eventually crossed.

(ix) Prototroph Analysis

In a cross involving two linked markers in the 'trans' position, the recombination frequency between the markers can be determined by prototroph analysis. The frequency of prototrophs formed is half the recombination frequency. This technique was employed in analyzing

strains from cross 195 since this method appeared to be the most economical as regards both materials and time.

Strains from cross 195 which involved cross-over events between hist-2 and ad-3B were selected. The derived hist-2 strains were crossed to a low frequency recombination ad-3B strain, and the derived ad-3B strains crossed to a low frequency recombination hist-2 strain.

A spore suspension was made in 1 ml of 0.1% agar in the crossing tube. 0.1% agar was used in preference to distilled water since it was found that the spores remained in suspension longer due to the greater viscosity of the 0.1% agar. No sedimentation of spores was observed after 45 minutes.

The 1 ml of spore suspension was transferred to a further 4 ml of 0.1% agar, and the resulting 5 ml of spore suspension was vortexed vigorously for 1-2 minutes. The number of spores in a drop of suspension from a pasteur pipette was counted, and an appropriate number of drops to give about 1000 spores was added to a large tube containing 10 ml of molten, unsupplemented, sorbose-glucose-fructose medium which had been maintained at 60°C for at least 30 minutes prior to using. The tube was briefly vortexed, and placed in a water-bath at 60°C for a further 45 minutes to heat-shock the spores, and kill any conidia or mycelium present. After heat-shocking, the

tubes were removed from the water-bath, vortexed briefly, and plates poured, one from each tube. A shake-plate method was employed to spread the spores more evenly in the agar, and to cover the surface of the plate. The plate was rocked not too vigorously 3 times in each direction indicated in fig. 7, in the order indicated by the numbers:

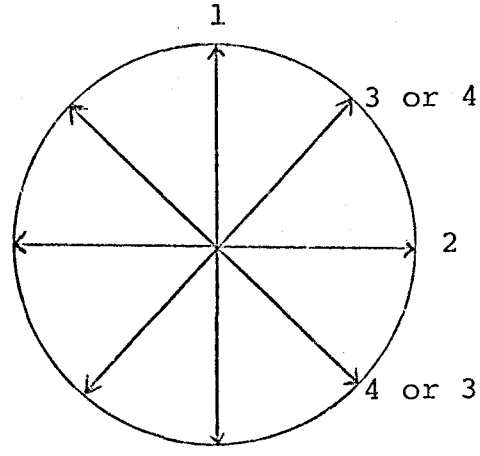


Fig. 7. Shake-plate method employed

Five replicates were made for each strain tested.

The plates were allowed to solidify, and were incubated at 25°C for two days, after which time they were scored for: number of spores plated, number of spores germinated, and number of prototrophs. The number of spores plated and germinated were calculated in the the following way. Ten fields under 35X magnification were scored for the number of spores present and the number of spores germinated.

One field is 6.2 mm diameter (35X magnification). Each plate is 85 mm diameter.

Hence there are $\frac{42.5 \times 42.5 \times \pi}{3.1 \times 3.1 \times \pi}$ fields per plate

= 187.95 fields per plate (assuming constant thickness of agar)

Thus the spore numbers counted in 10 fields, variance < 10%, were multiplied by a factor of 18.8 times to give the total numbers per plate. Hence the prototroph frequency could be calculated.

The above method, although crude in many ways, produced reasonably consistent results. No growth due to conidia or mycelium was observed, which might have obscured results.

(x) Calculations

The distance between two markers when calculated on data from random spores is given by:

$$d = \frac{x \times 100}{n}$$

where x = no. of recombinant spores

n = total no. of spores analyzed.

When dealing with asci, the map distance between two markers was calculated by:

$$\frac{\frac{1}{2} a \times 100}{n}$$

where a = no. recombinant asci

n = total no. asci analyzed.

Where multiple crossovers were involved, twice the number of 2-strand double and the number of 3-strand double crossovers was subtracted from the number of recombinant asci.

$\therefore$ map distance = $d = \frac{\frac{1}{2}(a - 2b - c) \times 100}{n}$ map units (m.u.s)

where b = no. of 2-strand doubles

c = no. of 3-strand doubles.

Occasionally it was not clear how many strands were involved in a crossover event. In these events, e.g. only half an ascus germinating, the number of asci was divided equally between the two possibilities.

Standard error, S.E., is calculated from the formula:

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

n = no. of asci scored or no. random spores scored

map distances are given as $d \pm S.E.$ m.u.s.

When no recombination was observed between two markers in n asci, the upper 95% confidence limit is given by $(1 - \frac{d}{y})^n = 0.05$ where y = distance between two markers within which it is known to lie

(Catcheside and Austin, 1971)

d = distance between the two markers.

Due to poor germination of a particular marker, unequal ratios of recombinant and parental classes may be observed. To overcome this difficulty, a correction factor was employed to adjust the parental class so that it is is line with the other parental class.

$$\text{corrected parental type} = P_1 = \frac{r_1 P_2}{r_2}$$

where P_2 = no. of other parental type

r_2 = recombinant type with P_2 marker under consideration

r_1 = recombinant type with P_1 marker under consideration.

In comparing two crosses, a G test (Sokal and Rohlf, 1969) was used rather than a contingency X^2 since it is more exact being a logarithmic function.

Suppose the following is a contingency table:

Type	Cross C_1	Cross C_2	Cross C_3	Totals
T_1	a_1	b_1	C_1	Σa_1
T_2	a_2	b_2	C_2	Σa_2
T_3	a_3	b_3	C_3	Σa_3
Totals	Σa	Σb	ΣC	$\Sigma a + \Sigma b + \Sigma c = N$

Then $G = 2\ ([a_1 \ln a_1 + b_1 \ln b_1 + c_1 \ln c_1 \ldots\ldots c_3 \ln c_3 + N \ln N] - [\Sigma a \ln \Sigma a + \Sigma b \ln \Sigma b + \Sigma c \ln \Sigma c + \Sigma a_1 \ln \Sigma a_1 + \Sigma a_2 \ln \Sigma a_2 + \Sigma a_3 \ln \Sigma a_3])$.

To compare the means of two samples and to investigate whether two samples are the same, a 't' test was used.

't' is given by the formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma_d}$$

where $\bar{x}_1$ = mean of first sample

$\bar{x}_2$ = mean of second sample

σ_d^2 = variance of the difference of the means.

σ_d^2 may be calculated by:

$$\sigma_d^2 = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}$$

$$\sigma_d = \pm \sqrt{\sigma_d^2}$$

σ_1^2 = variance of first sample

σ_2^2 = variance of second sample

n_1 = number in first sample

n_2 = number in second sample.

The variance of a sample, σ^2, may be calculated from the formula

$$\sigma^2 = \frac{\Sigma(x^2) - \frac{(\Sigma x)^2}{n}}{n}$$

n = no. of observations in the sample

x_1, x_2 etc. are the values of the observations

$\Sigma x = x_1 + x_2 + \ldots\ldots x_n$

$\Sigma(x^2) = x_1^2 + x_2^2 + \ldots\ldots x_n^2$

Section 3:

RESULTS

(i) Location of rec-s.

From work performed by Newcombe (1969) it is known that rec-s lies somewhere in the centromere proximal region of linkage group I. Until the present study, it was unknown whether rec-s was right or left of the centromere, or whether it was the centromere itself that caused the effect attributed to rec-s. Crosses were set up using markers close to the centromere of linkage group I. The markers involved were mt, ad-5, hist-2 and cr. The order of these markers and their positions relative to the centromere are given in Fig. 8.

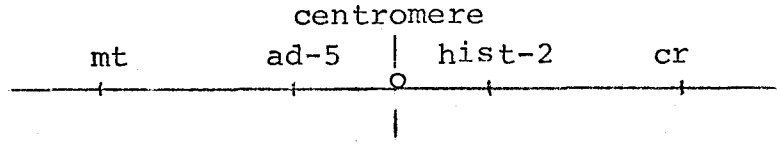


Fig. 8: The relative positions of markers used on linkage group I (not to scale).

A strain of Neurospora crassa (202-1) with these markers, and known to carry rec-c, was crossed to another strain (30JA-5) which was wild type, and known to carry

rec-s. Crossovers between hist-2 and the centromere occur with a low frequency, so if asci are selected which have a crossover in this region, and each spore pair is tested for the presence of rec-s, it should be possible to determine whether rec-s is to the right or the left of the centromere, or if it is the centromere itself.

Cross 1 was set up (Table 2) and two asci which exhibited second division segregation for hist-2 were selected.

TABLE 2

Data from cross 1

30JA-5 x 202-1

A, rec-s a, ad-5, hist-2, cr, rec-c

No. of asci isolated: 102
No. of asci analyzed: 72
Percentage germination[1]: 89.06%

	Crossover classes				
	Singles[2]	Doubles[3]		Triples	Map distance ± S.E.
		2-strand	3-strand		
ad-5 -o-	3	-	-	-	4.2 ± 2.4 m.u.s
-o- hist-2	3	-	-	-	3.5 ± 2.2 m.u.s
hist-2 cr	45	-	-	-	34.0 ± 5.6 m.u.s
ad-5 hist-2	48	-	-	-	6.3 ± 2.9 m.u.s
-o- cr	48	1	-	-	35.4 ± 5.6 m.u.s
ad-5 cr	51	2	1	1 (3-strand)	36.8 ± 5.7 m.u.s

[1]Analyzed asci only

[2]Crossovers from doubles and triples not included

[3]Crossovers from triples not included

As a control cross, cross 2, homozygous for rec-c, was set up (Table 3).

TABLE 3

Results from Cross 2:

Cross 2: ORA-1-1 x 202-1

A, rec-c a, ad-5, hist-2, cr, rec-c

No. of spores isolated: 500
No. of spores germinated: 283
% Germination: 56.60

Crossover class	Genotype	No. of spores
Parentals	+ , + , +	175
	ad-5, hist-2, cr	89
Recombinants	+ , hist-2, cr	2
	ad-5, + +	5
	+ , + , cr	8
	ad-5, hist-2, +	4

Map distances ± S.E.

Region	Uncorrected	Corrected	Data from CCl Newcombe (1969)
ad-5 hist-2	2.5 ± 0.9	2.5 ± 1.0	2.3 ± 0.7 m.u.s
hist-2 cr	4.2 ± 1.2	4.4 ± 1.2	3.8 ± 0.9 m.u.s
ad-5 cr	6.7 ± 1.5	6.9 ± 1.5	4.9 ± 1.0 m.u.s
mt - ad-5	-	-	11.9 ± 1.5 m.u.s

Thus from cross 1, two asci (1-11 and 1-77) were chosen, each of which has a crossover between hist-2 and the centromere. The genotypes of the spores from the asci are as follows, those given in parenthesis being the presumed genotypes of the spores which did not germinate:

1-11-1	A,	ad-5,	hist-2,	cr	1-77-1	A,	+ ,	hist-2, cr
2	(A,	ad-5,	hist-2,	cr)	2	A,	+ ,	hist-2, cr
3	(a,	ad-5,	+ ,	+)	3	a,	+ ,	+ , +
4	a,	ad-5,	+ ,	+	4	a,	+ ,	+ , +
5	a,	+ ,	hist-2,	cr	5	A,	ad-5,	hist-2, cr
6	a,	+ ,	hist-2,	cr	6	A,	ad-5,	hist-2, cr
7	A,	+ ,	+ ,	+	7	(a,	ad-5,	+ , +
8	A,	+ ,	+ ,	+	8	a,	ad-5,	+ , +

Spores which had germinated were crossed to known rec-c strains to ascertain which strains were rec-c and which were rec-s. Crosses set up to determine this are shown in Table 4. The prediction is, of course, that the rec-c x rec-c crosses will show the low crossover frequency, and the rec-c x rec-s crosses will show the high crossover frequency.

Results from the crosses described in Table 4 are summarized in Table 5.

TABLE 4

Test crosses to determine which strains are rec-c and which are rec-s

Cross #	Spore	Genotype	Tester-strain	Genotype
48	1-11-1	A, ad-5, hist-2, cr	ORa-1-3	a, rec-c
153	1-11-4	a, ad-5	PPr14	A, cr, rec-c
22	1-11-6	a, hist-2, cr	ORA-1-1	A, rec-c
53	1-11-7	A	202-1	a, ad-5, hist-2, cr, rec-c
54	1-11-8	A	202-1	a, ad-5, hist-2, cr, rec-c
20	1-77-2	A, hist-2, cr	ORa-1-3	a, rec-c
51	1-77-3	A	202-1	a, ad-5, hist-2, cr, rec-c
52	1-77-4	A	202-1	a, ad-5, hist-2, cr, rec-c
35	1-77-8	a, ad-5	PPr8	A, cr, rec-c

TABLE 5

Map distances from test crosses

Cross #	Spore #	No. spores Analyzed	% Germination	ad-5 hist-2	hist-2 cr	ad-5 cr
48	1-11-1	94	94.00	-	6.4 ± 2.5	-
153*	1-11-4	91	91.00	-	-	32.2 ± 4.3 (corrected)
22	1-11-6	218	72.67	-	8.7 ± 1.9	-
53	1-11-7	97	97.00	3.1 ± 1.8	30.9 ± 4.7	34.0 ± 4.8
54	1-11-8	96	96.00	6.3 ± 2.5	36.5 ± 4.9	40.6 ± 5.0
20	1-77-2	195	65.33	-	3.6 ± 1.7	-
51	1-77-3	92	92.00	4.4 ± 2.1	44.6 ± 5.2	48.9 ± 5.2
52	1-77-4	82	82.00	3.7 ± 2.1	40.2 ± 5.4	43.9 ± 5.5
35	1-77-8	180	60.00	-	-	38.3 ± 3.6

*Also from cross 153: mt - ad-5 16.8 ± 3.5; mt - cr 45.8 ± 4.6 } corrected (see section 2, x)

It can be seen that the following strains are rec-s

1-11-4	1-77-3
1-11-7	1-77-4
1-11-8	1-77-8

Whereas the following are rec-c

1-11-1	1-77-2
1-11-6	

Unfortunately spores 1-77-5 and 1-77-6 died before they were tested, but by inference they were rec-c.

The crossovers involved in the two asci are shown in Figs. 9(a) and 9(b).

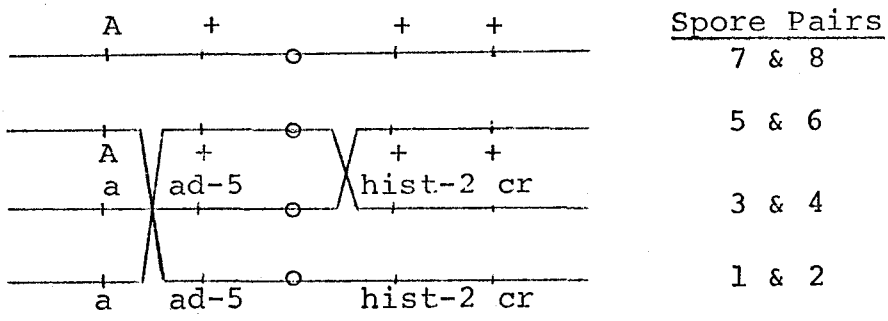


Fig. 9(a): Ascus 1-11 a 3-strand double crossover.

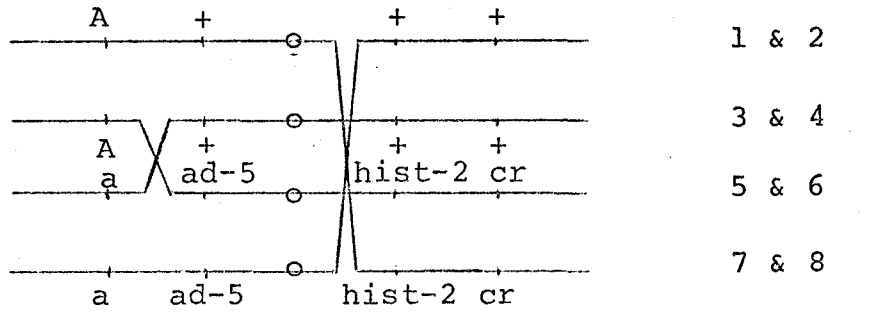


Fig. 9(b): Ascus 1-77 a 4-strand double crossover.

Fig. 9: Diagrammatic representation of crossovers involved in the selected asci (map distances are not to scale).

The common region between spores 7 and 8, and 4 of ascus 1-11 is to the right of the crossover which occurred between hist-2 and the centromere. Similarly, this is the case with spores 1-77-3 and 1-77-4, and 1-77-8. Thus it can be seen that crossing over has separated rec-s from the centromere in these instances, indicating that rec-s can only lie to the right of the centromere.

The previous results gave no indication as to which side of hist-2 rec-s may be located. To ascertain the location of rec-s more precisely, a cross was set up (cross 195) involving a hist-2 ad-3B strain. Ad-3B is much closer to hist-2 than is cr, hence studies on asci involving a crossover between hist-2 and ad-3B should provide more accurate information on the position of rec-s than studies on a cross involving hist-2 and cr.

Cross 195 30JA-5 x C162-461R

A, rec-s a, hist-2, ad-3B, rec-c

The order of the markers is given in Fig. 10.

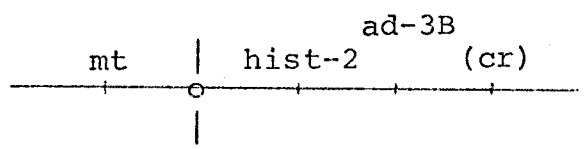


Fig. 10: Order of markers used in cross 195 (not to scale). (cr was not used in this cross, but its position is included for completeness)

Two hundred and four asci were dissected from cross 195, of which 193 were analyzed. Asci which exhibited a crossover between hist-2 and ad-3B were selected and backcrossed to known rec-c tester strains of similar origin to C162-461R.

Thus:

a, hist-2 strains were crossed to 2-17-114A (A, ad-3B)

A, hist-2 strains were crossed to 2-17-114a (a, ad-3B)

a, ad-3B strains were crossed to C162-9R (A, hist-2)

A, ad-3B strains were crossed to C442 R_9 (a, hist-2)

The recombination frequency between hist-2 and ad-3B in the above test crosses was determined by prototroph analysis, the frequency of prototroph formation being half the recombination frequency.

Cross 162, C443 R_9 X 2-17-114A, was used as a control since it is known to be a rec-c x rec-c cross.

Results of the prototroph analysis are given in Table 7.

Prototroph frequencies given in Tables 6a and 6b were further analyzed by expressing them in terms of histograms. Thus the prototroph frequencies from the foregoing tables were plotted as histograms of the number of strains vs. prototroph frequency. The frequencies formed when crossing ad-3B strains derived from cross 195 with known rec-c hist-2 strains was plotted separately from those frequencies formed when crossing hist-2 strains

derived from cross 195 with known rec-c ad-3B strains (Figs. 11 and 12) respectively. When both products of the crossover were present, the prototroph frequencies from the two strains was summed, and a third histogram, Fig. 13,was plotted as the number of pairs of crossover products vs. summed prototroph frequency.

TABLE 6

Recombination data from Cross 195

a) Both meiotic products of the crossover present

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
3-4	A + ad-3B	89.97	1248.32	47.20	3.78	13.15
-5	a hist-2 +	91.31	755.76	70.80	9.37	
5-7	A + ad-3B	87.10	913.68	41.60	4.55	10.40
-1	a hist-2 +		1605.52	94.00	5.85	
9-3	A + ad-3B	94.41	1079.12	40.60	3.76	12.69
-7	A hist-2 +	90.91	1081.00	93.25	8.63	
13-7	A + ad-3B	73.28	319.60	15.80	4.94	13.68
-3	a hist-2 +	89.63	1105.44	96.60	8.74	
14-1	A + ad-3B	85.89	778.32	27.00	3.47	11.41
-7	a hist-2 +	85.62	962.56	76.40	7.94	
18-6	A + ad-3B	90.14	1203.20	30.50	2.53	10.09
-1	a hist-2 +	79.00	650.48	49.20	7.56	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
22-7	A + ad-3B	92.96	992.64	102.00	10.28	11.12
-4	A hist-2 +	87.01	830.96	7.00	0.84	
26-7	A + ad-3B	94.35	1116.72	60.40	5.41	11.15
-3	a hist-2 +	93.07	706.88	40.60	5.74	
28-7	A + ad-3B		616.64	79.40	12.88	13.52
-3	A hist-2 +	92.26	1030.24	6.60	0.84	
33-5	A + ad-3B	90.44	924.96	8.80	0.95	14.26
-1	a hist-2 +	93.25	883.60	117.60	13.31	
35-1	A + ad-3B		259.44	10.40	4.01	13.41
-7	a hist-2 +	89.59	906.16	85.20	9.40	
37-1	A + ad-3B		379.76	18.80	4.95	14.92
-6	a hist-2 +	81.10	774.56	77.20	9.97	
39-3	a + ad-3B	93.47	861.04	56.40	6.55	15.20
-5	a hist-2 +	84.25	804.64	69.60	8.65	
46-7	A + ad-3B	81.73	605.36	66.20	10.94	11.76
-1	a hist-2 +	91.81	970.08	8.00	0.82	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
49-1	A + ad-3B	93.31	1049.04	46.20	4.40	13.32
-7	a hist-2 +		477.52	42.60	8.92	
52-5	A + ad-3B	96.40	1206.96	133.20	11.04	12.32
-7	A hist-2 +	93.46	1342.32	17.20	1.28	
53-7	A + ad-3B	94.69	752.00	99.40	13.22	14.02
-4	A hist-2 +	94.52	778.32	6.20	0.80	
54-3	A + ad-3B	94.44	447.44	19.40	4.34	11.15
-7	a hist-2 +	90.43	1030.24	70.20	6.81	
57-1	A + ad-3B	93.16	819.68	33.20	4.05	12.66
-7	a hist-2 +	94.91	770.80	66.40	8.61	
58-3	A + ad-3B		1391.20	71.40	5.13	14.13
-5	a hist-2 +	86.28	733.20	66.00	9.00	
62-4	A + ad-3B		1146.80	150.00	13.08	13.91
-5	A hist-2 +	92.91	887.36	7.40	0.83	
69-7	A + ad-3B	96.76	1237.04	55.40	4.48	11.97
-1	a hist-2 +	99.18	913.68	68.40	7.49	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
72-5[1]	A + ad-3B	93.10	380.70	10.75	2.82	10.06
-3	a hist-2 +	93.49	742.60	53.75	7.24	
73-1	A + ad-3B	84.04	1233.28	141.20	11.45	12.41
-5	a hist-2 +	91.07	958.80	9.20	0.96	
75-3	A + ad-3B	96.19	853.52	87.60	10.26	11.09
-7	A hist-2 +	91.31	566.48	4.69	0.83	
76-5	A + ad-3B	87.84	488.80	10.80	2.21	16.07
-3	a hist-2 +		533.92	74.00	13.86	
78-7	A + ad-3B	86.39	1718.32	159.00	9.25	10.33
-1	a hist-2 +	85.02	432.63	4.67	1.08	
80-3	A + ad-3B	89.57	774.56	31.20	4.03	11.42
-5	a hist-2 +		725.68	53.60	7.39	
81-3	A + ad-3B	97.14	1022.72	37.90	3.71	12.50
-7	a hist-2 +		691.84	60.80	8.79	
85-3	A + ad-3B		861.04	38.00	4.41	13.98
-5[1]	A hist-2 +	90.87	888.30	85.00	9.57	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
90-7	a + ad-3B	96.82	1030.24	114.20	11.08	12.27
-3	A hist-2 +	94.58	721.92	8.60	1.19	
96-7	A + ad-3B	87.59	876.08	120.60	13.77	14.66
-1	a hist-2 +	88.42	947.52	8.40	0.89	
98-1	A + ad-3B		1628.08	26.00	1.60	13.92
-5	a hist-2 +		594.05	73.20	12.32	
100-3	A + ad-3B	85.93	436.16	23.00	5.27	12.11
-5	a hist-2 +		763.28	52.20	6.84	
102-1	A + ad-3B	73.51	1097.96	32.80	2.99	14.06
-5	a hist-2 +	87.50	605.36	67.00	11.07	
105-5	A + ad-3B	91.24	744.48	81.8	10.99	12.27
-1	a hist-2 +	94.74	812.16	10.4	1.28	
109-3	A + ad-3B		1488.96	66.60	4.47	10.77
-7	a hist-2 +	92.83	876.08	55.20	6.30	
116-5	A + ad-3B	92.43	642.96	25.20	3.92	11.92
-4	a hist-2 +	92.15	1015.20	81.20	8.00	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
118-7	A + ad-3B	94.66	1000.16	102.60	10.26	10.94
-3	a hist-2 +	93.52	1030.24	7.00	0.68	
120-1^1_1	a + ad-3B	93.85	789.60	35.00	4.43	13.60
-6^1_1	A hist-2 +	90.95	850.70	78.00	9.17	
121-2	A + ad-3B		1259.60	46.60	3.70	15.64
-5	a hist-2 +	91.49	646.72	77.20	11.94	
130-1	A + ad-3B	85.06	834.72	24.80	2.97	13.01
-3	a hist-2 +		1090.04	109.40	10.04	
134-1	A + ad-3B	82.33	770.80	36.80	4.77	12.64
-5	A hist-2 +	93.54	924.96	72.80	7.84	
135-1	A + ad-3B	74.27	1052.80	47.60	4.52	12.11
-7	A hist-2 +	92.64	1515.28	115.00	7.59	
152-5	A + ad-3B		1391.20	56.40	4.05	12.76
-3	A hist-2 +	90.12	883.60	77.00	8.71	
155-5	A + ad-3B	82.84	1252.08	129.00	10.26	11.43
-3	A hist-2 +	90.65	592.20	7.50	1.27	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
161-5	a + ad-3B	90.96	1210.72	57.00	4.71	12.11
-1	A hist-2 +	94.30	808.40	59.80	7.40	
166-5	A + ad-3B	95.34	691.84	39.00	5.64	12.91
-1	a hist-2 +		533.92	38.80	7.27	
170-1[1]	A + ad-3B	94.29	744.48	39.40	5.29	11.53
-5[1]	a hist-2 +	89.32	864.80	54.00	6.24	
175-3	a + ad-3B	95.24	1128.00	14.80	1.31	13.36
-7	a hist-2 +	82.18	624.16	75.20	12.05	
177-1	A + ad-3B	94.42	1398.72	44.60	3.19	13.76
-5	a hist-2 +		556.48	58.80	10.57	
179-5	A + ad-3B	98.85	1613.04	155.20	9.62	10.53
-3	a hist-2 +	97.76	985.12	9.00	0.91	
180-7	A + ad-3B	97.22	1052.80	47.80	4.54	10.84
-2	a hist-2 +	92.98	797.12	50.20	6.30	
181-5	A + ad-3B	79.39	1173.12	23.80	2.03	12.41
-1	a hist-2 +	75.09	770.80	80.00	10.38	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
182-4	A + ad-3B	90.15	1135.52	131.80	11.61	12.65
-5	a hist-2 +	97.48	1018.96	10.60	1.04	
183-1	A + ad-3B	95.05	650.48	10.00	1.54	12.16
-7	a hist-2 +	90.24	1007.68	107.00	10.62	
188-1	A + ad-3B	82.13	838.48	28.60	3.42	14.10
-5	a hist-2 +	88.00	909.92	97.20	10.68	
191-7	A + ad-3B	80.77	1184.40	9.20	0.78	11.32
-3	a hist-2 +	87.71	972.90	102.50	10.54	
194-7	A + ad-3B	85.80	1067.84	15.60	1.46	11.59
-3	a hist-2 +		748.24	75.80	10.13	
195-5	A + ad-3B	87.50	1210.72	60.80	5.02	11.68
-3	a hist-2 +	93.75	733.20	48.80	6.66	
197-6	A + ad-3B	97.90	1575.44	155.20	9.85	10.27
-2	a hist-2 +	93.48	808.40	3.40	0.42	
200-3	A + ad-3B	81.62	1252.08	78.60	6.28	11.95
-5	a hist-2 +	85.71	571.52	32.40	5.67	

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs	Total % prototrophs
202-3	A + ad-3B	97.28	1342.32	68.00	5.07	11.71
-6	a hist-2 +	92.64	804.64	53.40	6.64	

b) One meiotic product of the crossover present

44-4	A + ad-3B	94.48	1094.16	47.40	4.33
61-3	a + ad-3B	94.88	906.16	50.00	5.52
89-7[1]	A + ad-3B	93.75	564.00	26.50	4.70
92-3	A + ad-3B		1985.28	99.00	4.99
160-8	A + ad-3B	75.23	913.68	29.00	3.18

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs
163-5	a + ad-3B	93.75	1240.80	19.40	1.56
176-7	A + ad-3B	79.49	1064.08	37.60	3.53
1-7	a hist-2 +	83.72	1605.50	170.00	10.59
6-1	A hist-2 +	85.20	714.40	76.40	10.69
32-3	A hist-2 +	93.42	1067.84	75.00	7.02
50-3	a hist-2 +	91.02	876.08	7.60	0.87
64-7[1]	A hist-2 +	84.95	371.30	2.25	0.61
65-5[1]	A hist-2 +	92.66	770.80	79.75	10.35
74-5	A hist-2 +	95.87	785.84	83.40	10.61
93-1[1]	a hist-2 +	92.13	385.40	1.50	0.39
112-3	A hist-2 +	85.69	564.00	59.00	10.46
150-5	A hist-2 +	95.32	774.56	68.40	8.83

Ascus & spore #'s	Genotype	% Germination	Ave. no. germinated/ plate	Ave. no. prototrophs/ plate	Ave. % prototrophs
151-5	a hist-2 +		774.56	63.80	8.35
153-3	A hist-2 +	95.29	1293.44	9.20	0.71
169-7	a hist-2 +		703.12	58.00	8.25
187-1	a hist-2 +	94.13	315.84	18.20	5.76
192-1	a hist-2 +	97.13	763.28	58.20	7.62

Control: % Germination: 91.68
Average # germinated/plate: 1214.48
Average # prototrophs/plate: 7.00
Average % prototrophs: 0.58

[1]On 4 plates only.

Fig. 11: Frequency of prototroph formation when ad-3B strains derived from cross 195 are crossed to rec-c hist-2 strains. (Parameters as in Fig. 12)

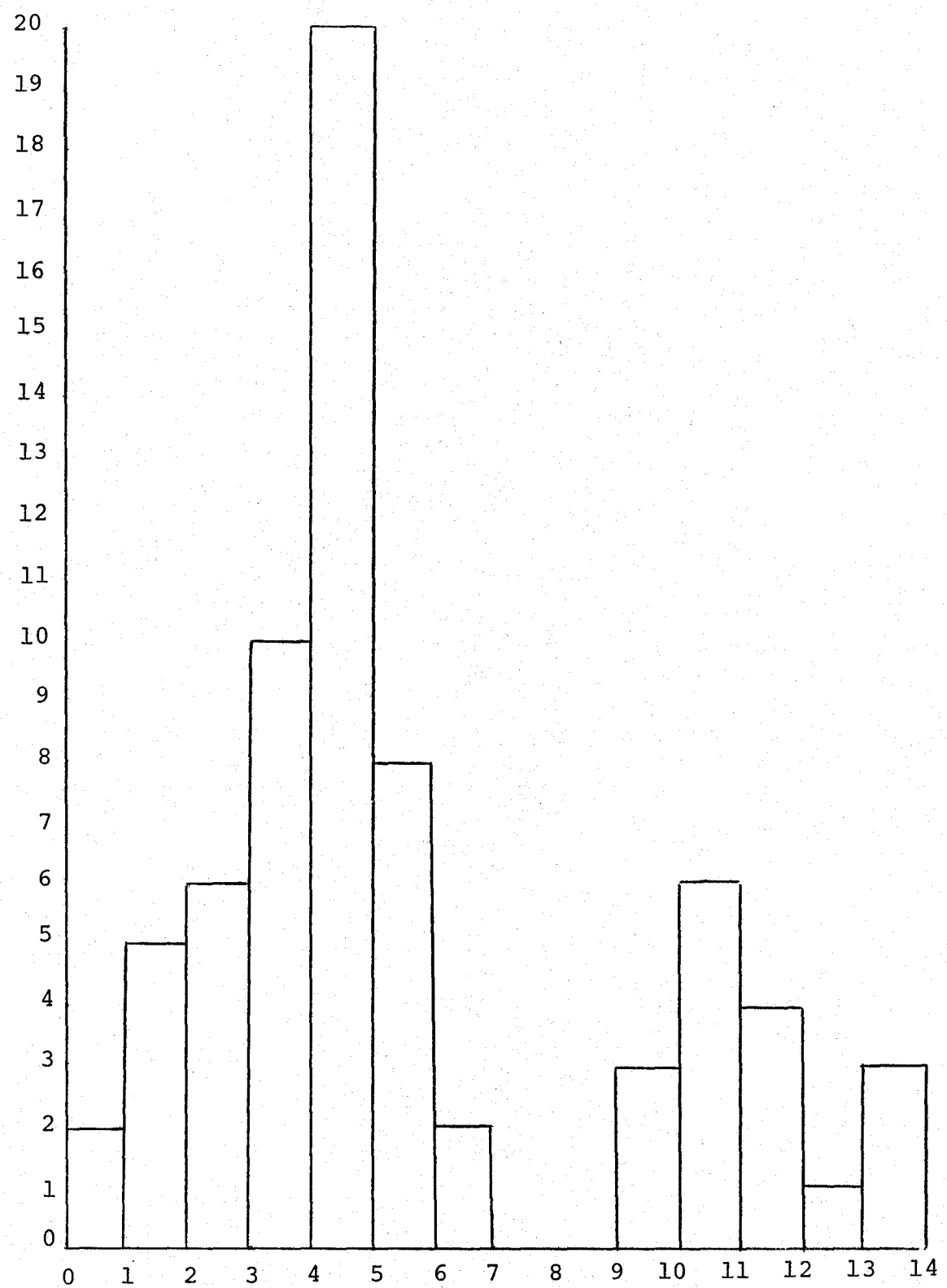

20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 11

Fig. 12: Frequency of prototroph formation when hist-2 strains derived from cross 195 are crossed to rec-c ad-3B strains.

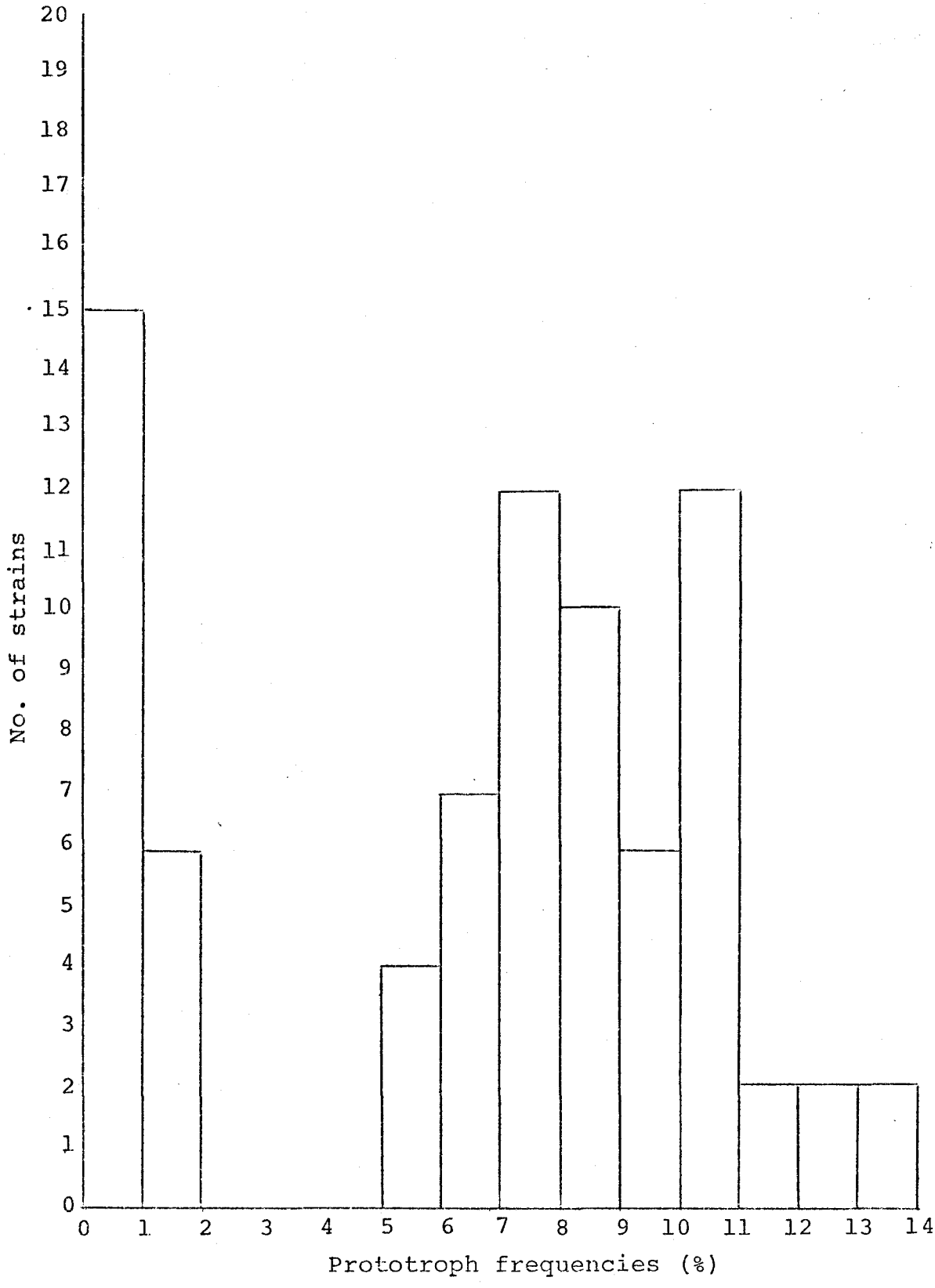
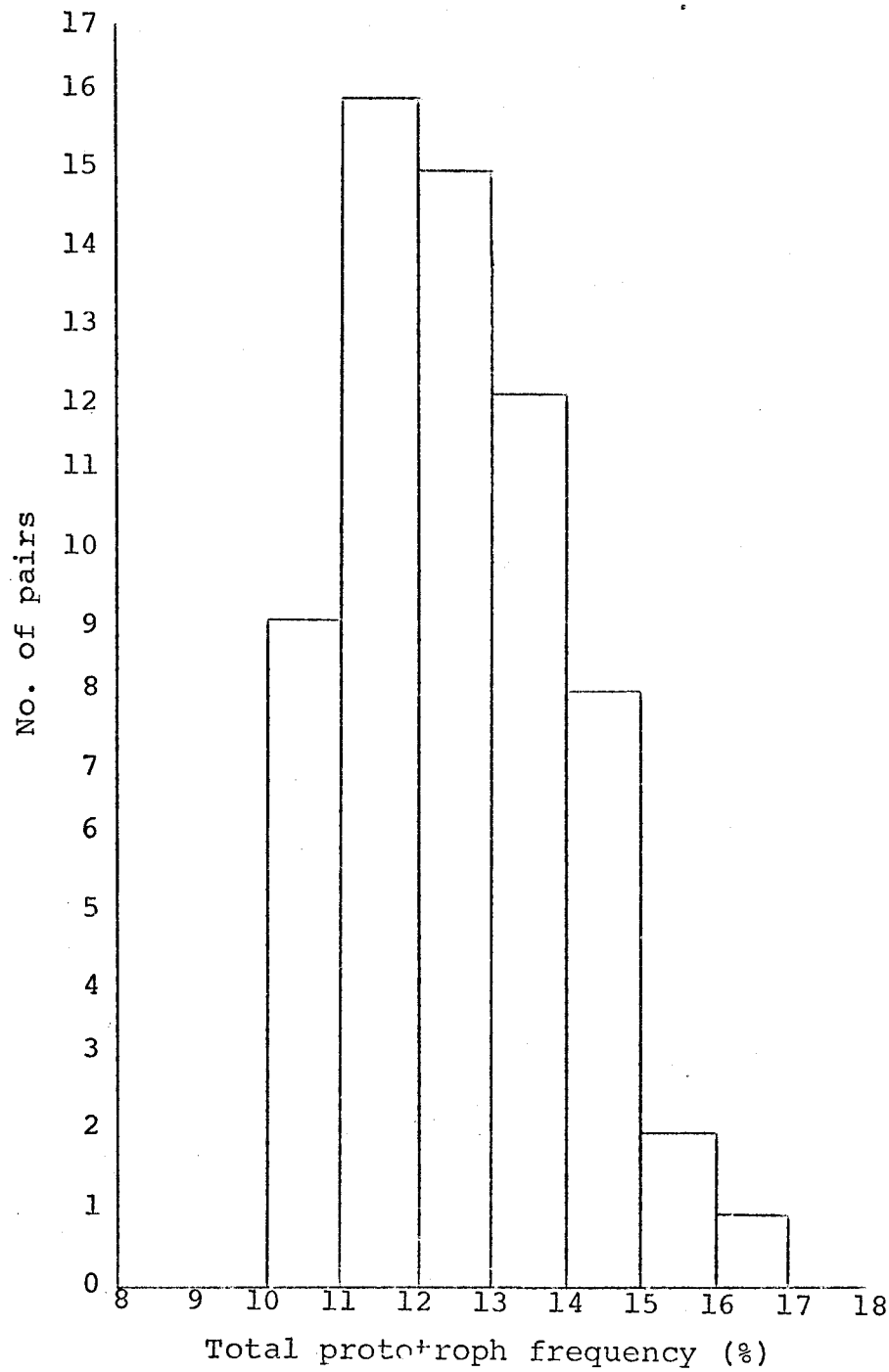


Fig. 13: Summed prototroph frequency formed when both products of the crossover between hist-2 and ad-3B are present, and back-crossed to known rec-c strains.



From the histogram of Fig. 13, it can be seen that a single normal distribution is formed. (Variance = 1.96, standard deviation = ± 1.40.) This is a strong indication that the prototroph frequencies formed from the meiotic products of the crossovers between hist-2 and ad-3B are additive.

The histograms shown in Figs. 11 and 12 obviously do not indicate a single normal distribution of prototroph frequencies in either case.

For the ad-3B strains: variance = 11.34, standard deviation = ± 3.37

For the hist-2 strains: variance = 15.29, standard deviation = ± 3.91.

It can be seen from the shapes of the two histograms (Figs. 11 and 12) that ad-3B strains and hist-2 strains fall into two groups. There is a clear gap between each group, this falling between 7% and 9% prototroph frequency for the ad-3B strains, and between 2% and 5% for the hist-2 strains. Each portion of each histogram under consideration exhibits a normal distribution, except for the higher hist-2 group which has a fairly high variance (3.77).

The divisions of the groups are given in Table 7.

Two types of comparisons were made between these groups, where relevant, (i) within genotypes and (ii) between genotypes. Details of these comparisons are

TABLE 7

Division of groups of strains

Group	Prototroph frequencies	Variance
low *ad-3B*	0-7%	1.84
high *ad-3B*	9-14%	1.14
low *hist-2*	0-2%	0.96
high *hist-2*	5-14%	3.77
medium *hist-2*[1]	5-9%	1.01
high *hist-2*[1]	9-14%	1.44

[1]On the basis of variance and the appearance of the histogram given in Fig. 12, the high *hist-2* group was subdivided into two groups.

shown in Table 8.

TABLE 8

't' - test to determine if the means of the histograms (Figs. 11 and 12) are significantly different

(a) Within genotypes

Group	Variance (σ^2)	't'	d.f.	P.
high ad-3B low ad-3B	1.14 1.84	22.94	68	<< 0.001
low hist-2 medium hist-2	0.96 1.01	23.59	52	<< 0.001
low hist-2 high hist-2	0.96 1.44	30.00	43	<< 0.001
medium hist-2 high hist-2	1.01 1.44	10.76	55	< 0.001

(b) Between genotypes

low ad-3B low hist-2	1.84 0.96	10.68	72	< 0.001
low ad-3B medium hist-2	1.84 1.01	13.74	84	<< 0.001
high ad-3B medium hist-2	1.14 1.09	12.09	48	< 0.001
high ad-3B high hist-2	1.14 1.44	1.34	39	> 0.1 not significant

All groups except the high hist-2 and high ad-3B groups show highly significantly different means as is demonstrated by the 't' tests given in Table 9. Since the low ad-3B group has a significantly different mean from the low hist-2 group, there is a strong suggestion that there are two different groups of prototroph frequency, one with a prototroph frequency of 0-2%, and with a frequency of 2-7%. Similarly, the other significant differences between the means suggest in total that there are four groups:

0-2%
2-7%
5-9%
9-14%

Summary of results of section 3(i)

(a) Rec-s has been located to the right of the centromere. The conclusion was drawn from cross 1 where second division segregation for hist-2 also produced second division segregation for rec-s in the two asci analyzed.

(b) It is possible to divide rec-s into more than one factor. Variable recombination frequencies are found when there is a crossover event between hist-2 and ad-3B, suggesting that components of rec-s may be separated by crossing over.

(c) As a corollary to (a) and (b), it may be deduced that rec-s lies completely or partially between

hist-2 and ad-3B.

(ii) Further mapping of Linkage Group I when rec-s is present

Although it was known before this present study that rec-s has a considerable effect on recombination frequencies between hist-2 and cr, increasing the recombination between them by eight-fold, it was not known over which region rec-s has the greatest effect. Location of very high increases in recombination frequency may be an indication of the location of rec-s or its components.

The following markers were used: mt, hist-2, hist-3, ad-3A, ad-3B, cr, al-2. Although al-2 does not lie between hist-2 and cr, it was convenient to use strains involving this locus. The gene order of the markers is given in Fig. 14, and a list of the crosses analyzed is given in Table 9.

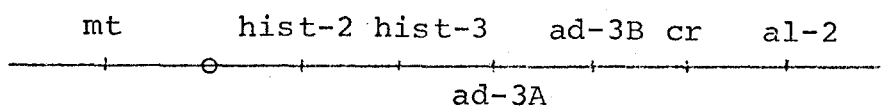


Fig. 14: Order of markers used for mapping of linkage group I when rec-s is present (not to scale).

TABLE 9

Crosses analyzed during mapping studies

Cross #	Protoperithecial Parent	Conidial Parent	No. of asci analyzed	No. of random spores analyzed
162	C443 R_9 a, *hist-2*, *rec-c*	2-17-114A a, *ad-3B*, *rec-c*	-	428
180	30JA-5 A, *rec-s*	C1220 R_{229} a, *ad-3A*, *rec-c*	195	-
195	30JA-5 A, *rec-s*	C162-461R a, *hist-2*, *ad-3B*, *rec-c*	193	-
197	ORA-5-1 A, *rec-c*	C1219 A_{43-7} a, *ad-3A*, *cr*, *al-2*, *rec-c*	-	175
198	30JA-5 A, *rec-s*	C1219 A_{43-7} a, *ad-3A*, *cr*, *al-2*, *rec-c*	-	287
204	180-4-7 a, *rec-s*(?)	C1220 R_{227} A, *hist-3*, *ad-3A*, *rec-c*	196	-
276	C1038,R_{24} A, *hist-3*, *rec-c*	C1219 A_{50-8} a, *ad-3A*, *cr*, *al-2*, *rec-c*	96	-

Data from these crosses are summarized in Tables 10-19.

TABLE 10

Data from cross 162

C443 R_9 x 2-17-114A

a, hist-2, rec-c A, ad-3B, rec-c

No. of spores isolated = 520
No. of spores germinated = 428
% Germination = 82.31%

Crossover Class	Genotype	No. of Spores
Parentals	hist-2 +	211
	+ ad-3B	211
Recombinants	+ +	3
	hist-2 ad-3B	3

Map distance between hist-2 and ad-3B = 1.4 ± 0.6 m.u.s

By comparison of cross 162 (Table 10) with cross 195 (Table 11), it can be seen that there is an increase of about sixteen fold in the distance hist-2 - ad-3B of rec-s x rec-c crosses over rec-s x rec-c cross. Cross 195 which is rec-s x rec-c gives a distance of 22.8 ± 3.0 m.u.s compared with 1.4 ± 0.6 m.u.s in cross 162 which is rec-c x rec-c.

TABLE 11

Results from cross 195

30JA-5 x C162-461R

A, rec-s a, hist-2, ad-3B, rec-c

No. of asci isolated = 204
No. of asci scored = 193
% Germination[1] = 89.77

Region	Crossover classes						Map distance ± S.E.
	Singles[2]	Doubles[3]			Triples		
		2-strand	3-strand	4-strand	2-strand	3-strand	4-strand	
mt -o-	51	-	-	-	-	-	-	24.1 ± 3.1 m.u.s
-o- hist-2	5	-	-	-	-	-	-	3.1 ± 1.3 m.u.s
hist-2 ad-3B	44	-	-	-	-	-	-	22.8 ± 3.0 m.u.s
mt hist-2	56	1	-	1	-	-	-	25.1 ± 3.1 m.u.s
-o- ad-3B	49	1.5	-	0.5	-	-	-	24.0 ± 3.1 m.u.s
mt ad-3B	100	10.5	21	9.5	1	1	1	37.1 ± 3.5 m.u.s

[1,2,3]For footnotes see Table 2.

TABLE 12

Data from cross 180

30JA-5 x C1220 R229

A, rec-s a, ad-3A, rec-c

No. of asci isolated = 201
No. of asci scored = 195
% Germination[1] = 86.73

Region	Crossover classes				Map distance ± S.E.
	Singles[2]	Doubles			
		2-strand	3-strand	4-strand	
mt -o-	62	-	-	-	23.6 ± 3.0
-o- ad-3A	47	-	-	-	12.1 ± 1.1
mt ad-3A	109	6	14.5	9.5	36.5 ± 3.5

[1,2]For footnotes see Table 2.

TABLE 13

Data from cross 204

180-4-7 x C1220 R227

a, rec-s(?) A, hist-3, ad-3A, rec-c

No. of asci isolated = 208
No. of asci scored = 196
% Germination[1] = 84.18
(mt was not scored in this cross)

Region	Singles[2]	Crossover classes: Doubles			Map distance ± S.E.
		2-strand	3-strand	4-strand	
-o- hist-3	43	-	-	-	11.2 ± 2.3
hist-3 ad-3A	10	-	-	-	2.8 ± 1.2
-o- ad-3A	53	-	1	-	13.8 ± 2.5

[1,2]For footnotes see Table 2.

TABLE 14

Data from cross 276

C1038 R_{24} x C1219 A_{50-8}

A, hist-3, rec-c a, ad-3A, cr, al-2, rec-c

No. of asci isolated = 98
No. of asci scored = 96
% Germination[1] = 92.58

Regions	Crossover classes				Map distance ± S.E. m.u.s
	Singles[2]	Doubles			
		2-strand	3-strand	4-strand	
-o- hist-3	3	-	-	-	1.6 ± 1.3
hist-3 ad-3A	-	-	-	-	< 0.08
ad-3A cr	-	-	-	-	1.0 ± 1.0
cr al-2	51	-	-	3	30.7 ± 4.7
-o- ad-3A	3	-	-	-	1.6 ± 1.3
hist-3 cr	-	-	-	-	1.0 ± 1.0
ad-3A al-2	51	0.5	1	0.5	30.2 ± 4.7
-o- cr	3	-	-	-	2.6 ± 1.6
hist-3 al-2	51	0.5	1	3.5	30.2 ± 4.7
-o- al-2	54	1	1	3.5	31.8 ± 4.8

[1,2]For footnotes see Table 2.

TABLE 15

Comparison of crosses 204 and 276 in the -o- ad-3A region

	Cross 276	Cross 204	Totals
Non-crossovers	93	142	235
-o- hist-3	3	43	46
hist-2 ad-3A	0	10	10
Doubles	0	1	1
Totals	96	196	292

G = 52.106 d.f. = 3 P < 0.001

Table 15 indicates that there is a highly significant difference between a rec-c x rec-c cross (cross 276) and a rec-s x rec-c cross (cross 204) in the region between the centromere and ad-3A.

From Table 18 it can be seen that there is a significant difference between a rec-c x rec-c cross (cross 197) and a rec-s x rec-c cross (cross 198). So it can be deduced that the effect of rec-s extends beyond ad-3A at least as far as to cr. Whether it extends beyond cr is uncertain since it is doubtful whether the increase in map distance between cr and al-2 is significant.

TABLE 16

Data from cross 197

ORA-5-1 x C1219 A_{43-7}

A, rec-c a, ad-3A, cr, al-2, rec-c

No. of spores isolated = 208
No. of spores scored = 175
% Germination = 84.13

Crossover class	Genotype	No. of Spores
Parentals	ad-3A, cr, al-2	49
	+ + +	72
Recombinants	ad-3A, cr, +	27
	+ + al-2	22
	ad-3A, + +	2
	+ cr al-2	2
	ad-3A + al-2	0
	+ cr +	1

Map distances:

Region	Uncorrected	Corrected
ad-3A cr	2.9 ± 1.2	2.7 ± 0.4
cr al-2	28.6 ± 3.4	28.8 ± 3.2
ad-3A al-2	30.3 ± 3.5	31.6 ± 3.4

TABLE 17

Data from cross 198

30JA-5 x C1219 A_{47-3}

A, rec-s a, ad-3A, cr, al-2, rec-c

No. of spores isolated = 312
No. of spores germinated = 287
% Germination = 91.99

Crossover class	Genotype	No. of Spores
Parentals	ad-3A, cr, al-2 / + + +	80
		82
Recombinants	ad-3A, cr, + / + + al-2	40
		43
	ad-3A + + / + cr al-2	14
		14
	ad-3A + al-2 / + cr +	7
		7

Map distances: ad-3A cr = 14.6 ± 2.1

cr al-2 = 33.8 ± 2.8

ad-3A al-2 = 38.7 ± 2.9

TABLE 18

Comparison of crosses 197 and 198

	Cross 197 (corrected)	Cross 198	Totals
Non-crossover	129	162	291
ad-3 cr	4	28	32
cr al-2	49	86	135
ad-3 al-2 doubles	1	14	15
Totals	183	290	473

G = 23.302 d.f. = 3 P < 0.001

This distance is 33.8 in the *rec-s* x *rec-c* cross and 29.8 in the *rec-c* x *rec-c* cross.

Comparisons of map distances have been tabulated, and presented in Table 19. Some results have been derived from section 3(i) and a few have come from other sources (see note at bottom of Table 19).

It can be seen from Table 19 that, to the left of the centromere, the frequency of recombination between *ad-5* and the centromere is unaffected (4.2 and 6.7 m.u.s from *rec-s* x *rec-c* crosses respectively do not appear to be significantly different), and the frequency of

TABLE 19

Comparison of map distances in the presence and absence of rec-s

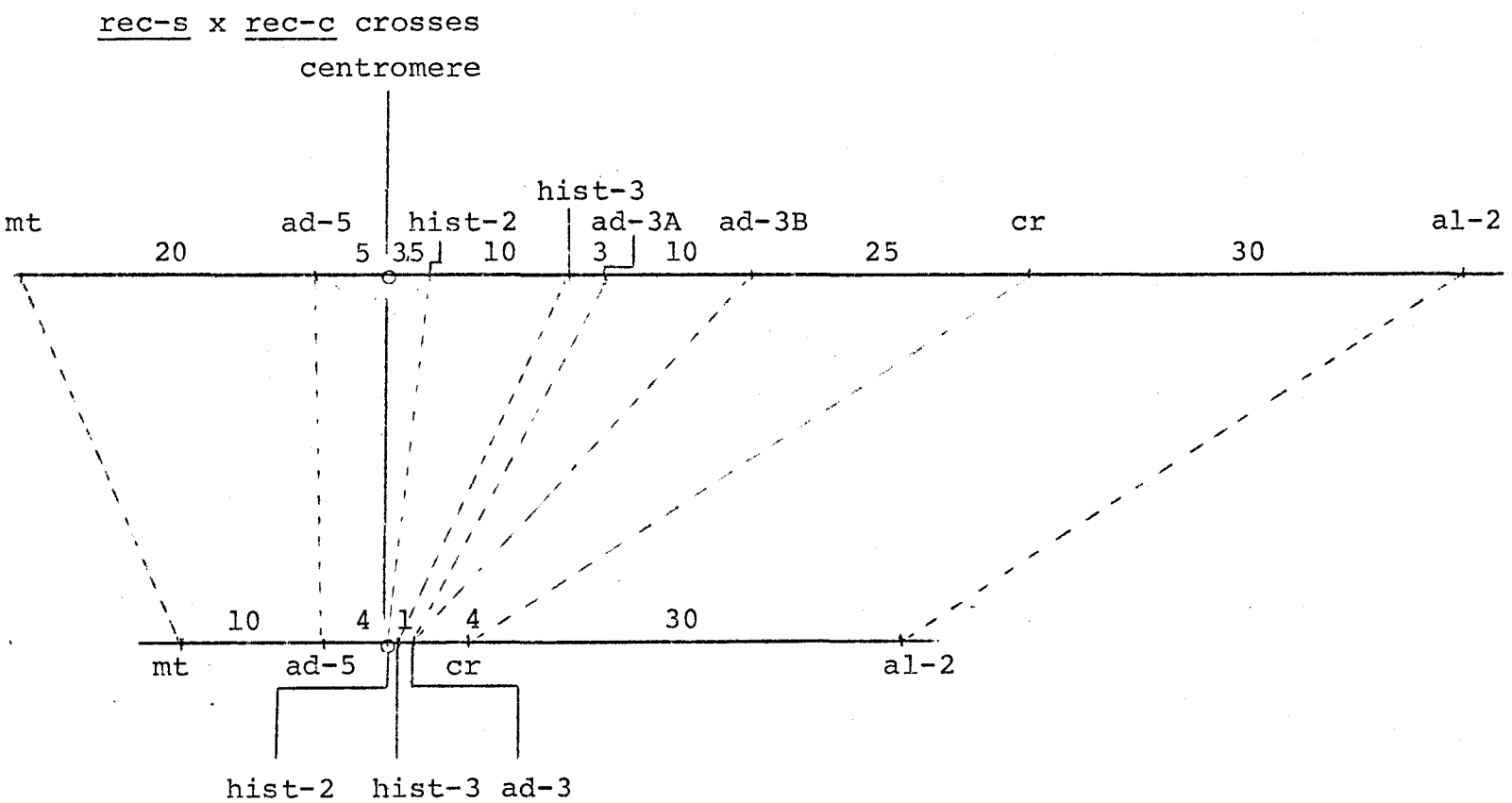
Region	rec-s present (heterozygous)	rec-s absent (homozygous rec-c)	Increase when rec-s present
mt ad-5	13.1-22.4[2]	11.9[1]	1.1-1.9
mt -o-	23.7	10.9	2.2
mt hist-2	25.1	12.2	2.1
mt ad-3B	37.1		
mt ad-3A	36.5		
mt cr		13.4[1]	
ad-5 -o-	4.2	6.7[3]	0.6
ad-5 hist-2	6.3[3]	2.5[3]	2.5
ad-5 cr	36.8[3]	6.9	5.3
-o- hist-2	3.3	< 0.2[2]	> 16.5
-o- hist-3	11.2	1.6	7.0
-o- ad-3A	13.0	1.6	8.1
-o- ad-3B	24.0		
-o- cr	35.4[3]	2.6	13.5
hist-2 ad-3B	22.8	1.4	16.3
hist-2 cr	34.0[3]	4.4[3]	7.7
hist-3 ad-3A	2.8	< 0.08	> 35.0
hist-3 cr		1.0	
hist-3 al-2		30.2	
ad-3A cr	14.6	1.0	14.6
ad-3A al-2	38.7	31.2	1.2
cr al-2	33.8	29.8	1.1
-o- al-2		31.8	

[1]Data from Ferraro (1971)

[2]Data from Newcombe (1969)

[3]Derived from section 3(i)

Fig. 15: A comparison of maps of linkage group I over the region mt - al-2 of rec-s x rec-c and rec-c x rec-c crosses.



recombination between mt and ad-5 in rec-s x rec-c crosses is variable, varying from a one- to two fold increase over rec-c x rec-c crosses. Increases to the right of the centromere as far as cr are not uniform varying from a sevenfold increase between the centromere and hist-3 to a more than thirty-five fold increase between hist-3 and ad-3A. Outside the region between the centromere and cr, rec-s appears to have little effect since the recombination frequency between cr and al-2 is practically unaffected. However, any effect immediately to the right of cr may go undetected due to the distance between cr and al-2.

Summary of Section 3(ii)

Rec-s has been shown to cause an increase in the recombination frequency in all regions studied between the centromere and cr, although the increases are not uniform.

A comparison of maps from rec-s x rec-c and rec-c x rec-c crosses is given in Fig. 15.

(iii) A short note on Female Sterility

As mentioned in section 2 (Materials and Methods) some problems were encountered with female sterility, particularly in cross 195. One member of each spore pair in each scoreable ascus was tested for female

sterility once such a problem was encountered. In some cases, all 8 spores were tested. In most cases, the spores were discovered to be weakly fertile rather than completely sterile. In a few cases they were completely female sterile, and in a few cases, normally fertile. It was also noted that this weak fertility (or female sterility) was associated with the hist-2 mutation:

No. of asci in which sterility segregated with hist-2:	183
No. of asci in which sterility did not segregate with hist-2:	13

In 11 of the 13 asci where sterility did not segregate with hist-2, the ratios were aberrant, and in all except 2 of these 11 asci, it was the hist-2 which became female fertile. In the other two asci, not enough data was present to determine whether they had aberrant ratios or not.

Type of ascus	No.
$fs:fs^+ = 3:1$	2
$fs:fs^+ = 1:3$ (or 3:5)	9
Uncertain	2

Section 4

DISCUSSION

In section 3(i) it was demonstrated that rec-s is located to the right of the centromere of linkage group I, and probably entirely to the right of hist-2. To resume, the rationales for these conclusions are:

(1) Two asci which showed second division segregation for hist-2 also showed second division segregation for rec-s.

(2) In a cross involving hist-2 and ad-3B which is heterozygous for rec-s, crossover events between hist-2 and ad-3B yielded variable recombination frequencies of the strains involved in such events.

On the basis of the data presented in the histograms of prototroph frequencies from crosses of hist-2 and ad-3B strains (Figs. 12 and 11, respectively) rec-s appears to be divided into at least two factors, or groups of factors, two of which lie between hist-2 and ad-3B. There appear to be four distinct classes of recombination frequency. These are:

low	< 4% recombination between hist-2 and ad-3B
medium-low	mode of 9% recombination, mainly associated with ad-3B strains
medium-high	mode of 15% recombination, mainly associated with hist-2 strains
high	> 18% recombination

This could be accounted for if rec-s was divided into two factors, or groups of factors, rec-s-1 nearer the hist-2 locus, and rec-s-2 nearer the ad-3B locus. Then rec-s-1 could result in 9% recombination and rec-s-2 in 15% recombination. The approximate positions of the two portions of rec-s are shown in Fig. 16.

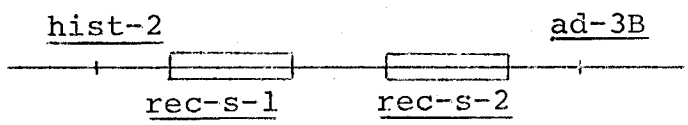


Fig. 16: The positions of rec-s-1 and rec-s-2.

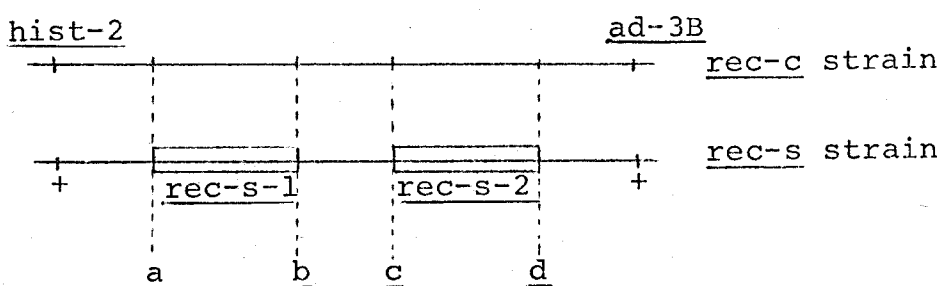


Fig. 17: Pairing of rec-c and rec-s strains

If rec-c and rec-s strains pairs as in Fig. 17, and a and b represent the proximal and distal ends of rec-s-1 respectively, and c and d represent the proximal and distal ends of rec-s-2 respectively, then: a crossover between hist-2 and a would give a high frequency

recombination hist-2 strain and a low frequency recombination ad-3B strain; a crossover between b and c would result in a medium-low frequency recombination ad-3B strain and a medium-high frequency recombination hist-2 strain; a crossover between d and ad-3B would result in an ad-3B strain with a recombination frequency between low and medium low and a hist-2 strain with a recombination frequency between medium-high and high; a crossover between c and d would result in a hist-2 strain with a recombination frequency between medium-high and low, and an ad-3B strain with a recombination frequency between medium low and high.

From the amount of recombination rec-s-1 and rec-s-2 are found to produce, it can be deduced that rec-s-2 is either longer or more efficient than rec-s-1.

An approximation of the locations of rec-s-1 and rec-s-2 may be calculated using the following procedure:

(a) Scoring the number of asci with hist-2 strains exhibiting high prototroph frequency (> 9%) and ad-3B strains exhibiting low prototroph frequency (< 2%). This would give an estimate of the distance between hist-2 and rec-s-1.

(b) Scoring the number of asci with hist-2 strains exhibiting low prototroph frequency and ad-3B strains exhibiting high prototroph frequency. This would

give an estimate of the distance between rec-s-2 and ad-3B.

(c) The number of asci with hist-2 strains exhibiting medium high prototroph frequency (7-8%) and ad-3B strains exhibiting medium low prototroph frequency (4-5%) would give an estimate of the distance between rec-s-1 and rec-s-2.

(d) The number of asci with hist-2 strains exhibiting > 7% prototroph frequency and ad-3B strains exhibiting < 5% frequency of prototroph formation would give an estimate of the distance between hist-2 and rec-s-2.

(e) The number of asci with hist-2 strains exhibiting < 8% frequency of prototroph formation and ad-3B strains exhibiting > 4% frequency of prototroph formation would give an estimate of the distance between rec-s-1 and ad-3B.

There are 25 asci in (a)
19 asci in (b)
27 asci in (c)
28 asci in (d)
30 asci in (e)

A total of 85 asci were scored.

In this cross, cross 195, the map distance between hist-2 and ad-3B is known to be 22.8 m.u.'s (Table 11). Thus the

map distance given by (a), (b), (c), (d) and (e) may be calculated from the formula:

$$\text{map distance} = \frac{\text{no. of asci}}{85} \times 23 \text{ m.u.'s}$$

This gives the following map distances:

hist-2 - rec-s-1	7 m.u.s
rec-s-2 - ad-3B	5 m.u.s
rec-s-1 - rec-s-2	7 m.u.s
hist-2 - rec-s-2	15 m.u.s
rec-s-1 - ad-3B	15 m.u.s

A diagrammatic representation of this situation is given in Fig. 18.

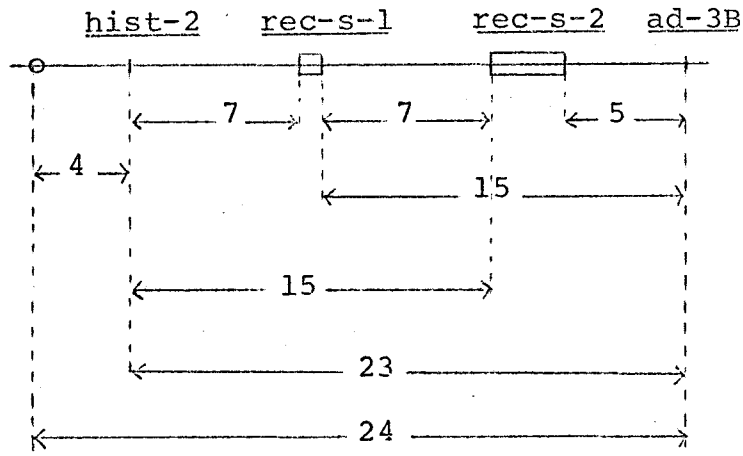


Fig. 18: Map of hist-2 - ad-3B region showing positions of rec-s-1 and rec-s-2. (Distances given in m.u.s)

By subtraction, it may be seen that rec-s-1 has a length of 1 map unit, and rec-s-2 a length of 3 map units.

In section 3(ii) the map distances between the centromere and hist-2, and the centromere and hist-3 are given as 3.3 m.u.s and 11.2 m.u.s when rec-s is present. This gives a distance approximately 8 m.u.'s between hist-2 and hist-3. Similarly, the distance between ad-3A and ad-3B may be estimated to be (23 - 13) = 10 m.u.s. This places hist-3 almost immediately right of rec-s-1, and ad-3A about 4 m.u.s left of rec-s-2. A map of the region of linkage group I from the centromere to ad-3B is given in Fig. 19.

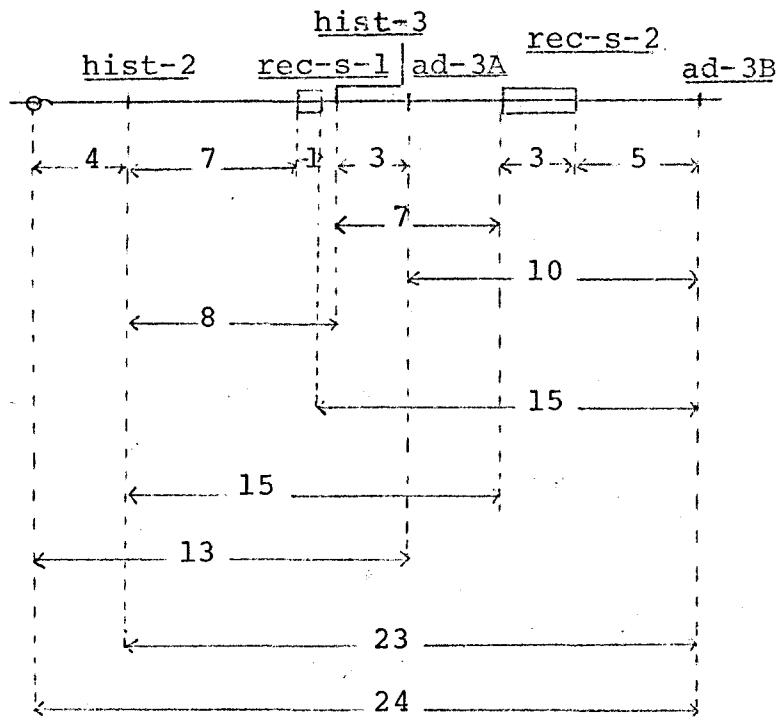


Fig. 19: Map of the centromere - ad-3B region showing positions of rec-s-1 and rec-s-2 in relation to other markers. (Distances given are in m.u.s)

Most of the distances in the above map are necessarily approximate. Many have been obtained by subtraction of distances of different crosses. The positions of rec-s-1 and rec-s-2 are approximate since not all pairs of strains could be fitted precisely into classes (a), (b), (c), (d) and (e) (pages 83 and 84). The preceeding calculation also rests on the hypothesis that the length of chromosome between hist-2 and ad-3B is the same in both rec-s and rec-c strains, i.e. there is good pairing between the homœologous portions of this linkage group.

The evidence from this calculation is that rec-s-2 is both longer and more efficient per unit length than rec-s-1.

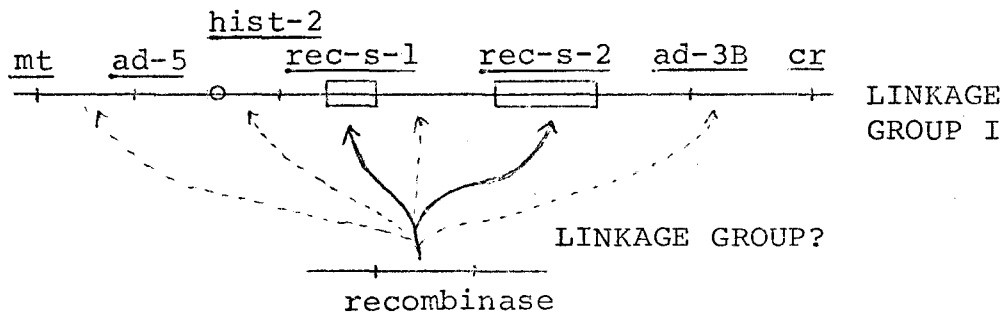
The question of whether or not these two portions of rec-s are the only two still remains to be answered. Studies on recombination frequencies between ad-3B and cr would be useful in determining whether there is another portion to the right of ad-3B. The increase in map distance when rec-s is present over that when rec-s is absent is estimated to be about fourfold between ad-3B and cr. This suggests there may be a small portion of rec-s present between ad-3B and cr. However, this may be due to the apparently massive effect of rec-s-2.

Possible modes of action of rec-s

One model which might explain the results obtained is that of differential attraction of a general recombinase, or some molecule necessary for recombination. It is known that protein 32 from phage T_4 which opens up DNA molecules by complexing with the strands of the helix (Alberts and Frey, 1970) is more attracted to a strand where there is already at least one molecule of protein 32 attached than to one which has none attached. Protein 32 is necessary for both replication and recombination of the phage genetic material. In the succeeding model it is necessary to postulate that, for the particular regions of linkage group I under consideration that either the DNA itself or some protein associated with the DNA has more affinity for a recombinase than in other regions. This is not incompatible with the hypothesis of Schaap (1971) of positive and negative correlation of controls of recombination discussed in Section 1.

It is suggested that a recombinase in produced by an apparently unlinked gene, or group of genes, and this is attracted preferentially to the factors comprising rec-s. This produces an overall increase in recombination in that region. The increase between mt and ad-5, and also between ad-3B and cr could be explained on the

basis that some of the recombinase becomes trapped in these regions before it reaches rec-s. The effect of rec-s causes more of the recombinase to be trapped in regions close to it than in the rest of the genome. This situation is represented diagrammatically in Fig. 20.



——→ Attraction of recombinase by rec-s-1 and rec-s-2.

- - - - → Residual attraction of recombinase affecting surrounding regions.

Fig. 20: Diagrammatic representation of differential attraction of a recombinase.

This hypothesis makes two predictions:

(1) If the region from hist-2 - cr is separated from the mt - ad-5 region, for example, in a translocation strain, and such a strain is crossed to another one bearing an identical translocation, but carrying rec-s, then the mt - ad-5 region will be unaffected by rec-s.

(2) There will be variable frequencies of recombination between mt and ad-5 when rec-s is present, and possibly between ad-3B and cr.

Prediction (1) has yet to be investigated, but there is a certain amount of evidence for prediction (2). Ferraro (1971) has obtained frequencies of recombination between mt and ad-5 varying from 13.1 to 22.4 m.u.s.

There are some criticisms which may be levelled against this hypothesis:

(1) It would have to be postulated that the same recombinase affecting this region is produced by both Neurospora crassa and Neurospora sitophila.

(2) It does not explain the lack of effect of rec-s in the region ad-5 - rg. To explain this, the effect of heterochromatin or the centromere may have to be invoked.

Other models which might be proposed to explain the effect of rec-s involve pairing mechanisms. Pritchard (1960) proposed a model involving effectively paired segments where recombination only takes place. Chromosomes could appear paired on cytological examination, but would only be discontinuously effectively paired. If regions rec-s-1 and rec-s-2 were regions of effective pairing additional to those already present, this would account for the increase in recombination frequency in the regions where the components of rec-s are located. However, there are three criticisms which may be levelled against such an

hypothesis:

(1) There is no explanation offered indicating how an effectively pairing segment right of the centromere would affect the recombination in a region at least 14 m.u.s distant from it, and on the other side of the centromere.

(2) It is surprising that a region of N. crassa will pair more effectively with a region of N. sitophila rather than with a homologous region of another N. crassa strain.

(3) It would be very difficult to test such a model since effectively pairing segments cannot be detected cytologically, and have only been hypothesized on the basis of interference studies.

The desynaptic model of Ferraro (1971) has been excluded as a pairing hypothesis since this required that rec-s is left of hist-2.

The effect of heterochromatin also appears to be an unlikely possibility unless rec-s-1 and rec-s-2 cause the involvement of heterochromatin in recombinational events. Heterochromatin has been shown in some organisms not to undergo recombination, e.g. in tomato (Barton, 1951) Tulbaghia (Dyer, 1963) and in other organisms to undergo very little recombination, e.g. Drosophila melanogaster (Mather, 1939) and the

frequency of recombination appears to be dependent on its location. Centromeric heterochromatin undergoes more recombination when it is relocated away from the centromere (Baker, 1958).

There may be two regions of heterochromatin, one between hist-2 and hist-3, and one between ad-3A and ad-3B in N. crassa, and two regions of euchromatin in corresponding positions in N. sitophila which have an effect on the heterochromatin in N. crassa. The lack of recombination between ad-5 and the centromere could, in this case, be attributed to the centromere or some uninvolved regions of heterochromatin. However, there appears to be no explanation for the increase in recombination between mt and ad-5.

It seems unlikely that rec-s-1 and rec-s-2 are rec genes as postulated by Catcheside et al. (see Section 1 for discussion of rec genes) since the rec genes so far identified are not contiguous with the regions they affect. There is the possibility that they are cog genes, each being affected by a different recombinase. However, once again it would have to be postulated that N. sitophila and N. crassa produced identical recombinases. Also this would not explain the effect rec-s has outside the region between the centromere and ad-3B since mt and ad-5 are several map units away from this region.

The region of effect of rec-s-1 and rec-s-2 has

yet to be determined, but, from their calculated positions, it might be expected that rec-s-1 has an effect from the centromere to right of hist-3, and rec-s-2 from left of hist-3 to right of ad-3B. However, more extensive studies would have to be performed using appropriate strains derived from cross 195. Such an investigation could be used to determine whether rec-s-1 or rec-s-2, or both, have an effect left of the centromere between mt and ad-5.

Section 5

SUMMARY

(i) Rec-s has been located distal to hist-2 on the basis of tetrad analysis.

(ii) Rec-s has been divided into two factors, rec-s-1 and rec-s-2. Other portions of rec-s may remain to be identified.

(iii) Both rec-s-1 and rec-s-2 have been approximately located.

(iv) Possible modes of action of rec-s have been discussed, but no entirely satisfactory one has been proposed.

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