TO THE BEAST

A DOMINANT CONTROL OF RECOMBINATION IN *N. CRASSA*

AN INVESTIGATION OF THE CONTROL OF RECOMBINATION IN *NEUROSPORA CRASSA* BY A DOMINANT FACTOR, OR FACTORS, FROM *N. SITOPHILA*

by

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

September 1971

MASTER OF SCIENCE (1971)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: An Investigation of the Control of Recombination in *Neurospora crassa* by a Dominant Factor, or Factors, from *N. sitophila*.

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NUMBER OF PAGES: xi, 185

SCOPE AND CONTENTS:

The phenomenon of genetic recombination is of fundamental importance to the evolution and adaptation of species, and is a valuable laboratory aid to the biological scientist. Probable mechanisms of control of recombination are largely unknown, due partly to the difficulty of obtaining artificial mutants affecting the process. The studies reported here avoid this difficulty by the use of different factors controlling recombination which occur naturally in the species *Neurospora crassa* and *N. sitophila*. Studies of hybrid *N. crassa* strains carrying factors from *N. sitophila* are described, and some models for the control of genetic recombination are discussed.

PREFACE

This thesis describes studies carried out in the Department of Biology, McMaster University, from September 1969 to September 1971. 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. F. H. Threlkeld, for allowing me enough freedom to learn from experience, yet being ready with help and advice when needed. Thanks are also due to Dr. O. Mylyk, Mr. K. Newcombe and Miss J. Hargrave for many helpful discussions, strains, and permission to use methods or data, and to the Department of Biology for financial support. Special thanks are extended to Mrs. S. Thomas for her patience with much of the biochemical testing, and to Mrs. L. Dowd for her perseverance with the typing.

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Chapter I

INTRODUCTION

The phenomenon of genetic recombination is basic not only to the study of genetics but also to the evolution of species throughout the biological phyla, yet it remains one of the least understood of biological phenomena.

The possibility of recovering all the products of meiosis has placed the ascomycete fungi, and particularly those which produce linear asci, in the forefront of recombination research. It is mainly from evidence obtained from gene conversion in such fungi that the various models proposed for the molecular mechanism of recombination in eukaryotes have come, e.g. Holliday (1964), Whitehouse and Hastings (1965), and Paszewski (1970). The common features of these models are that: recombination is initiated at single DNA strand breaks; disruption of base pairing adjacent to the breaks follows, with formation of hybrid DNA between recombining chromatids; ligases rejoin remaining breaks, and any mismatched base pairs resulting from mutations being included in the hybrid region may be corrected. Details of the models vary; Whitehouse and Paszewski postulate DNA synthesis, distinct from any synthesis necessary for correction of

mismatched base pairs, and degradation of some DNA. Whitehouse and Hastings have interpreted the data on polarity of intragenic recombination as indicating that recombination is initiated at specific sites. That such models may have general validity for eukaryotes is suggested by recent observations of gene conversion in Drosophila melanogaster (Smith et al., 1970), (Ballantyne and Chovnick, 1971) and possibly maize (Salamini and Lorenzoni, 1970).

The geneticist's normal method of studying a process by isolation and study of mutants affecting that process has been sadly unproductive in the field of eukaryotic recombination. Possibly such mutants disrupt meiosis to the point of sterility, allow a lethal accumulation of mutations or cause extensive DNA damage. In bacteria and phage the method has been more productive, see reviews by Davern (1971) and Echols (1971), and Barbour et al. (1970).

As an alternative to the artificial production of mutants, geneticists studying recombination have depended mainly on natural variants, e.g. the meiotic mutants of Drosophila melanogaster (Sandler et al., 1968), the fine controls of recombination in Schizophyllum commune (Simchen, 1967), (Simchen and Connolly, 1968), (Stamberg, 1968), (Simchen and Stamberg, 1969a), and (Schaap, 1971),

and in yeast (Simchen et al., 1971), and the rec genes in Neurospora crassa (Jessop and Catcheside, 1965), (Catcheside, 1966), (Jha, 1967, 1969). The observation that the Neurospora rec genes affect the polarity of intragenic recombination events (Smith, 1966), (Thomas and Catcheside, 1969), (Smyth, 1971) is consistent with the hypothesis (Catcheside and Austin, 1969) that a rec^+ gene produces a repressor capable of binding at a specific site, or sites (Catcheside and Austin, 1971), and inhibiting the initiation of recombinase action. It is also consistent with the hypothesis (Threlkeld, personal communication) that rec^- genes are deficient in production of specific ligases which would be expected to result in a high frequency of single strand breaks. Such a mechanism has been observed recently in T4 phage (Krisch et al., 1971). The cog mutant controlling intragenic recombination at the hist-3 locus and intergenic recombination in the hist-3 to ad-3 region of Neurospora crassa, and postulated by Angel et al. (1970) to be a mutant binding site for a recombinase, might then correspond to some feature of DNA replication.

From their work on Schizophyllum, Simchen and Stamberg (1969b) have suggested a general classification of recombination controls into coarse and fine. A

modification of their Table I is given below:

Table I

SOME PROPERTIES OF COARSE AND FINE CONTROLS OF RECOMBINATION

	Coarse control	Fine control
Magnitude of effect	extreme	small
Extent of effect	entire chromosomes	localised regions
Natural occurrence	rare	very common
Selective advantage	harmful	beneficial
Functions affected	overall mechanisms of synapsis, recombination, separation, etc.	specificity of coarse functions
Observed in	pro- and eukaryotes	eukaryotes
Examples	bacterial *rec* mutants (Davern, 1971)	Schizophyllum fine controls
	Drosophila meiotic mutants (Sandler *et al.*, 1968)	Neurospora *rec* genes
	Tomato desynaptic mutants (Moens, 1969b)	
	Fungal *uvs* mutants (Schroeder, 1970)	

One natural variant of Neurospora seems to lie somewhere between these definitions in that it appears to affect a large, about 15%, portion of only linkage group I. Fincham (1951) studied the relative controls for recombination in this chromosome in N. crassa and N. sitophila using mutant loci transferred from N. crassa to N. sitophila by repeated backcrossing. Marked regions close to the N. sitophila centromere showed a large increase in recombination frequency relative to the N. crassa controls, but the effect decreased with distance from the centromere. Fincham suggested the linkage group I centromere was responsible for control of recombination in adjacent regions. Later work by Threlkeld (1961a) and Scott-Emuakpor (1965a) confirmed Fincham's work in general, but failed to show an effect for markers closely linked to the centromere (Fig. 2b, Chapter 3, section XV) leading Scott-Emuakpor to suggest factors close to the centromere, rather than the centromere itself, were responsible. Both workers showed some changes in recombination frequency for other linkage groups with markers transferred to N. sitophila, but no pattern could be discerned.

Since comparisons were being carried out between species, Fincham, Threlkeld and Scott-Emuakpor were unable to exclude the possibility of different amounts of DNA between the markers in the two species, or of heterozygosity

in the N. sitophila crosses. Using a hist-2 mutant to mark the centromere of N. crassa, and the hist-2^{+} allele for the N. sitophila centromere, Newcombe (1969) attempted to transfer the linkage group I centromere proximal region from N. sitophila to N. crassa. The resulting hybrid showed recombination frequencies similar to those obtained previously in N. sitophila (Table 49, Chapter 3, section XIV), and confirmed that some dominant controlling factor(s) had been transferred from N. sitophila.

This study was undertaken to characterise Newcombe's hybrids further, and if possible suggest a mechanism for the control. Two main lines of investigation were followed:

I. a further study of changes in recombination frequency, particularly with respect to centromere distances and effects on other linkage groups, caused by the factor(s) originating in N. sitophila.

II. a search for possible effects of the N. sitophila factor(s) on recombination between N. crassa genomes when the hybrid and normal genomes are combined in a heterokaryon and crossed to a marked N. crassa strain.

The rationale for the second experiment was that a recombination frequency for the normal N. crassa x N. crassa component similar to that for hybrid x N. crassa controls would demonstrate the synthesis of a diffusible gene

product by the controlling N. sitophila factor(s). To avoid the possibility of excluding cytoplasmic determinants of recombination from this experiment the hybrid, or hybrid/N. crassa heterokaryon, was used as protoperithecial parent. Three types of perithecia were expected to be recovered from this experiment: those containing asci of exclusively N. crassa x N. crassa type crosses, those containing asci of exclusively hybrid x N. crassa type crosses, and those containing both types (genetically mixed perithecia). See Chapter 3, section X, for a review of observations by other works on genetically mixed perithecia.

It was decided to use dissection of asci as a general means of study, rather than the less time consuming isolation of random spores, since this would provide: more accurate centromere distances than could be obtained by use of hist-2 as a centromere marker; a more reliable means of following the segregation of the factors influencing recombination; a check on chromosome aberrations; a means of following chromatid interference, and would help to avoid errors due to differential germination of mutant spores.

The term "rec-s" has been used in place of more cumbersome alternatives to denote the specific factor(s) controlling recombination which originated in N. sitophila; "rec-c" specifies the alternative factor(s) from N. crassa.

No strains of exclusively N. sitophila origin were used in this study.

Chapter 2

MATERIALS AND METHODS

Section I:

Notations and Abbreviations

To avoid the introduction of errors the notation used during this work has been retained in this thesis.

Crosses are denoted by C followed by the cross number, e.g. C1070. Where crosses are described in the format, strain A x strain B, the first strain is the protoperithecial parent.

Original Fungal Genetics Stock Centre (FGSC, Humboldt State College, Arcata, California 95521) strains are denoted by their stock number. See Table 2, e.g. FGSC 667.

Strains derived by persons other than the author carry the original designation. See Table 3, e.g. CC2 R_{128}.

Strains isolated by the author as random spores carry the cross number followed by R and the spore number. e.g. C1070 R_{33}.

Strains isolated by the author from asci carry the cross number followed by A and the ascus and spore numbers. e.g. C1070 A_{17-7}.

Where strains originate as conidial isolates the appropriate notation is followed by a dash and conidial isolation number. e.g. FGSC 667-11, C1070 A_{17-7-5}.

The following abbreviations have been used:

in the text:

mt - the mating type locus.

m.u. - map units, a measure of the frequency of recombination between linked markers.

OR - an Oak Ridge strain or background.

p - probability, $0 < p < 1$, unless otherwise defined for special purposes.

SC - a rec-s x rec-c type cross, similarly CS, CC, SS.

SC (Het) - the SC component of a heterokaryon cross, similarly CC (Het).

-o- - a centromere. Used in e.g. mt -o- to denote the region of the chromosome between mating type and the centromere.

in tables only:

co. - crossover

n.co. - non crossover

n.s. - not significant, in statistical tests.

Other abbreviations are defined as necessary.

Section II: Strains used in this study.

The stock strains used in this study are shown in Tables 2, 3 and 4 below.

Stocks were maintained on solid glucose medium at -17°C, with the exception of the fluffy mating type testers which tend to die at low temperatures and which were maintained at 7°C.

The Oak Ridge standard wild types, OR_a and OR_A, were replaced during the study by OR_{a-1-3} and OR_{A-1-1} when they were shown to be heterokaryotic for female sterile mutants (Mylyk, 1971). OR_{A-1-1} was later replaced by OR_{A-5-1} when it was found to be heterokaryotic for osmotic mutations.

Section III: Media.

Double distilled water was used in all media.

Crosses were made on Westergaard and Mitchell (1947) medium consisting of 2% sucrose as carbon source, inorganic salt solution and 1.5% Bacto Difco agar. The inorganic salt solution, autoclaved and stored at 4x strength at 7°C consisted of:

KNO_3	4.0 g
KH_2PO_4	4.0 g
$MgSO_4.7H_2O$	2.0 g
$CaCl_2.6H_2O$	0.4 g
NaCl	0.4 g

TABLE 2

Original Fungal Genetics Stock Center (FGSC) strains used in this study.
See Neurospora Newsletter #16, June 1970

Stock #	Locus	Allele	Linkage group	mt	Ancestry[1.]	Notes
414	*N. sitophila* wild type	HSS f^1 32 aPC		A		
987	*N. crassa* wild type	74-OR23-1A		A	OR	= OR_A
460	*hist-3*	Y175M614	IR	a	SL→SL	Requires histidine
462	*hist-3*	Y155M261	IR	A	SL→SL	Requires histidine
667	*hist-2*	Y175M611	IR	a	SL→SL	Requires histidine
668	*hist-2*	Y152M43	IR	A	SL→SL	Requires histidine
678	*ad-5*	Y175M263	IL	a	SL→SL	Requires adenine
679	*ad-5*	Y112M192	IL	A	SL→SL	Requires adenine
1125	*leu-3*	$R15^p6$	IL	a	Mixed→SL2	Requires leucine
1690	*fl*	fl^p	IIR	a	SL	Fluffy
4	*lys-5*	DS6-85	VIL	a	Mixed	Requires lysine
	ylo-1	Y30539y	VIL			Yellow conidia
246	*ad-5*	Y152M40	IL	a	Mixed	Requires adenine
	hist-2	Y152M14	IR			and histidine
	cr	B123				Crisp

1. Mixed→SL2 denotes mutant originated in a strain of mixed ancestry and was backcrossed twice to St. Lawrence wild types.

TABLE 3

Stock strains derived prior to this study

Designation	Locus	Allele	Linkage group	mt	Obtained from[1]	Notes
ORa	*N. crassa* wild type			a	S.F.H.T.	An Oak Ridge wild type of uncertain origin.
2-17-186A	*ad-3 A*	2-17-186	IR	A	A.J.G.	Requires adenine. Excretes purple pigment. Induced in and backcrossed to OR.
D11	*arg-5*	27947	IIR	a	O.M.	7th Backcross to OR of FGSC 274. Requires arginine.
D12	*pyr-1*	H263	IVR	a	O.M.	5th Backcross to OR of FGSC 72. Requires a pyrimidine.
D25	*pan-2*	B3	VIR	a	O.M.	7th Backcross to OR of FGSC 469. Requires pantothenic acid.
D26	*ylo-1* *pan-2* *tryp-2*	Y30539y B3 75001	VIL VIR VIR	a	O.M.	Yellow conidia, requires pantothenic and anthranilic acids. 6th Backcross to OR of FGSC 469.
D35	*nic-3*	Y31881	VIIL	A	O.M.	8th Backcross to OR of FGSC 129. Requires nicotinamide.
D36	*nic-3*	Y31881	VIIL	a	O.M.	8th Backcross to OR of FGSC 129. Requires nicotinamide.
D41	*aur* *lys-3*	34508 4545	IR IR	a	R.E.S.	Synthesized from FGSC 540 and FGSC 17 and backcrossed to OR. Aurescent conidia, requires lysine.
1-3-a	fs	1-3-a	-	a	O.M.	Female sterile (Mylyk, 1971).
18-1-A	fs	18-1-A	-	A	O.M.	Female sterile (Mylyk, 1971).

Designation	Locus	Allele	Linkage group	mt	Obtained from[1]	Notes
202-1	ad-5	Y152M40	IL	a	K.D.N.	Requires adenine and histidine.
	hist-2	Y152M14	IR			
	cr	B123	IR			Morphological. 2nd Backcross to OR of FGSC 246. See Fig. 10.
P369	hist-2	Y152M14	IR	A	K.D.N.	Backcross to OR of 202-1.
	cr	B123	IR			
CC2 R_{128}	ad-5	Y152M40	IL	a	K.D.N.	Intercross between P369 and an ad-5 progeny also from 202-1 Backcross to OR.
K110xK4 #5	hist-2	?	IR	A	S.F.H.T.	Isolated as a contaminant.
	cr	B123	IR			See section V, Chapter 3.
14-5	al-2	15300	IR	a	S.F.H.T.	Albino, requires adenine and
	ad-1	3254	VIL			pantothenic acid. Of mostly
	pan-2	B5	VIR			OR background.
30JA-5	rec-s		I	A	K.D.N.	High recombination. 5th Backcross of FGSC 414 to N. crassa. See Fig. 10.
K4	ad-5	Y152M40	IL	A	K.D.N.	High recombination. 6th Backcross
	rec-s		I			of FGSC 414 to N. crassa. See
	cr	B123	IR			Fig. 10.

1. Initials used are:
A.J.G. - A. J. Griffiths
O.M. - O. Mylyk
K.D.N. - K. D. Newcombe
R.E.S. - R. E. Subden
S.F.H.T. - S. F. H. Threlkeld

TABLE 4

Stock strains derived during this study

Designation	Locus	Allele	Linkage group	mt	Notes
ORa-1-3	*N. crassa* wild type			a	Vegetative reisolate from ORa.
ORA-1-1	*N. crassa* wild type			A	Vegetative reisolate from FGSC 987.
ORA-5-1	*N. crassa* wild type			A	Vegetative reisolate from FGSC 987.
C481 R_{13}	*pyr-1*	H263	IVR	a	Backcross of D12 to ORA.
C484 R_{17}	*ylo-1*	Y30539y	VIL	a	Backcross of D26 to ORA.
	pan-2	B3	VIR		
	tryp-2	75001	VIR		
C595 R_{12}	*ad-5*	Y175M253	IL	a	FGSC 678 backcrossed to ORA and once to ORa.
C605 R_6	*ad-3 A*	2-17-186	IR	A	4th Backcross of 2-17-186 A to ORa.
C734 R_1	*hist-2*	Y175M611	IR	a	FGSC 667 Backcrossed twice to ORA and once to ORA-1-1.
C734 R_{12}	*hist-2*	Y175M611	IR	A	As for C734 R_1.
C833 R_1	*fl*	fl^p	IIR	A	Backcross of FGSC 1690 to ORA-1-1.
C834 R_{12}	*hist-2*	Y175M611	IR	a	FGSC 667 Backcrossed to ORA and twice to ORA-1-1.
C1038 R_{24}	*hist-3*	Y175M614	IR	A	Backcross of FGSC 460 to ORA-5-1.
C1100 R_{48}	*aur*	34508	IR	a	Backcross of D41 to ORA-5-1.
C1226 R_{46}	*hist-2*	Y152M43	IR	a	Backcross of FGSC 668 to ORa-1-3.
C860 R_{10}	*lys-5*	DS6-85	VIL	a	FGSC 4 Backcrossed once to ORA, once to ORa-1-3 and once to ORA-1-1.
C873 R_{20}	*leu-3*	R_{156}	IL	a	FGSC 1125 Backcrossed once to ORA-1-1 and once to ORa-1-3.

Biotin	16.0 µg
Trace element solution	1.0 ml
Distilled water up to	1000 ml

The trace element solution consisted of:

$Na_2B_4O_7.10H_2O$	0.01 g
$CuSO_4.5H_2O$	0.1 g
$FePO_4.2H_2O$	0.2 g
$MnSO_4.4H_2O$	0.02 g
$ZnSO_4.7H_2O$	2.0 g
$Na_2MoO_4.2H_2O$	0.02 g
Distilled water up to	250 ml

All other work was carried out on Vogel's medium N (Vogel, H. J., 1956). Trial crosses on Vogel's medium showed a tendency for some growth of the conidial parent. The Vogel's inorganic salt solution was made up at 50x strength and stored at room temperature without prior autoclaving. It consisted of:

$Na_3C_6H_5O_7.5\frac{1}{2}H_2O$	150 g
KH_2PO_4	250 g
NH_4NO_3	100 g
$MgSO_4.7H_2O$	10 g
$CaCl_2.2H_2O$	5 g
Trace element solution	5 ml
Biotin solution	250 µg
Chloroform	2 ml
Distilled water	750 ml

The trace element solution consisted of:

Citric acid.$1H_2O$	5.0	g
$ZnSO_4.7H_2O$	5.0	g
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1.0	g
$CuSO_4.5H_2O$	0.25	g
$MnSO_4.1H_2O$	0.5	g
H_3BO_3	0.5	g
$Na_2MoO_4.2H_2O$	0.5	g
Distilled water	95	ml

For vegetative growth Vogel's medium was supplemented with appropriate biochemicals and 2% glucose as carbon source and solidified with 1.5% Bacto Difco agar.

For tests for auxotrophic markers two media were used:

Low sorbose medium, for tests on strains carrying the crisp marker, consisting of Vogel's salt solution, 0.4% sorbose, 0.1% fructose, and 0.1% glucose solidified with 2% Bacto Difco agar.

High sorbose medium, for strains other than crisps, consisting of Vogel's salt solution, 1% sorbose, 0.05% fructose and 0.05% glucose solidified with 2% Bacto Difco agar.

The high sorbose medium was also used for prototroph plating. It has been shown (Brockman and de Serres, 1964) that the sorbose-fructose-glucose combination minimises

variations in sorbose toxicity.

All media was autoclaved at 15 lbs/sq.in. above atmospheric pressure for 15 minutes.

The following concentrations of biochemical supplements were used:

adenine	50 mg/l
L-arginine HCl	200 mg/l
anthranilic acid	100 mg/l
cytidine	100 mg/l
L-histidine HCl	100 mg/l
L-leucine	100 mg/l
L-lysine HCl	100 mg/l
nicotinamide	100 mg/l
calcium pantothenate	50 mg/l

Section IV:

METHODS

a) Crosses

Crosses were carried out in 150 x 15 mm test tubes on 5 ml slopes of Westergaard and Mitchell medium. An inoculum of the protoperithecial parent was allowed to grow for 7 days at 25°C before addition of the conidial parent and a few drops of sterile distilled water to aid in spreading the conidia. Where a crisp strain was used as the protoperithecial parent 11 days were allowed before conidiation.

For crosses using a heterokaryon as protoperithecial parent the heterokaryon was formed either directly on the minimal crossing medium, or transferred to it after formation on glucose minimal medium. No attempt was made to control nuclear ratios in heterokaryons, though there is some evidence (Pittenger and Atwood, 1956) that the final nuclear ratio may be influenced by extreme input ratios. However, since pan-2 spores from crosses on medium lacking pantothenic acid are pale and germinate less well than dark pan-2 spores (Threlkeld, 1965) the later heterokaryon crosses, C1170 and C1248, were conidiated with the addition of 1 mg of calcium pantothenate in the spreading water, which might be expected to affect nuclear ratios immediately prior to

fertilisation.

Crosses were incubated at 25°C.

b) Isolation of random spores

In crosses from which recombination data was sought from random spores the spores were left on the walls of the cross-tube to mature for at least 4 weeks after conidiation. Spores were transferred from the tube to the surface of a block of 4% agar using a loopful of sterile 10% sucrose solution. Under 35x magnification from a binocular microscope, an 18 s.w.g. tungsten wire needle, sharpened in molten sodium nitrite, was used to transfer each spore to its own 1 ml slope of vegetative medium. To ensure random selection of spores, all the spores in one region of the agar were transferred before proceeding to another region. The isolates were heat-shocked immediately in a 60°C water bath for 40 minutes to kill any conidia transferred and to induce germination of the spores. Crisp spores sometimes germinated only after several heat shocks. The isolates were grown at 25°C.

c) Isolation of asci

Crosses varied from 2 to 10 days in the length of time taken to shoot off the majority of their spores. For this reason isolation of asci was started as soon as possible after spores were first noticed on the wall of

the cross tube, even though immature ascospores sometimes show poor germination.

Perithecia were isolated to individual pools of sterile 10% sucrose solution on a flamed microscope slide and were cut open using tungsten wire needles. Rosettes of asci possessing mature, black ascospores were transferred away from the perithecial debris to fresh pools of sucrose solution using the capillary action of a Pasteur pipette. The rosettes were then broken up into sections of up to 10 asci, each section being transferred by Pasteur pipette to a block of 4% agar and left to dry. At the stage of dryness when the asci suddenly clumped together individual asci were separated from the clump and dragged to an adjacent spot on the agar. Individual ascospores were then isolated in order from each ascus starting at the end distal from the base of the rosette and using very sharp needles at 100x magnification. Each spore was transferred to its own 1 ml slope of vegetative medium.

A record was kept of the spore colours in each ascus, of the perithecium from which it originated and of the presence of aborted or colourless spores when they occurred.

Racks of asci were stored at room temperature in polythene bags for 14 days to mature before being

heat-shocked in a 60°C water bath for 40 min. Further heat shocks were given as necessary and the cultures grown up at 25°C.

d) Isolation of prototrophs

Several authors have described methods of obtaining prototrophs which give statistically reproducible results (e.g. Griffiths and Threlkeld, 1966; Catcheside, 1970). In this work prototroph analysis has been used only during the characterisation of K110 x K_4 #5, in which the occurrence, rather than the frequency, of prototrophs in crosses to hist-2 alleles was important. For this reason a relatively crude method was employed.

Ascospores were left to mature for at least a month after fertilisation. They were then transferred to 2 ml of sterile 0.1% agar using loopfuls of the agar solution until a concentration of about 4000 spores per ml was reached. Spore concentration was estimated from the number in one drop from a sterile Pasteur pipette. The suspension was vortexed vigorously for 2 minutes to break up spore clumps and ensure homogeneity. An aliquot of approximately ½ ml of suspension was added to each of several flasks containing 20 ml minimal liquid high sorbose medium maintained at 60°C in a water bath. After thus being heat shocked for 45 minutes the suspension was poured into a petri dish and allowed to solidify.

After 24 hrs an estimate of the number of spores per plate and percentage germination was obtained for each plate by observation of several fields under the microscope. After 72 hrs prototrophs were scored.

e) Tests for auxotrophic markers

Cultures were tested for their biochemical requirements by inoculating them onto appropriately supplemented Petri plates of sorbose medium. All biochemical mutants tested unambiguously with the exception of ad-5 and ad-1 which showed a slight additional requirement for histidine, see appendix II, and tryp-2 which become leaky after 48 hrs. With experience, and the introduction of the low sorbose medium for crisps, accurate scoring became routine with these mutants.

f) Complementation tests

Complementation tests were carried out on sorbose plates, minimal with respect to the markers being tested, divided into 4 quadrants. Two quadrants were each inoculated at the same point with both strains while each of the other quadrants was inoculated with one of the strains as a control. Plates were scored after 4 days.

g) Mating type tests

Mating types were determined using the aconidial fluffy testers FGSC 1690a and C833 R_1 A. Westergaard

and Mitchell sucrose plates with 2% agar were inoculated with either FGSC 1690 or C833 R_1 and incubated for 5 to 10 days at 25°C. Cultures to be tested were then inoculated onto fluffy lawns of both mating types using a slight spreading motion. The plates were incubated for a further 3 to 10 days until perithecia were abundant when the mating type of the culture tested was scored by production of perithecia on only one of the two plates.

It was found that fluffy strains maintained by serial sub-culture tend to become female sterile so several vegetative lines were maintained. One guide to the presence of female sterility was found to be the increase in heavily pigmented protoperithecia-like bodies in the fluffy lawn.

Where it was necessary to test other strains for female sterility Mylyk's method was used (Mylyk, 1971). The strain to be tested was grown for 7 days on a 1 cc slope of appropriately supplemented Westergaard and Mitchell sucrose medium, and was then conidiated with a suspension in sterile distilled water of both 1-3-a and 18-1-A female sterile testers. The absence of perithecia after 7 days confirmed the female sterility.

Chapter 3

RESULTS

Section I:

Method of presentation of data and their statistical analysis

The results consist of data from 21 main crosses which have been analysed by asci, and a number of subsidiary crosses analysed by asci, random spores or prototrophs. The main crosses have been arranged in 6 types: rec-s x rec-c SC, rec-c x rec-s CS, rec-s x rec-s SS, rec-c x rec-c CC, rec-c x rec-c component of heterokaryon crosses CC (Het) and rec-s x rec-c component of heterokaryon crosses SC (Het). See Table 5.

The recombination frequency between two linked markers in Neurospora depends on the genetic background of the strains used and on the conditions of crossing. The pooling of data from several crosses of one type which differ slightly in the origin of their parents should give recombination frequencies more representative of the type than might be expected from any single cross. However, particularly when relatively few crosses are pooled, it is important to avoid a strong bias in the totals due to one highly aberrant cross. The presence of such a cross may be tested for by a statistical test for heterogeneity. However, in the crosses presented

there is no a priori reason to assume the cross causing heterogeneity is any less representative of that type of cross than are the homogeneous crosses. For this reason the bias due to heterogeneity has been discussed as required rather than the aberrant cross rejected automatically.

For each cross type a table is given of the total crossovers observed in each crossover region of each cross, and of the map distance, ± standard error, based on the total crossovers for that region from all crosses of that type (Tables 6, 10, 16, 17, 25, 29, 44). The dotted boxes indicate groups of data for which heterogeneity tests are given. For these tests the data have been arranged to give cross totals in the margin equal to the number of asci scored for that cross (see legend opposite Table 7).

The traditional test for heterogeneity has been the X^2 heterogeneity test. However, the application of X^2 methods to tables larger than 2 x n, where n = 1, is extremely tedious and the X^2 values obtained are only approximately additive and approximately distributed as χ^2. The G test largely overcomes these objections and has been used throughout this work. Since G tests have been little used until recently an outline of their principle is given below.

The basis for most statistical tests is the comparison of some function of the data with an expected function, which may be assumed a priori or calculated from the data. The G_H (H for heterogeneity) test uses such a comparison between observed probabilities, calculated from the data on the basis of the multinomial distribution, and expected probabilities, calculated from the marginal totals of the data on the basis of the multinomial distribution. Thus a liklihood ratio L may be defined as:

$$L = \frac{\frac{n!}{f_1!\ f_2!\ \ldots f_a!}\ P_1^{f_1}\ P_2^{f_2}\ \ldots\ Pa^{fa}}{\frac{n!}{f_1!\ f_2!\ \ldots f_a!}\ \hat{P}_1^{f_1}\ \hat{P}_2^{f_2}\ \ldots\ \hat{P}a^{fa}}$$

$$= \prod_{i=1}^{a} \left(\frac{Pi}{\hat{P}i}\right)^{fi}$$

where: P_1, P_2 ... Pa are observed proportions of the various classes.

$\hat{P}_1$, $\hat{P}_2$ ... $\hat{P}a$ are expected proportions of the various classes.

and f_1, f_2 ... fa are frequencies of the various classes.

G is defined as 2 ln L and is distributed as χ^2 with the same degrees of freedom as would be used in a conventional X^2 test. It can be shown that the above formula for G reduces to the more convenient computational form:

$$G = 2 \left[\sum_{i=o}^{a} f_i \ln f_i - \sum_{i=o}^{a} f_i \ln \hat{P}_i - n \ln n \right]$$

(Sokal and Rohlf, 1969, p. 654)

The precise method of calculation is given in Appendix I.

In 2 x 2 G tests of the form:

a	b	a+b
c	d	c+d
a+c	b+d	n

a Yates correction has been applied: if ad > bc, ½ has been added to b and to c and ½ subtracted from a and from d, if ad < bc, ½ has been added to a and to d and ½ subtracted from b and c. Grizzle (1967) has shown that the Yates correction in 2 x 2 X^2 tables leads to an unduly low frequency of type I error - low frequency of rejection of homogeneous data as heterogeneous, and high type II error - high frequency of acceptance of heterogeneous data as homogeneous. Because of the normally high variation between Neurospora crosses Yates correction has been used in 2 x 2 G tests, but not in larger tables since it leads to loss of additivity of G values.

Each cross was checked for extreme variations in recombination frequencies between perithecia, though in most cases sample sizes were too small for statistical analysis. In addition the total data from randomised

asci from each cross were checked for reciprocality of crossover classes and for 1:1, mutant to wild type, allele ratios. Details have not been presented except as anomalies require. In many cases such anomalies can be accounted for by what will be referred to as "an inflated sample size". Consider the ratio of the reciprocal classes for two unlinked mutants m_1 and m_2 in a sample of 20 randomised asci. Ideally the m_1m_2:m_1+:+m_2:++ ratio should be 40:40:40:40. If the segregation in one ascus were different, however, the ratio might become 36:44:44:36. Such inflated sample sizes will give inflated G values. The point is discussed further by Cooke (1966).

The pooled data from different cross types were compared using 2 x n G_H tests for each crossover region in turn.

Multiple crossovers, aborted spore pairs and the mixed perithecia from the heterokaryon crosses are considered separately.

Section II:

SC type crosses

Data from SC type crosses are summarised in Table 6.

Randomised asci from cross C651 showed apparent linkage of ad-5 and arg-5$^+$, $p < 0.001$. Since arg-5 is

TABLE 5

Crosses Analysed by Asci

Cross	Type	Protoperithecial parent	Conidial parent	Asci isolated
C651	SC	C419 A_{1-7} A, ad-5, rec-s, cr	D11 a, rec-c; arg-5	15
C661	SC	C419 A_{1-8} A, ad-5, rec-s, cr	D11 a, rec-c; arg-5	60
C663	SC	C419 A_{1-8} A, ad-5, rec-s, cr	D36 a, rec-c; nic-3	18
C666	SC	C419 A_{1-8} A, ad-5, rec-s, cr	C481 R_{13} a, rec-c; pyr-1	20
C703	CS	D25 a, rec-c; pan-2(B3)	C419 A_{1-8} A, ad-5, rec-s, cr	32
C967	CC	K110 x K4 #5 A, hist-2, rec-c, cr	C484 R_{59} a, rec-c; ylo-1, pan-2(B3), tryp-2	104
C1078	CS	As for C703	As for C703	56
C1085	SC	(ORa x K4) A_{9-1} a, ad-5, rec-s	C1009 R_{15} A, hist-2, rec-c, cr, al-2	46

Cross	Type	Protoperithecial parent	Conidial parent	Asci isolated
C1128	Het.	(C973 R_6 + C703 A_{22-1}) a, hist-2, rec-c, cr; ad-1, pan-2(B5) + a, rec-s, cr; pan-2(B3)	C967 A_{78-4} A, rec-c; ylo-1, tryp-2	CS 10 CC 75
C1130	Het.	As for C1128	As for C1128	CC 19
C1148	Het.	As for C1128	As for C1128	CC 78
C1170	Het.	As for C1128	As for C1128	CS 23 CC 199
C1182	CS	C860 R_{10} a, rec-c; lys-5	C703 A_{26-1} A, rec-s, cr	26
C1183	CS	C873 R_{20} leu-3, a, rec-c	C703 A_{26-1} A, rec-s, cr	104
C1198	SS	30JA-5 A, rec-s	C703 A_{25-5} a, ad-5, rec-s, cr; pan-2(B3)	130
C1199	CS	C1100 R_{48} a, rec-c, aur	C703 A_{3-7} A, ad-5, rec-s, cr; pan-2(B3)	98
C1206	CC	C973 R_6 a, hist-2, rec-c, cr; ad-1, pan-2(B5)	C967 A_{78-4} A, rec-c; ylo-1, tryp-2	177

Cross	Type	Protoperithecial parent	Conidial parent	Asci isolated
C1207	SC	C703 A^{22-1} a, rec-s, cr; pan-2(B3)	C967 A^{78-4} A, rec-c; yl0-1, tryp-2	50
C1219	CC	C1096 A^{1-1} a, rec-c, ad-3 A	C1085 A^{35-7} A, ad-5, hist-2, rec-c, cr, al-2	60
C1248	Het.	As for C1128	As for C1128	Mixed[1]. 23 CC 83
C1253	SC	As for C1207	As for C1207	64

1. Total asci from genetically mixed perithecia.

close to the linkage group II centromere, linkage could only be explained by the phenomenon of affinity of centromeres originating in the same parent. Affinity has been found in mice (Wallace, 1953) and possibly in cotton and tomatoes (Wallace, 1960a and b). In fungi it has been demonstrated in Saccharomyces cerevisiae (e.g. Shult and Lindegren, 1962), in Ascobolus immersus where the locus controlling affinity was separable from the centromere (Surzycki and Paszewski, 1964), and in Neurospora crassa (Prakash, 1963). The C651 data are not supported by C661, however, and are probably a result of inflated sample size. A similar effect is seen with C703 (section III), $p < 0.001$, which is also unsupported by its replica C1078. Since affinity does not seem to be specifically related to rec-s it will not be considered further.

C1207 and C1253 both show a high frequency of asci containing aborted spores (see section XII). In the absence of C1085, discussed below, Table 8 shows a trend towards heterogeneity, $0.1 > p > 0.05$, which seems to be due to the high -o- cr distances of C1207 and C1253, 35.9 ± 7.7 m.u. and 38.1 ± 6.1 m.u. respectively, as compared to the "without C1085" SC type overall value of 33.2 ± 3.3 m.u.

LEGEND FOR TABLES 6, 10, 16, 17, 25, 29, 44

1. Asci containing one or more aborted or colourless spore pairs. Details are presented in section XII. Where scorable they have been included in the appropriate crossover class.
2. Calculated as the % germination of spores from scored asci.
3. Numbers in brackets have been calculated from the sum of the crossover asci for the two separate regions, less twice the number of two strand double crossovers, less the number of three strand double crossovers. For each two or three strand double crossover 1½ is subtracted, for each three or four strand ½.
4. In some crosses al-2 strains have not been backcrossed to determine the presence of ylo-1. Hence asci scored for ylo-1 may be fewer than asci scored for other markers.
5. The total asci, n, scored from all crosses for that crossover region.
6. Map units, p, ± standard error, calculated as ½ x 100 x total crossover asci/total asci scored, n $\pm\sqrt{\frac{p(100 - p)}{n}}$.
7. Where no second division asci have been observed for a marker amongst n asci, the upper limit given is the upper 95% confidence limit p, where p is given by $(1 - \frac{2}{100} p)^n = 0.05$ (modified from Catcheside and Austin, 1971).

TABLE 6

REC-S X REC-C TYPE CROSSES

TOTAL CROSSOVERS BY REGIONS

Cross	C651	C661	C663	C666	C1085	C1207	C1253	Totals	Asci[5]	m.u.±S.E.[6]
Perithecia	-	13	-	-	6	9	8	> 39		
Asci isolated	15	60	18	20	46	50	64	273		
Asci scored	14	58	16	19	45	39	63	253		
Aborted asci[1]	2	4	2	2	0	5	6	21		
% germination[2]	75	90	86	86	79	61	86	81		
Crossovers										
mt - ad5	6	24	8	10	20			68	152	22.4±3.4
ad5 -o-	3	5	4	4	14			30	152	9.9±2.4
-o- cr	6	37	10	10	(14)	28	48	153	254	30.1±2.9
mt -o-	(7)[3]	(28)	(11)	(13)	(34)	15	29	137	254	27.1±2.9
-o- hist2					4			4	45	4.4±3.1
hist2 -cr					10			10	45	11.1±4.7
cr -al2					21			21	45	23.3±6.3
ylol -o-						7	7	14	102	6.9±2.5
-o- pan2						4	6	10	102	4.9±2.1
pan2 - tryp2						8	17	25	102	12.3±3.2
Miscellaneous	-o- arg5 4	-o- arg5 8	-o- nic3 9	-o- pyr1 3						

Group A

Group B

Group C

The rec-s parent of C1085 is most dissimilar to the rec-s parents of other SC crosses. This may be the cause of the trend towards heterogeneity, $0.1 << p < 0.05$, shown by C1085 in Table 7. Both the high mt -o- distance, 37.8 ± 7.2 m.u., and the low -o- cr distance, 15.6 ± 5.4 m.u., of C1085 relative to the overall SC values of 27.1 ± 2.9 m.u. and 30.1 ± 2.9 m.u. contribute to this heterogeneity.

The extreme overall G_H value for Table 8, $p < 0.001$, is thus due to the opposing effects of C1085 and C1207 and C1253, even though no one of the three crosses shows extreme deviations from the overall map distances. The pooled totals of Table 6 are therefore probably representative of SC type crosses.

Section III:

CS type crosses

Data from CS type crosses are summarised in Table 10.

Cross C1199 causes significant heterogeneity, $0.01 < p < 0.001$, in both Tables 11 and 12. The heterogeneity is apparently due to low crossing-over in the mt to centromere regions, and high crossing-over in -o- cr. One possible explanation is that the aur parent originates from only the second backcross to OR of an aur, lys-3 strain of predominantly Lindegren background.

LEGEND FOR HETEROGENEITY G TESTS

TABLES 7, 8, 9, 11, 12, 18, 19, 20, 21, 26, 27, 30

To take into account the total asci scored for each cross it is necessary to arrange for the marginal total for each cross to be equal to the total asci scored.

The asci from a cross with three crossover regions, 1, 2 and 3, may be distributed in the following 8 classes: non-crossover, region 1, region 2, region 3, regions: 1+2, 2+3, 1+3, 1+2+3. The sum of the classes will be the total asci scored. No information seems to be available on the minimum size permissible for any cell in a heterogeneity G test table. As a safety precaution any class with several cells < 5 has been pooled in such a way as to preserve both the distribution of crossovers and the marginal total of the cross. In the above example a single ascus in class 1+2+3 would be distributed as 1/3 to each of classes 1, 2 and 3. The data in the G_H test tables have been obtained in this manner.

TABLE 7

TABLE 6, GROUP A, HETEROGENEITY G TEST

Cross	C651	C661	C663	C666	C1085	Totals
n.co.	4	6	1	4	5	20
mt - ad5	2.5	11	3.333	5.333	16	38.166
ad5 -o-	2	4.5	2.833	1.833	11.5	22.666
-o- cr	2.5	24.5	4.833	3.833	8.5	44.166
mt - ad5, -o- cr	3	12	4	4	4	27
Total Asci	14	58	16	19	45	152

G_H = 22.28, d.f. = 16, n.s.

TABLE 8

TABLE 6, GROUP B, HETEROGENEITY G TEST

Cross	C651	C661	C663	C666	C1085	C1207	C1253	Totals
n.co.	5	6	1	4	5	7	8	36
mt -o-	3	15	5	5	26	4	7	65
-o- cr	2	24	4	2	7	17	26	82
mt -o- cr	4	13	6	8	7	11	22	71
Total asci	14	58	16	19	45	39	63	254

$G_H = 52.17$, d.f. = 18, $0.001 > p$

Without C1085: $G_H = 24.96$, d.f. = 15, n.s.

TABLE 9

TABLE 6, GROUP C, HETEROGENEITY G TEST

Cross	C1207	C1253	Totals
n.co.	3	5	8
mt -o-	4.75	8	12.75
-o- cr	12.083	23.166	35.25
ylol -o-	3.416	2.75	6.166
-o- pan2	2.833	2.166	5
pan2 - tryp 2	1.916	6.916	8.833
mt -o- cr	7	11	18
-o- cr, pan2 - tryp2	4	4	8
Total asci	39	63	102

$G_H = 3.22$, d.f. = 7, n.s.

TABLE 10

REC-C X REC-S TYPE CROSSES

TOTAL CROSSOVERS BY REGIONS

Cross	C703	C1078	C1199	C1182	C1183*	C1183	Totals		
Perithecia	-	-	7	3	7	10	> 29		
Asci isolated	32	56	98	26	51	53	316		
Asci scored	31	51	93	24	49	34	282		
Aborted asci[1]	2	2	1	1	6	3	15		
% germination[2]	91	86	88	74	82	49	81		
Crossovers								Asci[5]	m.u.±S.E.[6]
	Group A								
mt - ad5	14	18	14				46	175	13.1±2.6
ad5 -o-	8	9	11				28	175	8.0±2.1
-o- pan2	2	4	6				12	175	3.4±1.4
	Group B								
-o- cr	22	34	69	13	32	29	199	282	35.3±2.8
mt -o-	(21)[3]	(27)	(24)	10		16	98	233	21.0±2.7
cr - aur			53				53	93	28.5±4.7

*The first 51 asci of C1183 were scored only for crisp.

See p. 34 for legend.

TABLE 11

TABLE 10, GROUP A, HETEROGENEITY G TESTS

Cross	C703	C1078	C1199	Totals
n.co.	3	10	14	27
mt - ad5	4.333	3	2.666	10
ad5 -o-	3.333	4.333	5.666	13.333
-o- cr	8.666	12.833	53.5	75
-o- pan2	0.666	1.833	3.166	5.666
mt - ad5, -o- cr	8	15	7	30
ad5 -o- cr	3	4	6	13
Total Asci	31	51	92[1]	174

G_H = 28.88, d.f. = 12, 0.01 > p > 0.001.

Without C1199: G_H = 3.66, d.f. = 6, n.s.

[1] One ad5 -o- OR -o- cr 4sd ascus has not been included.

TABLE 12

TABLE 10, GROUP B, HETEROGENEITY G TESTS

Cross	C703	C1078	C1199	C1182	C1183	Totals
n.co.	3	10	16	6	4	39
mt -o-	7	7	8	5	1	28
-o- cr	8	14	53	8	14	97
mt -o- cr	13	20	16	5	15	69
Total asci	31	51	93	24	34	233

G_H = 32.29, d.f. = 12, 0.01 > p > 0.001

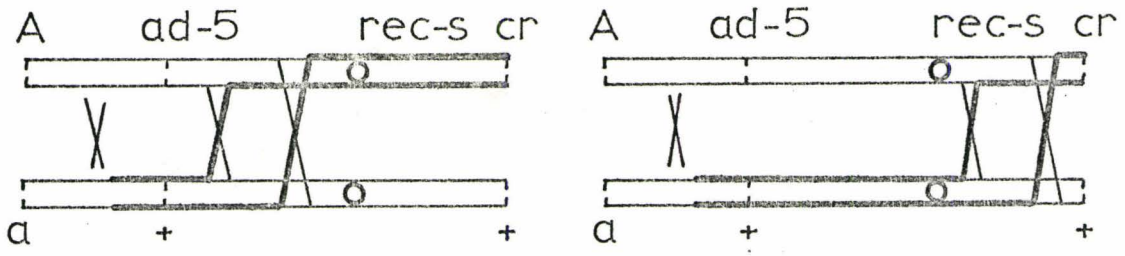
Without C1199: G_H = 13.50, d.f. = 9, n.s.

The aur mutant was chosen as being part of the albino complex (Subden and Threlkeld, 1970) so that map distances involving it would be comparable to distances obtained with al-2. Recent evidence, however, places aur about 2.5 m.u. distal to al-2 with arg-6, and possibly hs, in between (Perkins, 1971). The resulting error has been taken into account when comparing different cross types, section VIII.

In addition to heterogeneity tables 11 and 12 a check was made for heterogeneity in the -o- cr data between the first 51 asci of C1183, which were scored only for cr, and the last 53 asci, which were scored for cr and mt: G_H (Yates) = 3.29, d.f. = 1, not significant.

The randomised data from C703 show possible centromere affinity, $p < 0.001$, between linkage groups I and VI, but this is not supported by the replica cross, C1078, and is probably due to an inflated sample size. See previous section.

C703 A_{30} has a crossover between mt and ad-5 and a four strand double crossover between either ad-5 and centromere or centromere and cr. If rec-s is assumed to lie right of the centromere (see section XI) it becomes possible to distinguish the two possible four strand double crossovers, see diagram below, by backcrossing.



Both crisp spores must carry rec-s

Both, one, or neither of the crisp spores carry rec-s.

The thickly drawn strands are the two strands backcrossed.

From the C1229 (Table 13) and C1230 (Table 14) backcrosses alone it seems that C703 A_{30-2} carries rec-c and A_{30-4} carries rec-s placing the double crossover in the -o- cr region. The only crosses from this work available for comparison with C1229 and C1230 are C1219, which appears to have the high (section V) mt - ad-3A crossover frequency for a CC cross of 20.8 ± 5.2 m.u., and the CC cross C1220 (Table 15) which is also high. Further, C1229 has low germination and a trend towards a high frequency of double crossovers. This is suggestive of a chromosome aberration which might be responsible for the low recombination, but no evidence of aborted spores was found in the cross. The possible role of aberrations in controlling recombination is further discussed in section IX. Hargrave has obtained a value of 36.5 ± 3.4

TABLE 13

C1229: C703 A_{30-2} Backcross

C703 A_{30-2} x C1164 R_{29}

a, cr A, ad-3A, aur

Crossover type	Genotype	Random spores
Parentals	a + cr +	30
	A ad3 + aur	27
mt - ad3	a ad3 + aur	2
	A + cr +	0
ad3 - cr	a + + aur	0
	A ad3 cr +	1
cr - aur	a + cr aur	9
	A ad3 + +	5
mt - ad3 cr - aur	a ad3 + +	1
	A + cr aur	0
ad3 - cr cr - aur	a + + +	2
	A ad3 cr aur	1
		78 (germination 75%)

Region	Total crossovers	m.u. ± S.E.
mt - ad3	3	3.8 ± 2.2
ad3 - cr	4	5.1 ± 2.5
cr - aur	18	23.1 ± 4.8

TABLE 14

C1230: $C703_{30-4}$ Backcross

C703 A_{30-4} x C1164 R_{53}

A, cr a, ad-3A, aur

Crossover type	Genotype	Random spores
Parentals	A + cr +	22
	a ad3 + aur	27
mt - ad3	A ad3 + aur	5
	a + cr +	10
ad3 - cr	A + + aur	3
	a ad3 cr +	8
cr - aur	A + cr aur	13
	a ad3 + +	11
mt - ad3 cr - aur	A ad3 + +	1
	a + cr aur	1
ad3 - cr cr - aur	A + + +	1
	a ad3 cr aur	0
		102 (germination 98%)

Region	Total crossovers	m.u. ± S.E.
mt - ad3	17	16.7 ± 3.7
ad3 - cr	12	11.8 ± 3.2
cr - aur	27	26.5 ± 4.4

TABLE 15

CC TYPE CROSS: C1220

C1096 A_{1-1} x C1038 R_{24}

a, ad-3A A, hist-3

Crossover type	Genotype	Random spores
Parentals	A hist3 +	98
	a + ad3	97
mt - hist3	A + ad3	19
	a hist3 +	18
hist3 - ad3	A hist3 ad3	1
	a + +	3
		236 (germination 86%)

Region	Total crossovers	m.u. ± S.E.
mt - hist3	37	15.7 ± 2.4
hist3 - ad3	4	1.69 ± 0.84
mt - ad3	41	17.4 ± 2.5

for mt - ad-3A from an SC type cross (section XIII) but, if aberrations are excluded, the C1229 - C1230 comparison is more likely to be meaningful than comparisons with crosses of very different origins so the conclusion of a -o- cr four strand double is probably correct. It has been included as such in the data.

Section IV:

SS type cross C1198

TABLE 16

REC-S X REC-S TYPE CROSS C1198

TOTAL CROSSOVERS BY REGIONS

Perithecia	25	
Asci isolated	130	
Asci scored	121	
Aborted asci[1]	7	
% germination[2]	82	m.u.±S.E.[6]
Crossovers		
mt - ad5	80	33.0±4.3
ad5 -o-	14	5.8±2.1
mt -o-	(88)[3]	36.4±4.4
-o- cr	63	26.0±4.0
-o- pan2	10	4.1±1.8

See p..34 for legend

Section V:

CC type crosses

Data from the main CC type crosses are summarised in Table 17.

An assumption inherent to the interpretation of heterogeneity tests between crosses analysed by asci is that recombination frequency does not vary with the stage of development at which the asci are isolated. The relationship between recombination frequency and the time of expulsion of unordered asci from the perithecium - which would be expected to be related to the degree of maturation and, therefore, choice of ordered asci to be dissected - has been studied by Strickland and Thorpe (1963). Using linkage group V markers they found one perithecium out of the 5 studied showing a significant change in recombination frequency with time. Lamb (1967), however, has shown that the observed changes in recombination frequency among shot asci with time for markers on linkage groups I, V and VI are due to differential maturation and bursting of asci, and may be overcome by early dissection of intact asci. In this study C1170 (section VI) and C1206 (Table 18) have been studied for changes in recombination frequency with time of isolation of asci. The significant change in C1170 with time may be due to one large aberrant perithecium.

TABLE 17

REC-C X REC-C TYPE CROSSES

TOTAL CROSSOVERS BY REGIONS

Cross	C967	C1206	C1219	Total	Asci[5]	m.u.±S.E.[6]
Perithecia	12	9	3	24		
Asci isolated	104	177	60	341		
Asci scored	91	174	60	325		
Asci for ylo1[4]	91	167	-	258		
Aborted asci[1]	8	3	0	11		
% germination[2]	76	80	83	80		
Crossovers						
mt - ad5			18	18	60	15.0±4.6
ad5 -o-			8	8	60	6.7±3.2
	Group A					
mt -o-	19	27	(25)	71	325	10.9±1.7
-o- hist2	0	0	0	0	325	< 0.46
hist2 - cr	10	14	(3)	27	325	4.2±1.1
-o- cr	(10)[3]	(14)	(3)	27	325	4.2±1.1
		Group B				
cr - al2		65	34	99	234	21.2±2.7
ylo1 - ad1		15		15	167	4.5±1.6
ad1 -o-		2		2	174	0.6±0.6
ylo1 -o-	10	(15)		25	258	4.8±1.3
-o- pan2	5	13		18	265	3.4±1.1
pan2 - tryp2	30	54		84	265	15.8±2.2
	Group C					
Miscellaneous			ad3-cr 3	ad3-cr 3	60	2.5±2.0

See p.34 for legend.

TABLE 18

C1206 HETEROGENEITY G TEST

Time[1]	0 hrs	65 hrs	113 hrs	141 hrs	Totals
n.co.	19	15	12	13	59
mt -o-	3.833	4.333	3.333	6.833	18.333
-o- cr	2.666	2	3.5	2.5	10.666
cr - al2	4.833	8.833	15.333	8.833	37.833
pan2 - tryp2	8.666	6.833	6.833	8.833	31.166
cr - al2, pan2 - tryp2	2	5	4	6	17
Total asci	41	42	45	46	174

G_H = 12.03, d.f. = 15, n.s.

[1] Time of opening of the perithecia, the first being opened at zero time.

Table 18 shows no change with time for C1206 frequencies. The C1206 total crossover data (not given) did, however, suggest an upward trend for the cr - al-2 frequency. This was analysed by transforming the recombination frequencies to arc sine (Sokal and Rohlf, 1969, p. 430) values and testing for the significance of the regression against time. No evidence for a change in recombination frequency with time was obtained.

The low germination of C967 is mainly due to low germination of crisp spores amongst the latter half of the cross. Recombination frequencies are similar between the two halves and the linkage group VI markers show the expected 1:1, mutant to wild type, segregation amongst the low germination half.

The protoperithecial parent of C967, K110 x K_4 #5, is, from the unambiguous evidence of C967, a histidine requiring, rec-c, crisp type strain. Yet it was isolated from a rec-s x rec-s type cross. Since C967 A_{78-4} has been used extensively in the heterokaryon crosses the origin of #5 has been investigated in detail. Its parent cross was:

K110	x	K_4
A, rec-s		a, ad-5, rec-s, cr

the rec-s character of both parents having been demonstrated by Newcombe (1969), and supported by this work. The K_4 ad-5

allele Y152M40 appears to have an additional, and highly variable requirement for histidine. This requirement is discussed in detail in Appendix II and it now appears highly unlikely that it is due to a separate gene. At the time of isolation of #5 several histidine requiring loci were in use in the laboratory, including hist-2's Y175M611, Y152M43 and Y152M14, the latter being the only hist-2 used in linkage with cr (P369), of which the only allele in use was B123. #5 failed to complement with any of the hist-2's and prototroph analysis of crosses between #5 and the hist-2 alleles gave the results:

Number of crosses	Known hist-2 parent	Allele	germinating spores	% germination	Prototrophs
2	FGSC 667-11	Y175M611	41,500	87	2
2	C1226 R_{46}	Y152M43	58,500	88	2
1	P369	Y152M14	20,000	90	0

No control crosses were done amongst the "known" parents and no data are available in the literature on the positions of these alleles within the hist-2 locus so no conclusions can be drawn other than that #5 is a hist-2.

The crisp allele of #5 was not tested for identity with B123 though it has been recently shown (Garnjobst and Tatum, 1970) that two other crisp loci are located on linkage group I. These, however, are distal to B123 and would be expected to give higher second division

frequencies than are found in C967. The most likely origin for #5 would seem to be contamination by P369.

Garnjobst and Tatum (1970) have also demonstrated a high spontaneous occurrence of modifiers of crisp - an observation supported by this study. C967 A_{10-6-4} was isolated from a A, hist-2, cr strain which reverted after 3 days of growth to a non-crisp, pale yellow and largely aconidial strain resembling Garnjobst's m-4. It was backcrossed to OR_{a-1-3} and 50 crisp, 63 wild type and 43 yellow-aconidials were recovered. Germination was 83%. Since C967 A_{10-6-4} was not necessarily homokaryotic this provides evidence only for a morphological mutant dominant to crisp.

C1219 appears to have an abnormally high mt to centromere crossover frequency (Tables 17 and 19) but normal -o- cr frequency. On the basis of the latter the cross has been classified as a type CC cross - in all cases where cross types are unambiguous a large increase in -o- cr distance is found in crosses with one or more rec-s parents as compared to known CC type crosses. The A, ad-5, hist-2, rec-c, cr, al-2 parent of C1219 inherits its ad-5 to centromere region from C1085, an SC cross which also shows abnormally high mt -o- crossover frequency (section II). Unfortunately the data obtained during this work do not provide further data for mt - ad-5 and ad-5 -o-

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TABLE 19

TABLE 17, GROUP A, HETEROGENEITY G TESTS

Cross	C967	C1206	C1219	Total
n.co.	64	134	32	230
mt -o-	18	26.5	25	69.5
-o- cr	9	13.5	3	25.5
Total asci	91	174	60	325

G_H = 17.47, d.f. = 4, 0.01 > p > 0.001

Without C1219: G_H = 1.39, d.f. = 2, n.s.

TABLE 20

TABLE 17, GROUP B, HETEROGENEITY G TESTS

	C1206	C1219	Totals
n.co.	110	29	139
cr - al2	64	31	95
Total asci	174	60	234

G_H (Yates) = 3.47, d.f. = 1, n.s.

TABLE 21

TABLE 17, GROUP C, HETEROGENEITY G TEST

Cross	C967	C1206	Total
n.co.	58	108	166
-o- pan2	4.5	12.5	17
pan2 - tryp2	28.5	53.5	82
Total asci	91	174	265

$G_H = 0.52$, d.f. = 2, n.s.

in CC crosses.

In C1206 and the heterokaryon crosses C1128, C1130 and C1148 al-2 strains from asci in which the arrangement of ylo-1 and ylo-1$^+$ markers was uncertain were backcrossed to OR wild types and 16 random spores isolated. In asci in which no ylo-1's could be detected by this method 6 al-2$^+$ ylo-1$^+$ spores were taken as sufficient evidence of ylo-1$^+$ in the parent.

A large amount of data on the cr - al-2 distance in CC type crosses was obtained from these backcrosses (Table 22). The G test shows significantly low cr

TABLE 22

cr - al-2 data from CC(Het) backcrosses

Genotype	Observed random spores	Expected
al cr	722	766.5
al +	254	236.5
+ cr	223	236.5
+ +	811	766.5
Total spores	2010	

$G = 14.97$, d.f. = 3, $0.01 > p > 0.001$

cr - al-2 map distance 23.73 ± 0.95 m.u.

germination but the similarity of the cr, al-2: cr^+, $al\text{-}2^+$ ratio (0.89) to the cr, $al\text{-}2^+$: cr^+, al-2 ratio (0.88) suggests that this will have little effect on the map distance.

The upper limit for -o- hist-2 map distance in Tables 17 and 25 is the upper 95% confidence limit. A total total of 3 apparent -o- hist-2 crossovers were observed in CC and CC(Het) crosses: one was also second division for mt, ad-1 and pan-2 in an asymmetric pattern and was therefore probably due to nuclear passing

(Howe, 1956); the other two asci contained an aborted spore pair and could therefore be explained by assuming the extra space in the ascus allowed slippage of spore pairs.

Data from two subsidiary CC crosses are given in Tables 23 and 24. C1009 map distances are in agreement with other CC crosses, though the significant deviation, $0.01 > p > 0.001$, from a 1:1, mutant to wild type, allele ratio for linkage group VI markers is unexplained. Linkage group I markers are unaffected. Since germination is 91% it seem unlikely to be due to differential viability. The reciprocal cross for C1009, C973, data not given, gave only 83% germination and a number of strains of abnormal morphology suggesting that P369 had accumulated cytoplasmic modifiers of the hyphal morphology, or that only certain regions of the culture had accumulated nuclear modifiers. The map distances given by C1009 are significantly different, $0.05 > p > 0.01$, from those given by C1107 illustrating again the variation between even closely related crosses.

Section VI:

CC type components of heterokaryon crosses from non-mixed perithecia

Data from the CC components of heterokaryon crosses is given in Table 25.

TABLE 23

Subsidiary cross C1107

C1009 R_{22} x C1009 R_{33}

A, rec-c, al-2 a, rec, cr; ad-1, pan-2 (B5)

Crossover type	Genotype					Random spores
Parental	A	+	al2	+	+	26
	A	+	al2	adl	pan2	24
	a	cr	+	+	+	30
	a	cr	+	adl	pan2	27
mt - cr	A	cr	+	+	+	8
	A	cr	+	adl	pan2	1
	a	+	al2	+	+	9
	a	+	al2	adl	pan2	4
cr - al	A	+	+	+	+	8
	A	+	+	adl	pan2	14
	a	cr	al2	+	+	14
	a	cr	al2	adl	pan2	15
adl - pan2	A	+	al2	adl	+	3
	A	+	al2	+	pan2	2
	a	cr	+	adl	+	4
	a	cr	+	+	pan2	1
mt - cr - al	A	cr	al2	+	+	4
	A	cr	al2	adl	pan2	1
cr - al; adl - pan2	a	cr	al2	adl	+	1
	a	cr	al2	+	pan2	2
						Total 198
						Germination 95%

.... Continued on next page

TABLE 23 continued

Region	Total crossovers	m.u. ± S.E.
mt - cr	27	13.6 ± 2.4
cr - al2	59	29.8 ± 3.3
adl - pan2	13	6.6 ± 1.8

TABLE 24

Subsidiary cross C1009

14-5 x P369

a, rec-c, al-2; ad-1, pan-2(B5) A, hist-2, rec-c, cr

Crossover type	Genotype						Random spores
Parental	A	hist2	cr	+	+	+	13
	A	hist2	cr	+	adl	pan2	13
	a	+	+	al2	+	+	21
	a	+	+	al2	adl	pan2	11
mt - hist2	A	+	+	al2	+	+	3
	A	+	+	al2	adl	pan2	4
	a	hist2	cr	+	+	+	3
	a	hist2	cr	+	adl	pan2	2
hist2 - cr	a	+	cr	+	+	+	3
	a	+	cr	+	adl	pan2	1
cr - al2	A	hist2	cr	al2	+	+	5
	A	hist2	cr	al2	adl	pan2	0
	a	+	+	+	+	+	6
	a	+	+	+	adl	pan2	1
adl - pan2	A	hist2	cr	+	+	pan2	1
hist2 - cr - al	A	hist2	+	+	+	+	2
mt - hist2 - cr	a	hist2	+	al2	+	+	1
mt - hist2, cr - al	A	+	+	+	+	+	2
hist2 - cr - al	a	+	cr	al	+	+	1
mt - hist; adl - pan2	a	hist2	cr	+	adl	+	1
mt - hist2 - cr adl - pan2	a	hist2	+	al	adl	+	1
							Total 95
							Germination 91%

TABLE 24 continued

Region	Total crossovers	m.u. ± S.E.
mt - hist2	17	17.9 ± 3.9
hist2 - cr	9	9.5 ± 3.0
cr - al2	17	17.9 ± 3.9
adl - pan2	3	3.2 ± 1.8

ad-1, *pan-2* 32: *ad-1*$^{+}$, *pan-2*$^{+}$ 60

$G = 8.65$, d.f. = 1, $0.01 > p > 0.001$

TABLE 25

HETEROKARYON CROSSES:

REC-C X REC-C TYPE, NON-MIXED PERITHECIA

TOTAL CROSSOVERS BY REGIONS

Cross	C1128	C1130	C1148	C1170	C1248	Totals	Asci[5]	m.u.±S.E.[6]
Perithecia	6	3	3	9	9	30		
Asci isolated	75	19	78	199	83	454		
Asci scored	74	18	77	185	82	436		
Asci for ylo[4]	74	18	75	107	56	330		
Aborted asci[1]	3	0	0	1	1	5		
% germination[2]	80	72	75	79	90	79		
Crossovers								
			Group A					
mt -o-	19	0	16	28	26	89	436	10.2±1.4
-o- hist2	0	0	0	0	0	0	436	<0.34
hist2 - cr	6	1	6	21	8	42	436	4.8±1.0
cr - al2	32	5	32	83	28	180	436	20.6±1.9
-o- cr	(6)[3]	(1)	(6)	(21)	(8)	42	436	4.8±1.0
ylo1 - ad1	6	4	3	11		24	354	3.4±1.0
ad1 -o-	1	0	0	4		5	354	0.7±0.4
			Group B					
ylo1 -o-	(7)	(4)	(3)	(12½)	4	30½	330	4.6±1.2
-o- pan 2	3	1	4	8	Group	16	354	2.3±0.8
pan2 - tryp2	15	4	29	71	C	119	354	16.8±2.0

See p.34 for legend.

Cl170 asci were isolated in two groups: the early group with 2 perithecia including one perithecium from which 108 asci were isolated, and the late group with 7 perithecia. The slight heterogeneity, $0.05 > p > 0.01$, in Table 26 given by the early group of Cl170 is illustrative of the bias which may be caused by one perithecium with many asci.

TABLE 26

TABLE 25, GROUP A, HETEROGENEITY G TESTS

Cross	Cl128	Cl130	Cl148	Early[1] Cl170	Late[1] Cl170	Cl248	Totals
n.co.	27	12	34	53	29	38	193
mt -o-	9.5	0	8.333	10	6	14.333	48.666
hist2 - cr	5.5	1	3.833	12	1	5.833	33.666
cr - al2	23	5	24.333	38.5	22	15.833	128.666
mt -o-, cr - al2	9	0	6	3	6	8	32
Total asci	74	18	77	121	64	82	436

$G_H = 36.02$, d.f. = 20, $0.05 > p > 0.01$.

Without early Cl170: $G_H = 20.91$, d.f. = 16, n.s.

[1]Early asci isolated April 3rd.
Late asci isolated April 11th to 14th.

TABLE 27

TABLE 25, GROUP B, HETEROGENEITY G TEST

Cross	C1128	C1130	C1148	C1170	C1248	Totals
n.co.	67	14	72	94.5	52	299.5
ylol -o-	7	4	3	12.5	4	30.5
Total asci	74	18	75	107	56	330

G_H = 6.78, d.f. = 4, n.s.

TABLE 28

TABLE 25, GROUP C, HETEROGENEITY G TEST

Cross	C1128	C1130	C1148	C1170	Totals
n.co.	57	13	45	109	224
-o- pan2	2.5	1	3.5	7.5	14.5
pan2 - tryp2	14.5	4	28.5	68.5	115.5
Total asci	74	18	77	185	354

G_H = 10.02, d.f. = 6, n.s.

Section VII:

SC type components of heterokaryon crosses from non-mixed perithecia

TABLE 29

HETEROKARYON CROSSES:

REC-S X REC-C TYPE, NON-MIXED PERITHECIA

TOTAL CROSSOVERS BY REGIONS

	C1128	C1170	Total	
Perithecia	1	1	2	
Asci isolated	10	23	33	
Asci scored	10	19	29	
Aborted asci[1]	1	2	3	
% germination[2]	69	76	74	
Crossover asci				m.u.±S.E.[6]
mt -o-	4	8	12	20.7±7.5
-o- cr	8	16	24	41.3±9.3
ylol -o-	2	1	3	5.2±4.1
-o- pan2	2	3	5	8.6±5.2
pan2 - tryp2	4	6	10	17.2±7.0

See p. 34 for legend.

TABLE 30

TABLE 29, HETEROGENEITY G TEST

Cross	C1128	C1170	Totals
n.co.	1	2	3
mt -o-	1.666	4	5.666
-o- cr	5.166	10.5	15.666
pan2 - tryp2	2.166	2.5	4.666
Total asci	10	19	29

$G_H = 0.40$, d.f. = 3, n.s.

Section VIII:

Comparisons of the different cross types

As pointed out in section I of this chapter, in comparing crosses it is necessary to consider heterogeneities within pooled crosses as they arise, and not merely eliminate the cross causing heterogeneity from the comparisons. For this reason Tables 31 to 40 give data for the various cross types with and without crosses previously shown to cause heterogeneity within a type. Not all possible correlations have been tested statistically: in many cases it is possible to deduce

significance, or lack of it, from the results of tests on limiting cases.

The cross types are compared one crossover region at a time. Three extra notations are used in this section, illustrated here by examples:

CS - C1199 denotes pooled data from all the CS crosses with the exception of C1199.

CS v. SC denotes a statistical test for heterogeneity between the pooled CS cross data and the pooled SC cross data (v. = versus).

(CS + SC) denotes the combined data from CS and SC crosses.

These notations may be combined in various ways:

e.g. CC v. CS - C1199 v. SC denotes the test for heterogeneity between CC, SC, and CS, without C1199, data.

TABLE 31

INTER-TYPE mt - ad-5 COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC (C1219)	18	42	60	15.0 ± 4.6
CS	46	129	175	13.1 ± 2.6
CS - C1199	32	50	82	19.5 ± 4.4
SC	68	84	152	22.4 ± 3.4
SC - C1085	48	59	107	22.4 ± 4.0
SS	80	41	121	30.0 ± 4.3
Totals	212	296	508	

Overall G_H = 51.29, d.f. = 3, 0.001 > p
CS v. SC G_H = 12.22, d.f. = 1, 0.001 > p
CS - C1199 v. SC G_H = 0.71, d.f. = 1, n.s.
CC v. CS - C1199 v. SC G_H = 4.03, d.f. = 2, n.s.
CS - C1199, v. SC v. SS G_H = 18.39, d.f. = 2, 0.001 > p

CS cross C1199 has been shown (section III) to have a low mt -o- recombination frequency, hence it is likely that no difference exists between CS and SC crosses. CC cross C1219 appears to have a high mt -o- recombination frequency, which may account for the lack of significant difference between it and CS - C1199 and SC crosses. It seems probable that recombination frequencies in this region are: SS >> SC = CS > CC.

TABLE 32

INTER-TYPE ad-5 -o- COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC (C1219)	8	52	60	6.7 ± 3.2
CS	28	147	175	8.0 ± 2.1
CS - C1199	17	65	82	10.4 ± 3.4
SC	30	122	152	9.9 ± 2.4
SC - C1085	16	91	107	7.5 ± 2.5
SS	14	107	121	5.8 ± 2.1
Totals	80	428	508	

Overall - C1199 G_H = 2.54, d.f. = 3, n.s.

The overall - CS cross C1199 G_H test is the closest to being significant for this table. In view of Newcombe's data (1969), see section XIV, on the ad-5 - hist-2 region the lack of significant differences between any of the cross types in Table 32 is unexpected. However, the comparisons for -o- hist-2 show that the increases for ad-5 - hist-2 shown by Newcombe can probably be attributed to increases in the -o- hist-2 region. This would be in agreement with the results of Scott-Emuakpor (1965a) who showed no change for arg-3 -o- after transfer of arg-3 from N. crassa to N. sitophila by Threlkeld (1961). A relationship CC = CS = SC = SS seems probable.

TABLE 33

INTER-TYPE mt -o- COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC	71	254	325	10.9 ± 1.7
CC - C1219	46	219	265	8.7 ± 1.7
CC (Het)	89	347	436	10.2 ± 1.4
CS	98	135	233	21.0 ± 2.7
CS - C1199	74	66	140	26.4 ± 3.7
SC	137	117	254	27.1 ± 2.9
SC - C1085	103	106	209	24.6 ± 3.0
SC (Het)	12	17	29	20.7 ± 7.5
SS	88	33	121	36.4 ± 4.4
Totals	495	903	1398	

CS v. SC v. SC (Het) G_H = 7.40, d.f. = 2, 0.05 > p > 0.01
CS v. SC - C1085 G_H = 2.32, d.f. = 1, n.s.
CS v. SC G_H = 6.88, d.f. = 1, 0.01 > p > 0.001
CC v. CS G_H = 26.09, d.f. = 1, 0.001 > p
SC v. SS G_H = 12.41, d.f. = 1, 0.001 > p

The apparent difference between CS and SC crosses may be explained by the high recombination frequency for SC cross C1085. Assuming the conclusion of no differences among cross types for the ad-5 -o- region is correct, this table, with its larger sample sizes, supports the supposition that for mt - ad-5 the recombination frequencies are SS > SC = CS > CC.

TABLE 34

INTER-TYPE -o- hist-2 COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E. or 95% upper limit
CC + CC (Het)	0	761	761	< 0.20
SC[1]	20	285	305	3.3 ± 1.0
Totals	20	1046	1066	

[1] Including data of Hargrave's (section XIII)

Although a G test is not meaningful for this table, the 95% confidence limit and the standard error strongly support the relationship SC >> CC for this region.

TABLE 35

INTER-TYPE -o- ad-3 COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E. or 95% upper limit
CC	0	60	60	< 4.9
SC[1]	169.5	218.5	388	21.8 ± 2.1

[1] Data from Hargrave, section XIII

A G test is again inapplicable, but the data strongly support SC >> CC.

ad-3 - cr region:

No table has been given for this since precise data are slight. C1219 gives a CC value for this region of 2.2 ± 2.0. From Tables 35 and 36 a rough estimate of ad-3 - cr in SC crosses would be in the range 8 to 13 showing SC > CC.

TABLE 36

INTER-TYPE -o- cr COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC	27	298	325	4.2 ± 1.1
CC - C1219	24	241	265	4.5 ± 1.3
CC (Het)	42	394	436	4.8 ± 1.0
CS	199	83	282	35.3 ± 2.8
CS - C1199	130	59	189	34.4 ± 3.5
SC	153	101	254	30.1 ± 2.9
SC - C1085	139	70	209	33.3 ± 3.3
SC (Het)	24	5	29	41.4 ± 9.1
SS	63	58	121	26.0 ± 4.0
Totals	508	939	1447	

CS v. SC G_H = 6.33, d.f. = 1, 0.05 > p > 0.01
SC v. SC (Het) G_H = 6.25, d.f. = 1, 0.05 > p > 0.01
SC v. SS G_H = 2.23, d.f. = 1, n.s.
SC - C1085 v. SS G_H = 6.68, d.f. = 1, 0.01 > p > 0.001
CC v. CC (Het) G_H = 0.40, d.f. = 1, n.s.

It has been suggested (section II) that SC cross C1085 has a low -o- cr recombination frequency but that this may be compensated for by other SC crosses. CS cross C1199 has been shown (section III) to be high in -o- cr crossovers. Since the degree of significance is low, since Neurospora crosses generally show a high level of variation, and since the effects of C1199 and C1085 are

uncertain, the higher crossing over in CS crosses than in SC crosses is probably spurious. There is unfortunately only one cross in the SS class so the validity of the SC - C1085 v. SS comparison is suspect, particularly as Newcombe (1969) (section XIII) finds no difference between CS and SS crosses. A relationship SS = SC = CS >> CC is suggested. The lack of difference between CC and CC (Het) indicates the lack of a demonstratable effect of a diffusible rec-s gene product in non-mixed perithecia. Since an effect cannot be shown in mixed perithecia (section X), and since a dilution effect might be expected in unmixed perithecia (Catcheside, 1968) this observation is not unexpected.

TABLE 37

INTER-TYPE *cr* - *al-2/aur* COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC	99	135	234	21.2 ± 2.7
CC - C1219	65	109	174	18.7 ± 3.0
CC (Het)	180	256	436	20.6 ± 1.9
CS (C1199)	53	40	93	28.5 ± 4.7
SC (C1085)	21	24	45	23.3 ± 6.3
Totals	353	455	808	

Overall G_H = 8.05, d.f. = 3, 0.05 > p > 0.01
(CC + CC Het) v. (CS + SC) G_H = 1.47, d.f. = 1, n.s.
CS v. CC (Het) G_H = 7.62, d.f. = 1, 0.01 > p > 0.001
CC - C1219 v. CC (Het) v. SC G_H = 1.52, d.f. = 2, n.s.

The overall significant G_H value seems to arise mainly from the high CS value. Since the marker in the CS cross is *aur* which may be up to 2.5 map units right of *al-2* (Perkins, 1971), the most that can be claimed for this region is a trend towards higher recombination frequencies in crosses involving rec-s.

Linkage group VI regions:

TABLE 38

INTER-TYPE ylo-1 -o- COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC	25	233	258	4.8 ± 1.3
CC (Het)	30½	299½	330	4.6 ± 1.2
SC	14	88	102	6.9 ± 2.5
SC (Het)	3	26	29	5.2 ± 4.1
Totals	72½	646½	719	

Overall G_H = 1.63, d.f. = 3, n.s.

TABLE 39

INTER-TYPE -o- pan-2 COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC	18	247	265	3.4 ± 1.1
CC (Het)	16	338	354	2.3 ± 0.8
CS	12	163	175	3.4 ± 1.4
CS - C1199	6	76	82	3.7 ± 2.1
SC	10	92	102	4.9 ± 2.1
SC (Het)	5	24	29	8.6 ± 5.2
SS	10	111	121	4.1 ± 1.8
Totals	71	975	1046	

Overall G_H = 8.54, d.f. = 4, n.s.

TABLE 40

INTER-TYPE pan-2 - tryp-2 COMPARISONS

Cross type	co. asci	n.co. asci	Total asci	m.u. ± S.E.
CC	84	181	265	15.8 ± 2.2
CC (Het)	119	235	354	16.8 ± 2.0
SC	25	77	102	12.3 ± 3.2
SC (Het)	10	19	29	17.2 ± 7.0
Totals	238	512	750	

Overall G_H = 3.25, d.f. = 3, n.s.

It is evident from these tables that rec-s has little effect on these regions of linkage group VI.

Section IX:

Multiple crossovers and interference

Positive chiasma interference between short intervals on the same side of the centromere, but not across the centromere, has been reported for both N. crassa and N. sitophila by many workers (see below). Similar results have been obtained in this study with both CC crosses and those with parents of sitophila

origin (Tables 41 and 42) though the latter show a trend to positive interference across the centromere.

The evidence for chromatid interference in N. crassa is conflicting: Lindegren and Lindegren (1942) found an excess of two strand double crossovers (2sd) across the linkage group I centromere and a trend towards an excess of four strand double crossovers (4sd) in the proximal, but not distal, region of one arm. Stadler (1956) failed to find chromatid interference across the linkage group VI centromere. Howe (1956) showed that an apparent excess of 2sd across the linkage group I centromere was due to nuclear passing, detected by a high correlation of asymmetric 2sd and second division for a centromere marker on a second chromosome, and suggested Lindegren's results could be explained in the same way. Rifaat (1956) supported Lindegren's results, as did Whitehouse (1958) who confirmed the proximal, but not distal, excess of 4sd. Strickland (1961) found an excess of 2sd within the right arm of linkage group V, but only in adjacent regions. Bole-Gowda et al. (1962, using a centromere marker on a second chromosome, found a trend towards an excess of 2sd both within one arm and across the linkage group I centromere. This was confirmed by Perkins (1962), but without the use of an extra centromere marker. Prakash (1964b), using a second centromere marker, also found an excess of 2sd within and

LEGEND FOR TABLES 41, 42 AND 43

1. The strand not involved in a 3sd crossover. Noted as S (strand of rec-s origin) or C (strand of rec-c origin) for CS, SC and SC (Het) crosses, and as hist-2 or + (hist-2$^+$) for CC crosses.

2. Triple crossovers have been included in two ways: as the two doubles with adjacent crossovers ("Adjacent" columns), or as all three doubles ("All" columns). An adjacent double does not necessarily have its two crossovers in contiguous marked regions, provided the intervening region shows no recombination.

3. The expected number of double crossovers is based on map distances calculated from the pooled results of those crosses capable of contributing to the double crossover class, including, where appropriate, the SS cross. Asterisks denote a significantly different value from the observed total (the highest where more than one total is given).

   * denotes $0.05 > p > 0.01$, ** denotes $0.01 > p > 0.001$, *** denotes $0.001 > p$, n.s. denotes not significant.

4. Four strand double crossovers within one region have not been included in the two-region double crossover classes. The expected numbers of double crossovers for the two-region classes have been calculated on ascus totals reduced by the number of one-region 4sd.

5. Data obtained by other workers have not been included in this section.

6. In Table 43 figures in brackets are the expected numbers of double crossovers assuming a 1:2:1 ratio for 2sd, 3sd and 4sd.

TABLE 41

DOUBLE CROSSOVERS IN CROSSES WITH ONE OR MORE REC-S PARENTS[5]

Crossover regions	Strand relationships	Free[1] strand (in 3sd)	CS, SC, SC (Het) crosses		CS, SC, SC (Het) and SS crosses		Expected[3] double crossovers
			Adjacent[2]	All[2]	Adjacent[2]	All[2]	
mt - ad5 ad5 -o-	2sd		1		3		
	3sd	S	1.5				
	3sd	C	2.5		6		
	4sd		1		3		
	Total		6		12		31**
mt - ad5 -o- cr	2sd		6	6	17	17	
	3sd	S	18	18			
	3sd	C	16	16	52	55	
	4sd		14	17	24	24	
	Total		54	57	93	96	114 n.s.
mt - ad5 cr - al2/aur	2sd		2	4			
	3sd	S	0	1			
	3sd	C	7	8			
	4sd		2	4			
	Total		11	17			18 n.s.
ad5 -o- cr	2sd		7		8		
	3sd	unknown	13.5		19.5		
	4sd		4.5		5.5		
	Total		25		33		42 n.s.

TABLE 41 (cont.)

Crossover regions	Strand relationships	Free[1] strand (in 3sd)	CS, SC, SC (Het) crosses		CS, SC, SC (Het) and SS crosses		Expected[3] double crossovers
			Adjacent[2]	All[2]	Adjacent[2]	All[2]	
-o- cr cr - al2/aur	2sd		10				
	3sd	S	12.5				
	3sd	C	9.5				
	4sd		9				
	Total		41				44 n.s.
ad5 -o- cr - al2/aur	2sd		0	1			
	3sd	S	2	3			
	3sd	C	3	4			
	4sd		5	5			
	Total		10	13			13 n.s.
mt -o- cr	2sd		12.5				
	3sd	unknown	39				
	4sd		12.5				
	Total		64				61 n.s.
mt - ad5[4]	4sd		0		1		21
cr - al2/aur[4]	4sd		2				10
ad5 -o- OR -o- hist2[4]	4sd		1				
ad5 -o- OR -o- cr[4]	4sd		2		3		
mt -o- OR -o- cr[4]	4sd		1				

TABLE 42

DOUBLE CROSSOVERS IN CC AND CC\ (Het) CROSSES[6]

Crossover regions	Strand relationships	Free[1] strand (in 3sd)	Adjacent[2]	All[2]	Expected[3] double crossovers
mt -o- hist2 - cr	2sd	hist2 +	4.5		
	3sd		1.5		
	3sd		2.5		
	4sd		2.5		
	Total		11		13 n.s.
mt -o- cr - al2	2sd	hist2 +	9	9	
	3sd		21	21	
	3sd		7	7	
	4sd		7	9	
	Total		44	46	47 n.s.
mt -o- cr - al2	2sd	hist2 +	2	2	
	3sd		0	0	
	3sd		3	4	
	4sd		2	2	
	Total		7	8	10 n.s.
hist2 - cr cr - al2	2sd	hist2 +	2		
	3sd		5		
	3sd		2		
	4sd		5		
	Total		14		25 *

TABLE 42 (cont.)

Crossover regions	Strand relationships	Free[1] strand (in 3sd)	Adjacent[2]	All[2]	Expected[3] double crossovers
ad5 -o- cr - al2	2sd 3sd 4sd Total	pooled	3 0 2 5	3 0 2 5	5 n.s.
mt - ad5 -o-	3sd	hist2	1		2
mt -o-[4]	4sd		3		6.5
cr - al2[4]	4sd		13		29 **

TABLE 43

CHROMATID INTERFERENCE WITHIN ARMS AND ACROSS THE CENTROMERE

Cross types	Crossover regions	Triples included	2sd	3sd	4sd	Significance
CC + CC (Het)	across centromere	all	18.5 (17.5)	37 (35)	16.5 (17.5)	n.s.
	within arms	all	2 (3.75)	8 (7.5)	5 (3.75)	n.s.
CS + SC + SC (Het) + SS	across centromere	all	42.5 (55.75)	129.5 (110.5)	51 (55.75)	G = 6.61, d.f. = 2, *
	within arms	all	13 (13.25)	28 (26.5)	12 (13.25)	n.s.
	across centromere	adjacent	39.5 (52.75)	122.5 (105.5)	49 (52.75)	G = 164.8, d.f. = 2, ***
CS + SC + SC (Het)	across centromere	all	30.5 (44)	102.5 (88)	43 (44)	G = 6.9, d.f. = 2, *
	across centromere	adjacent	26.5 (40.75)	98.5 (81.5)	38 (40.75)	G = 9.2, d.f. = 2, **

Figure 1

TRIPLE CROSSOVER ASCI ISOLATED DURING THIS STUDY

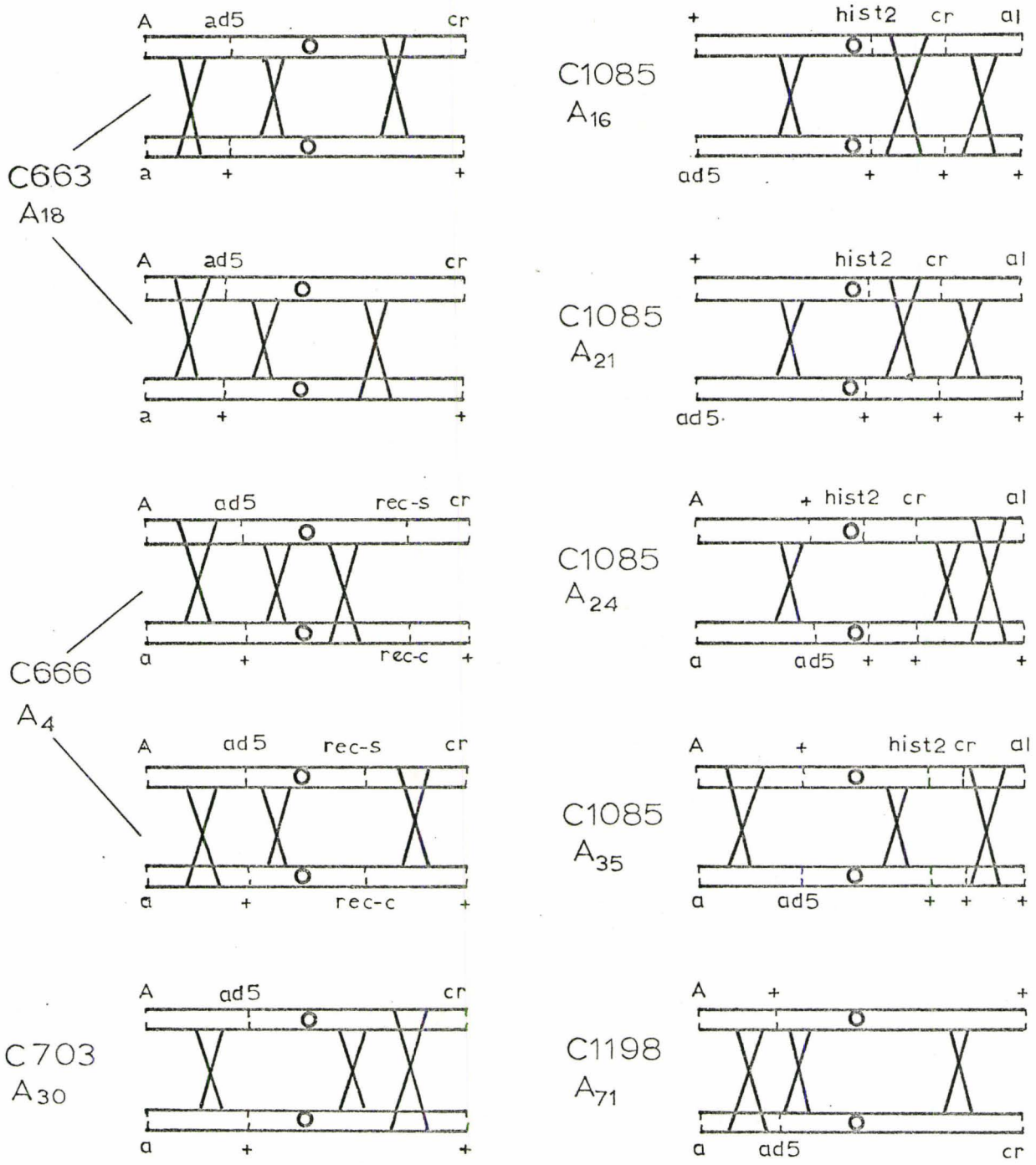


Fig. 1 cont.

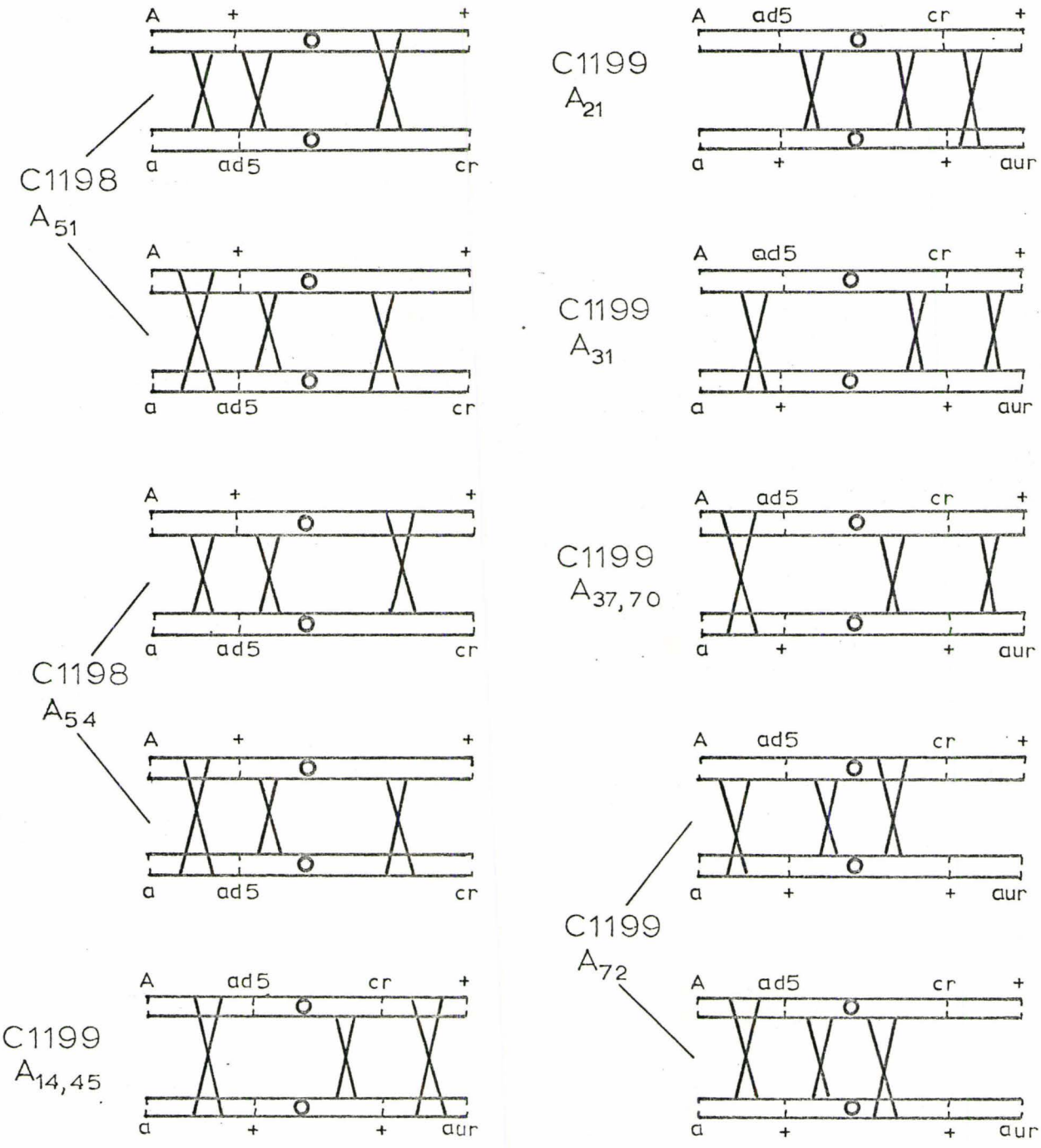
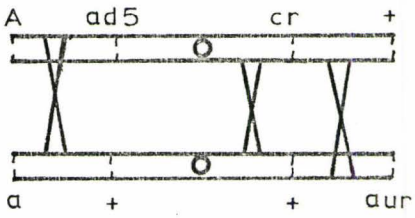
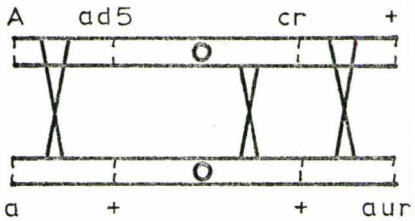

C1198
A51
C1199
A21
C1199
A31
C1198
A54
C1199
A37,70
C1199
A14,45
C1199
A72
A
ad5
cr
a
aur
+

Fig. 1 cont.

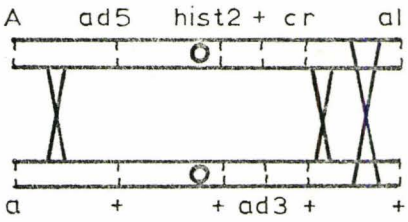
C1199
A_{84}



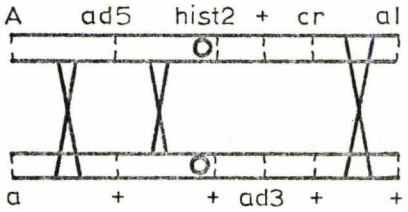
C1199
A_{89}



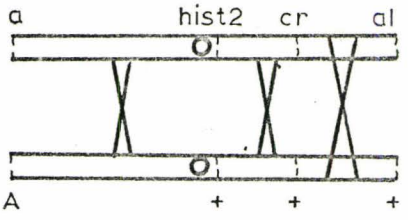
C1219
A_{45}



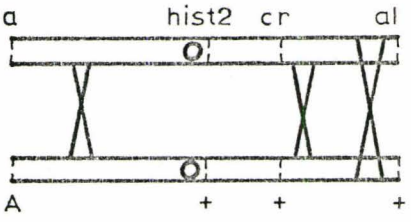
C1219
A_{60}



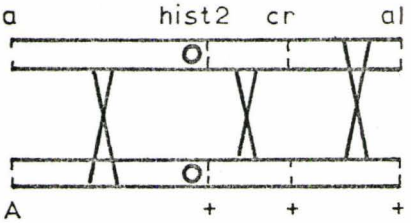
C1148
A_{34}



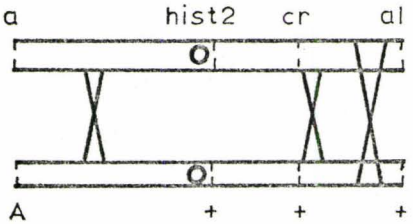
C1170
A_{137}



C1248
A_6



C1248
A_{142}



between linkage group I arms. His data do not support Stadler's for linkage group VI showing a lack of three strand doubles (3sd) and an excess of 2sd across that centromere. However, Prakash's data need to be considered separately since he alone claims (1964a) no chiasma interference within arms, and negative chiasma interference across the linkage group I centromere in some crosses. His centromere to hist-2 distance would seem to be about 1 map unit, which is very much larger than the 0.15 m.u. value given by Giles et al. (1957), and the value obtained during this study: < 0.20 (section VIII). Scott-Emuakpor (1965b) finds an excess of 2sd across the centromere of linkage group I in both N. crassa and N. sitophila, but an excess of 3sd within arms in N. sitophila only.

In this study it has been necessary to pool crosses to obtain information on chromatid interference. Whether this introduces any error is unknown. A second centromere marker has been used in many, but not all, crosses and shows no evidence of nuclear passing (but see section VIII on centromere-hist-2 crossovers in CC crosses). CC crosses show no significant chromatid interference overall either between or within arms, though data from the latter type are slight. The cause of the odd ratios for the CC mt -o- and cr - al-2 doubles

is not known. Crosses involving rec-s, with or without the SS cross C1198, show a strongly significant excess, $p < 0.001$, of 3sd and deficiency of 2sd across the centromere. Whilst triple crossovers have been included in the double crossover classes in two ways (see legend opposite Table 41) it would be expected that the inclusion of only adjacent doubles would give a more accurate picture of chromatid interference, and it is this method of inclusion of triples which gives the most significant excess of 3sd. The excess of 3sd is not reflected by an excess of either of the two free strand classes. The implications of these findings are not clear and, considering the variety of results found by other workers, they may be best ignored.

Section X:

Genetically mixed perithecia

All heterokaryon crosses were of the type:

(C973 R_6 + C703 A_{22-1}) x C967 A_{78-4}

a, *hist-2*, rec, *cr*; + *a*, rec-s, *cr*; *A*, rec-c;

ad-1, *pan-2* (B5) *pan-2* (B3) *ylo-1*, *tryp-2*.

The lack of a demonstrable effect of a rec-s gene product on the CC type component in non-mixed perithecia of heterokaryon crosses has been discussed in section VIII.

The frequency of genetically mixed perithecia observed by other workers in heterokaryon crosses varies greatly: 50% of 8 perithecia (Sansome, 1949), 23% of 752 perithecia (Weijer and Dowding, 1960), 2% of 2,778 perithecia (Nakamura and Egashira, 1961), none from 26 perithecia and 20% of 26 perithecia in two different crosses (Griffiths, 1967). In this study, two mixed perithecia were observed in 34 (6%). Conidial isolates from two independent cultures of the heterokaryotic female parent (C973 R_6 + C703 A_{22-1}) gave the following results:

	Homokaryotic rec-c (al)	Homokaryotic rec-s (al⁺ pan-2-3)	Heterokaryotic (al⁺ pan⁺ phenotype)
Set 1	36	77	70
Set 2	27	85	93

Although these frequencies may not directly reflect the true nuclear ratio in the heterokaryon, because of possible inhibition of growth of one homokaryon by biochemicals present in the medium necessary only for the other component (Atwood and Mukai, 1955), they suggest that the observed frequency of mixed perithecia is not due to a deficiency of the rec-s component in the heterokaryon. More likely explanations for why higher frequencies of

mixed perithecia were not obtained are that the chromosome aberration present in C703 A_{22-1} inhibits the development of SC asci in mixed perithecia when in competition with normal CC asci, or that the addition of pantothenate to the conidiating suspension causes a rapid breakdown in the heterokaryon. Other unknown explanations are also possible. In the former case it would be expected that some mixed perithecia might go undetected: in some perithecia classified as non-mixed asci of ambiguous origin, usually non-crossover asci in which only the hist-2^+, cr$^+$, al-2^+; ylo-1, ad-1^+, pan-2^+, tryp-2 had germinated, were obtained. In all cases, however, the frequency of these was comparable with the frequency of asci in which only the hist-2^+, cr$^+$, al-2^+; ylo-1^+, ad-1, pan-2, tryp-2^+ had germinated.

The ascus data from the two mixed perithecia isolated from C1248 are given in Table 44. The genotype data from the randomised CC component asci are given in Table 45. The corresponding SC data have not been given since no class had greater than 5 isolates in it and there was no evidence of a bias in germination. Table 45 shows no significant deviation from a 1:1, mutant to wild type, ratio. The significant correlation between hist-2 and pan-2 can be shown to be due to an inflated sample size.

TABLE 44

GENETICALLY MIXED PERITHECIA, TOTAL CROSSOVERS BY REGIONS

Perithecium	1		2		Totals	
Component	CC	SC	CC	SC	CC	SC
Asci isolated	4	1	14	4	17	5
Asci scored [4]	3	1	14	4	17	5
Asci for ylo [1]	1	1	9	4	10	5
Aborted asci [2]	0	0	2	0	2	0
% germination	87	100	87	84	87	87
Unassignable asci	0		2		2	
Crossovers						
mt -o- [7]	0	1	2	1	2	2
-o- cr	0	1	0	4	0	5
cr - al2 [8]	2	-	8	-	10	-
ylo1 -o-	0	0	2	1	2	1
-o- pan2	0	0	0	0	0	0
pan2 - tryp2 [9]	0	0	4	3	4	3
mt -o- cr 4sd [9]	0	1	0	1	0	2

For notes 1, 2 and 4 see legend opposite Table 6, p. 34.

7. For CC component: -o- hist-2 and hist-2 - cr regions.
8. For CC component: ylo-1 - ad-1 region. No crossovers in ad-1 -o- observed.
9. Included also in appropriate single crossover regions above.

TABLE 45

GENOTYPE DATA FROM RANDOMISED ASCI

OF CC COMPONENT OF MIXED PERITHECIA

Crossover type	Genotype[1]								Random spores
Parental	A	+	+	+ ;	ylol	+	+	tryp2	23
	A	+	+	+ ;	+	adl	pan2	+	17
	a	hist2	cr	al2;	ylol	+	+	tryp2	15
	a	hist2	cr	al2;	+	adl	pan2	+	22
mt - hist2	A	hist2	cr	al2;	+	adl	pan2	+	2
cr - al2	A	+	+	al2;	ylol	+	+	tryp2	5
	A	+	+	al2;	+	adl	pan2	+	6
	a	hist2	cr	+ ;	ylol	+	+	tryp2	5
	a	hist2	cr	+ ;	+	adl	pan2	+	5
ylol - adl	A	+	+	+ ;	ylol	adl	pan2	+	1
pan2 - tryp2	A	+	+	+ ;	ylol	+	+	+	3
	a	hist2	cr	al2;	+	adl	pan2	tryp2	1
cr - al2	A	+	+	al2;	+	+	+	tryp2	2
ylol - adl	a	hist2	cr	+ ;	ylol	adl	pan2	+	2

TABLE 45 cont.

Crossover type	Genotype[1]								Random spores
cr - al2	A	+	+	al2;	ylol	+	+	+	1
pan2 - tyrp2	a	hist2	cr	+	+	adl	pan2	tryp2	2
mt - hist2	A	hist2	cr	al2;	ylol	+	+	+	2
pan2 - tryp2	a	+	+	+ ;	ylol	+	+	+	2
	a	+	+	+ ;	+	adl	pan2	tryp2	2
									Total: 118
									Germination: 87%

[1] Where al-2 made scoring of ylo-1 impossible the ascus, for this table only, has been assumed to be non-recombinant for the ylo-1 - ad-1 region.

The two asci in perithecium 2 which could not be classified as SC or CC were a mt -o- cr 3/4 sd and a 2 colourless:4 dark:2 colourless spores aborted ascus with at least a -o- cr crossover. It seems unlikely that the only two asci to be unclassifiable are also the only asci in a CC component of 19 asci to have -o- cr crossovers. Since the aborted ascus is also more likely to originate from the SC component, it is probable that both asci are of SC type. They have not, however, been included in the SC crossover data.

Normal statistical methods cannot be applied to the low crossover totals in Table 44. However, from the 95% confidence limits given in Table 46 for the -o- cr region it seems certain that rec-s causes no substantial increase in the -o- cr recombination frequency of the CC component of mixed perithecia. No conclusions can be made concerning the possibilities that rec-s reduces recombination in the CC component, that rec-c increases recombination in the SC component or that rec-c decreases recombination in the SC component. Whilst models including these three hypotheses can be devised, they are inherently unlikely owing to the dominance of rec-s over rec-c, in non-heterokaryon crosses.

Griffiths and Threlkeld (1966) have demonstrated that one component of a heterokaryon can have an influence

TABLE 46

95% CONFIDENCE LIMITS FOR MAP DISTANCE,

IN MIXED PERITHECIAL DATA AND CONTROL CROSSES.

-o- cr REGION ONLY

	Mixed CC	Mixed SC	C1206 CC	C1207, C1253 SC
95% upper limit	8.1	93.8	6.9	42.0
Map distance	-	50.0	4.0	37.3
95% lower limit	-	6.2	1.1	32.5

on the recombination behaviour of the other component, but this could be explained (Griffiths, 1967) by a lasting effect of one component on the other, which would not require transmission of factors across the wall of a developing ascus. Moreover the effect was not limited to genetically mixed perithecia. Too little work has been carried out on the temporal sequence of recombination and of ascal development to predict what barriers to transmission of possible rec-s gene products exist, even if the time of action of the rec-s gene products were known. A more detailed discussion of meiotic events in Neurospora is given in Chapter 4, but

it seems that the data presented in this section provide, at least, only circumstantial evidence that rec-s does not produce diffusible products.

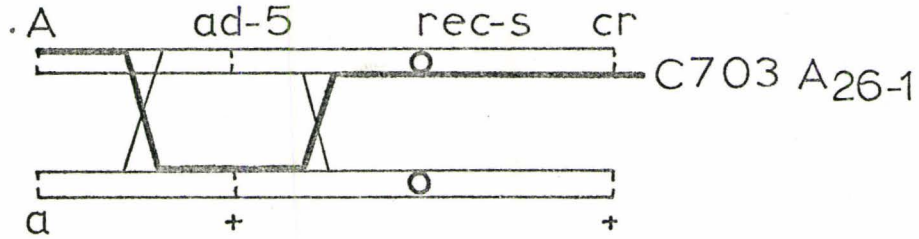
Section XI:

The location of rec-s on the genetic map

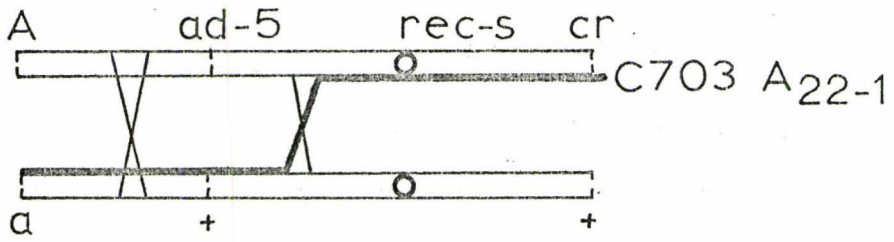
The mapping of the rec-s factor or factors responsible for high recombination has not been one of the primary aims of this study but some conclusions are possible.

From the general mode of segregation of rec-s in asci and from the method used in the construction of K_4 it is probable that rec-s is a factor located on the linkage group I chromosome between ad-5 and cr. By backcrossing spores from two -o- hist-2 crossover asci from an SC cross Hargrave has shown that rec-s is probably right of the centromere (personal communication, see section XIII). Data obtained in this study supports this conclusion:

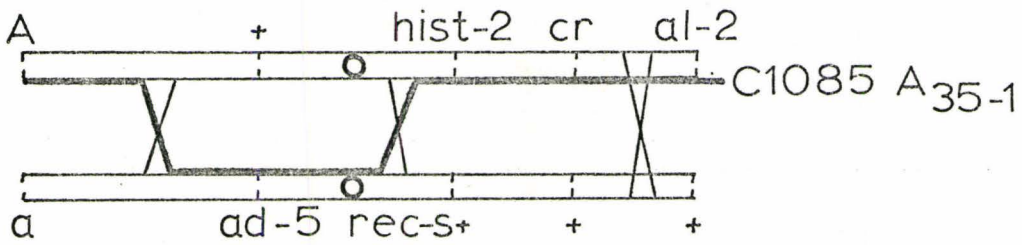
CS crosses C1182 and C1183 show high recombination (section III) and have C703 A_{26-1} as rec-s parent:



SC crosses C1207 and C1253 show high recombination (section II) and have C703A_{22-1} as rec-s parent:

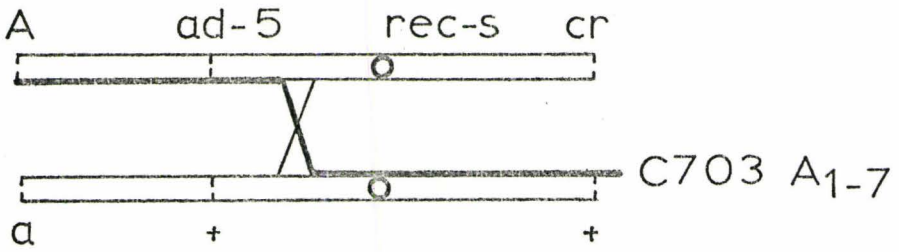


CC cross C1219 shows low recombination (section V) and has C1085 A_{35-7} as one rec-c parent.



Against the conclusion that rec-s is right of centromere is the C981 backcross of C703 A_{1-7} to C834 R_{12}, a, hist-2, rec-c, which from 183 random spores,

germination 88%, gives map distances of mt - ad-5



22.4 ± 3.1 and ad-5 - hist-2 11.5 ± 2.4 suggesting C703 A_{1-7} is rec-s. However, it has been shown in section VIII that the mt -o- region is an unreliable guide to rec-s character, and the C983 backcross of C703 A_{28-3}, which is of similar origin to C703 A_{1-7}, gives mt - ad-5 15.2 ± 2.5 and ad-5 - hist-2 5.1 ± 1.6 from 198 spores with 95% germination.

Section XII:

Aborted spore patterns in asci

Throughout this thesis the term "aborted spore" is used to included both colourless dot-like spores and normal sized colourless spores. Only asci with aborted spores in pairs have been recorded.

The importance of aborted spores in the characterisation of chromosome rearrangements was noted by McClintock (1945). Since aberrations might either

be the cause of, or modify, effects attributed to rec-s the tube walls of crosses used in this study were regularly checked for high levels of aborted spores. None were found. However, Barry (1967) has suggested that asci containing aborted spores (aborted asci) may mature more slowly leaving black asci to be preferentially discharged from the perithecium. This appears to be the case with C1182, C1183 and C1207, C1253 since on perithecial dissection many aborted asci were found though none were detected on the cross tube walls. A count was taken of aborted asci frequencies in C661 and $OR_a \times K_4$ (Table 47). The results for $OR_a \times K_4$ do not differ substantially from the estimate of 10% aborted asci in crosses of standard wild types to each other (Newmeyer and Taylor, 1967), but the C661 value is higher than expected. From the classification provided by Perkins (1966) the only simple type of aberration fitting the C661 data is a pericentric inversion with one break at a dispensable tip (e.g. Newmeyer and Taylor, 1967). However, the low frequency of 6d:2c asci, which result from single and 3sd crossovers between the centromere and the dispensable tip, indicates that this cannot be a linkage group I inversion where the distance to the tips is large. 16 asci (6 4d:4c and 10 6d:2c) were isolated from $OR_a \times K_4$ but the linkage group I crossover patterns, as deduced from the germinating

TABLE 47

ABORTED SPORE PATTERNS IN C661 AND OR_a x K_4

Cross	Perithecia analysed	Total asci	Spore patterns[1] 8d:0c	4d:4c	6d:2c	2d:2c:2d:2c[2]	% aborted asci
C661	15	163	126	11	21	5	23
OR_a x K_4	13	173	150	7	14	2	13

[1] xd:yc denotes x dark spores and y colourless spores.
[2] Including 2d:4c:2d.

spores, showed no correlation with the aborted spore patterns. C1182, C1183, C1207 and C1253 were not studied directly for aborted asci patterns, but they appeared to show a much higher frequency of 4d:4c asci than C661. The non-random sample of aborted asci included in the isolated asci from these, and other, crosses showed no correlation of aberration with any of the linkage group I or VI crossover regions.

Aborted asci may also be produced by non-disjunction events (Threlkeld and Stoltz, 1970), but in the case of linkage group I this would be expected to produce Dark Agar phenotypes in isolated aborted asci (Newmeyer and Taylor, 1967). These were not observed.

The similarity of the recombination data from crosses showing various levels of aberration suggests that, whatever the type and location of the rearrangements they neither modify, nor are responsible for, effects attributed to rec-s.

Section XIII:

The species origin of regions studied for recombination

The role of heterozygosity in controlling recombination has been studied extensively in N. crassa (Barratt, 1954), (Stadler and Towe, 1962), (Lavigne and Frost, 1964), (Cameron, et al., 1966). In general, repeated backcrossing, which might be expected to reduce heterozygosity, has resulted in higher recombination frequencies. de Serres (1958) and Cooke (1967) have studied the relative roles of heterozygosity within the recombination region studied and heterozygosity outside that region; the latter seems to exert the greater control. It follows that it is not possible to completely rule out heterozygosity as a basis for the effects attributed to rec-s in this study, though the magnitude of these effects is in some regions greater than the maximum previously attributed to changes in heterozygosity.

The probable species origin of regions, as deduced from Fig. 10 (foldout at back), gives a guide to the role of within-region heterozygosity.

The only crosses involving rec-s known to have a mt - ad-5 region of N. crassa origin, ignoring possible undetected 2 strand double crossovers, are C1207 and C1253 both of which have slightly low mt -o- distances, pooled value: 21.6 ± 4.1. This value, however, is still significantly greater, 0.001 > p, than the 10.5 ± 1.1 from pooled CC and CC (Het) crosses. It is probable that all SC and CS crosses involve a largely N. crassa mt - ad-5 region since all rec-s parents of these crosses are derived from K_4, which carries N. crassa mating type and ad-5 alleles but which, from its mode of construction, could still carry some N. sitophila genome in the mt - ad-5 region.

In all crosses involving rec-s at least some of each of the ad-5 -o- and -o- cr regions of the rec-s parent originates from N. sitophila.

From the number of backcrosses of rec-s strains to N. crassa it is highly probable that in rec-s strains regions left of mt and right of cr, thus including cr - al-2, and linkage groups other than I are predominantly N. crassa in origin. Assuming random assortment of chromosomes in the backcrosses an estimate may be given for C419 A_{1-8} as being 98.5% N. crassa except in the mt - cr region.

Section XIV:

Data obtained by other workers

This section gives data obtained by other workers which are of direct relevance to this study. It is referred to as necessary in other sections.

Data from Hargrave (personal communication):

a) Cross 1 (cf. Newcombe's CS4)

30JA-5 x 202-1

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

Total asci scored: 68

Region	ad5 -o-	-o- hist2	hist2 - cr
Crossover asci	5	4	45
m.u. ± S.E.	3.7 ± 2.3	2.9 ± 2.0	33.1 ± 5.7

b) Position of rec-s

Spores from two asci (1-11 and 1-77, shown below) recombinant for -o- hist-2 were backcrossed to rec-c strains to determine the segregation of rec-s in the asci.

Ascus 1-11

-1	A	ad5	hist2	cr
-2	-	-	-	-
-3	-	-	-	-
-4	A	ad5	+	+
-5	a	+	hist2	cr

Ascus 1-77

-1	A	+	hist2	cr
-2	A	+	hist2	cr
-3	a	+	+	+
-4	a	+	+	+
-5	A	ad5	hist2	cr

Ascus 1-11				
-6	a	+	hist2	cr
-7	a	+	+	+
-8	a	+	+	+

Ascus 1-77				
-6	A	ad5	hist2	cr
-7	-	-	-	-
-8	a	ad5	+	+

An estimate of the error caused in the recombination frequencies (Table 48) by the low germination of crisp in some crosses may be obtained from the ratios of parental cr/parental cr^+ to recombinant cr/ recombinant cr^+ given below:

1-11-6	0.90 : 0.73
1-77-2	0.66 : 0.38
1-77-8	0.66 : 0.30

Recalculating the 1-77-2 hist-2 - cr distance from twice the cr^+ recombinants gives a maximum value of 8.2 ± 2.0.

Assuming the absence of undetected multiple crossovers in 1-11 and 1-77 the data suggest that rec-s lies right of the -o- hist-2 crossovers, and is therefore separable from the centromere.

c) Crosses 180 and 181

30JA-5 x C1220 R_{229}

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

Total asci scored: 195

TABLE 48

Recombination frequencies in backcrosses of spores from 1-11 and 1-77

Backcrossed spore	Spores scored	% germination	ad5 - hist2	hist2 - cr	ad5 - cr
1-11-1	94	94		6.4 ± 2.5	6.4 ± 2.5
1-11-6	218	73		8.7 ± 1.9	
1-11-7	97	97	3.1 ± 1.8	30.9 ± 4.7	32.0 ± 4.7
1-11-8	96	96	6.3 ± 2.5	36.5 ± 4.9	32.3 ± 4.8
1-77-2	195	65		5.6 ± 1.7	
1-77-3	92	92	3.3 ± 2.1	44.6 ± 5.2	48.9 ± 5.2
1-77-4	82	82	3.7 ± 2.1	40.2 ± 5.4	41.5 ± 5.4
1-77-8	180	60			38.3 ± 3.6

Region	mt -o-	-o- ad3	mt - ad3
Crossover asci	92	77	
m.u. ± S.E.	23.6 ± 3.0	19.7 ± 2.9	36.5 ± 3.4

Multiple crossovers from crosses 180 and 181:

	2sd	3sd	4sd	Total
Observed	5.5	15.5	9	30
Expected	7.5	15	7.5	expected total 46.6

For total multiple crossovers: X^2 (Yates) = 5.56, 0.05 > p > 0.01, showing a significant reduction from the expected number of double crossovers.

d) Cross 195

30JA-5 x C162-461R

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

Total asci scored: 193

Region	mt -o-	-o- hist2	hist2-ad3	-o- ad3
Crossover asci	93	12	86	92.5
m.u. ± S.E.	24.1 ± 3.1	3.1 ± 1.3	22.8 ± 3.0	24.0 ± 3.1

Multiple crossovers for mt -o- and -o- ad-3 from cross 195, triple crossovers including 2sd in -o- hist-2 and hist-2 - ad-3 have been ignored:

	2sd	3sd	4sd	Total
Observed	10	21	9	40
Expected	10	20	10	Expected total 44.6

In contrast to cross 180 and cross 181 no deviation from the expected results is apparent.

Section XV:

Summary of results

a) A factor, or factors, originating in N. sitophila and referred to as rec-s in this study is able to control recombination in linkage group I of N. crassa.

b) Rec-s is dominant in crosses over the corresponding N. crassa factor(s), rec-c, except in the mating type - ad-5 region where it appears to be semi-dominant.

c) Rec-s does not appear to be maternally or cytoplasmically inherited, and maps on the right arm of linkage group I between centromere and crisp.

d) In crosses with (rec-s + rec-c) heterokaryons no effect of rec-s on the rec-c x rec-c component was detected, either in mixed or unmixed perithecia.

e) Positive chiasma interference within chromosome arms between adjacent short intervals was found in rec-c x rec-c crosses and in crosses involving rec-s. In contrast to rec-c x rec-c crosses, crosses involving

TABLE 49

DATA FROM NEWCOMBE (1970)[2]

Cross	Protoperithecial Parent	Conidial Parent	Spores Scored	% germination	mt - ad5	ad5 - hist2	hist2 - cr	ad5 - cr
CC1	987 A, rec-c	202-1 a, ad5, hist2, rec-c, cr	469	90	11.9 ± 1.5	2.3 ± 0.7	3.8 ± 0.9	4.9 ± 1.0
CC2	P367(?9) hist-2, rec-c, cr	P109 ad5, rec-c	471	91		3.2 ± 0.8	4.0 ± 0.9	4.7 ± 1.0
CS1	OR_a a, rec-c	K_4 A, ad5, rec-s, cr	444	85				38.7 ± 2.3
CS2[1]	B6 rec-s	B68 ad5, hist2, rec-c, cr	535	86		5.2 ± 1.0	33.1 ± 1.5	36.4 ± 2.1
CS3	B40 ad5, hist2, rec-c, cr	B38 rec-s	411	79		8.3 ± 1.4	32.0 ± 2.3	36.0 ± 2.4
CS4[1]	30JA-5 A, rec-s	202-1 a, ad5, hist2, rec-c, cr	383	73		9.9 ± 1.5	32.0 ± 2.3	34.2 ± 2.4

TABLE 49 cont.

Cross	Protoperithecial Parent	Conidial Parent	Spores Scored	% germination	mt - ad5	ad5 - hist2	hist2 - cr	ad5 - cr
SS1	K_4. A, ad5, rec-s, cr	K110 a, rec-s	357	69				37.0 ± 2.5
SS2	B56 A, rec-s, cr	B27 a, ad5, rec-s	340	85	32.1 ± 2.5			37.1 ± 2.6
02	987 A, rec-c	B44 a, ad5, hist2, rec-c, cr	351	68		2.0 ± 0.7	7.7 ± 0.5	9.7 ± 0.5
13	20JR-16 A, rec-s	202-1 a, ad5, hist2, rec-c, cr	333	64		9.6 ± 0.5	24.3 ± 2.4	31.9 ± 2.5

[1] Newcombe does not distinguish between CS and SC crosses.

[2] Corrected for errors in assignment of mating type to K_4 and K110.

rec-s showed an excess of three strand double crossovers, and a deficiency of two strand doubles, across the centromere.

f) No chromosome aberrations were detected which could account for the properties of rec-s.

g) Map distances from rec-c x rec-c and crosses involving rec-s are summarized for linkage group I in Fig. 2a. No effect of rec-s was found on the ylo-1 - pan-2 - tryp-2 region of linkage group VI. Fig. 2b (Scott-Emuakpor, 1965a), gives data for linkage group I markers in N. crassa and N. sitophila. Map distances are necessarily approximate.

FIGURE 2a

MAPS OF LINKAGE GROUP I IN REC-C x REC-C AND CROSSES INVOLVING REC-S

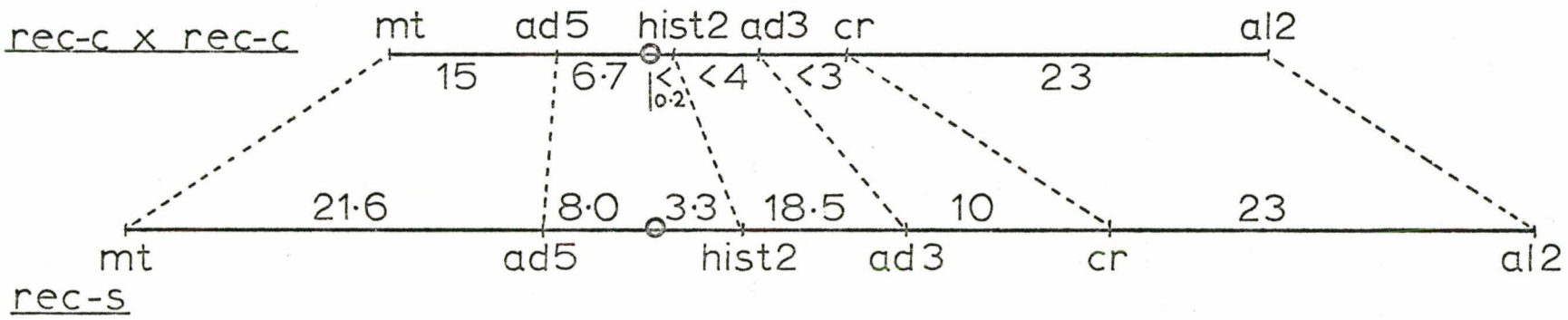
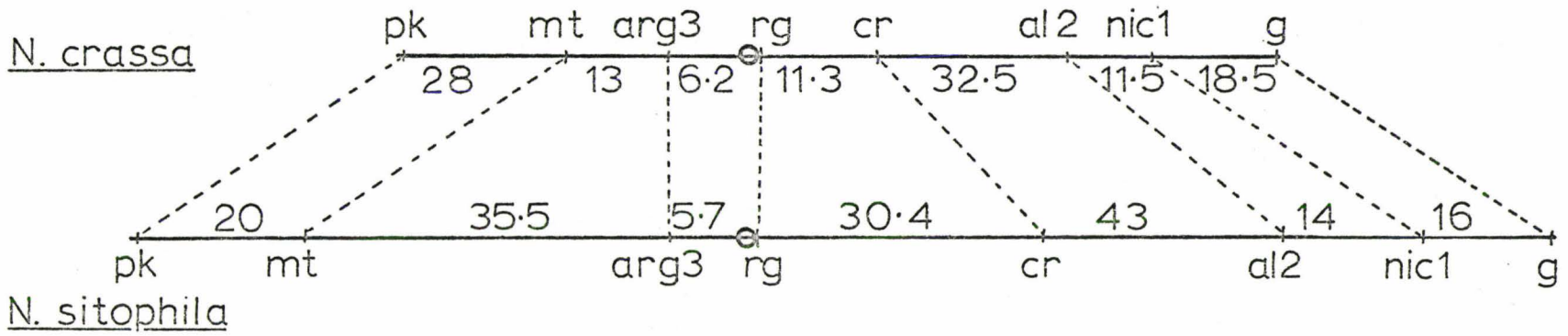


FIGURE 2b

MAPS OF LINKAGE GROUP I OF NEUROSPORA CRASSA AND N. SITOPHILA (Scott-Emuakpor, 1965a)



CHAPTER 4

DISCUSSION

Section I:

A brief review of some meiotic events

Any attempted explanation for the effects of rec-s must take into account what is known about the events leading up to, and the mechanism of, recombination. The probable mechanism of recombination has been considered briefly in Chapter 1; a brief review of meiotic events in Neurospora, possible mechanisms of chromosome pairing, and of the possible role of centric heterochromatin will be given here before considering possible models. For detailed reviews drawing on data from many more species see Grell (1969) and Henderson (1970).

Backus (1939) and Dodge (1935) have described the development of the protoperithecium and the process of fertilisation in Neurospora. The events immediately following fertilisation are not clear, but it is thought that in general a single pair of nuclei of opposite mating types undergo several conjugate mitotic divisions within the protoperithecium (Emerson, 1966). The final conjugate division for each resultant pair takes place in the tip of a crozier-shaped ascogeneous hypha, after which crosswalls are laid down isolating a pair of unlike nuclei which fuse

and enter meiosis (Singleton, 1953).

Meiosis in Neurospora has been studied by McClintock (1945), Singleton (1953) and Barry (1966,67,69). Immediately prior to nuclear fusion a heterochromatic blob is visible in each nucleus in a position corresponding to the pole of the previous mitotic anaphase. A similar arrangement can be seen at interphase I and II. This arrangement is lost after fusion as the chromosomes become distinct, contracted and scattered, with up to the 14 chromosomes visible. Synapsis is claimed to occur in this highly contracted early prophase state starting from the telomeres, but the published photographs are, at best, unconvincing. However, in view of the speed with which synapsis would have to occur to be completed and allow partial separation of homologues before prophase, it does seem likely that synapsis, whatever its direction, does take place in the contracted state. An alternative hypothesis is suggested by the work of Moens (1964). He also observed a clumped state in early prophase tomatoes, and claimed that the chromosomes were already paired. In the case of Neurospora it might be suggested that, since two clumps are visible - presumably centric heterochromatin, pairing takes place between the unclumped free chromosome ends which then condense and force the dissipation of the two clumps. An intermediate stage in this process would

appear to show up to 14 separate chromosomes - in reality 14 paired ends. During prophase the chromosomes elongate to a maximum at late pachytene when the bivalents lie slightly apart (up to 0.8 μ depending on fixative) and parallel to each other with few, if any, relational coils. No chiasmata are visible but considerable chromomere detail can be seen. A diffuse diplotene follows during which little chromosome detail is visible. A similar stage has been found in Neotiella rutilans (Rossen and Westergaard, 1966), Gelasinospora calospora (Lu, 1967), Coprinus lagopus (Lu and Raju, 1970), Plethodon c. cinereus (Kezer and Macgregor, 1971), and in at least one race of maize (Ghidoni, 1967). Moens (1964) has observed a similar stage in Lycopersicon and further suggests that in this species the so-called "zygotene" is in reality the beginning of homologue separation. He suggests the name schizonema and places it between pachytene and the diffuse diplotene. Whether this is likely in Neurospora is uncertain, but Mitchell (1965) has suggested that meiosis in Neurospora is more complex than previously thought.

The stage at which recombination takes place is less uncertain. No chiasmata are visible until diakinesis when univalents are rarely, if ever, seen and when linkage group I (chromosome I, Barry, 1967) regularly shows two or three chiasmata. Given the clarity of the pachytene chromosomes it would seem that recombination has not occurred by that time, placing recombination at the diffuse

diplotene stage or at schizonema (Barry, 1966,69). In the ascomycete Neotiella rutilans it is claimed from Feulgen spectrophotometry that the premeiotic DNA replication takes place before karyogamy, thus eliminating the possibility of recombination at that stage (Westergaard, 1966), (Rossen and Westergaard, 1966). However, Grell (1969) has drawn attention to possible errors in this work: N. rutilans cannot, as yet, be cultured in the laboratory; there are conflicting reports on the mode of reproduction and the chromosome number which throw doubt on the identity of the ascomycete and on which nuclei are proceeding to meiosis; Sueoka et al. (1967) have demonstrated DNA replication both before and after karyogamy in Chlamydomonas, no check on the latter being provided by Rossen and Westergaard. This last objection may not be valid since Chiang and Sueoka (1967) have shown that the majority of the vegetative portion of the Chlamydomonas life cycle is diploid and that the post-karyogamous replication either occurs after, or simultaneously with, crossing-over. In higher organisms Grell and Chandley (see review by Grell, 1967) attempted to determine the time of recombination by injecting Drosophila females with ^{3}H-thymidine and correlating the appearance of the label in the mature oocyte with the appearance of changes in frequency of recombinant progeny

induced by a heat shock. Henderson (1970) has criticised these experiments on grounds that the appearance of labelled oocytes may not reflect the time at which labelled eggs are laid, and that the length of heat shock may make the method too insensitive. Henderson's own experiments (1966) with Schistocerca gregaria, which also used heat and labelling but followed decreases in chiasmata frequency and placed crossing-over at, or after, late zygotene, well after DNA replication, have been criticised, seemingly invalidly, by Grell (1969). A limitation to both these and other similar experiments, but with particular relevance to Grell's, is that chemical or physical treatments may have effects well after their application. One example of this is found in Neurospora where Threlkeld (1961b) using u/v light, and both Mitchell (1957) and Landner (1970) using temperature, have shown pre-conidiation treatments can affect recombination frequency. Holliday (1971) has recently shown that γ-ray irradiation of Ustilago maydis diploids induces enzyme(s), possibly repair enzyme(s), responsible for mitotic recombination. It is possible that similar systems in Neurospora may account for at least the observations of Threlkeld. Landner claims to show that changes in temperature after 5 days after conidiation of the cross have no effect, and that centomere proximal regions remain

sensitive longest. Landner claims this would agree with Barry's placement of the time of crossing-over at diffuse diplotene, though he did not time his meioses after temperature shocks. Landner also seems to use the dubious criterion for sensitivity of a recombination frequency outside the range of either of his controls. Thus interference with the course of meiosis seems unreliable as a means of timing crossing-over. Fortunately, in Neurospora, the pachytene evidence seems clear. O si sic omnia!

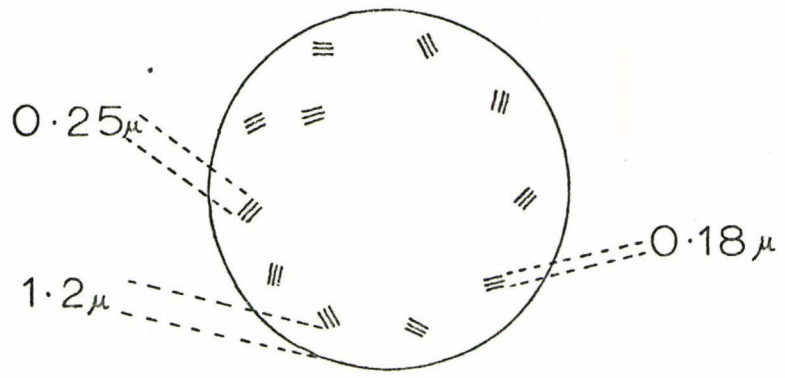
Lu (1969) has carried out similar experiments with temperature shocks on Coprinus lagopus, which possesses a synchronous meiosis and might be expected to give clearer results. He correlates the sensitive stage, zygotene - pachytene, with the formation of the synaptonemal complex (Lu, 1967,1970). The possible relationship of synapsis and crossing-over to the synaptonemal complex, which is in general observed only during meiotic prophase, has been reviewed by Moses (1968) and King (1970), and more generally by Grell (1969). The complex has been observed in most eukaryotes, including ascomycetes: yeast (Engels and Croes, 1968), (Rapport, 1971), Neotiella (von Wettstein, 1971) and recently Neurospora (Gillies, 1971). A general description of the synaptonemal complex is given based on Moens' observations

on Lilium (1968) and Locusta (1969a). Evidence of the complex is first found at leptotene when segments of electron-dense axial cores form in the chromatin fibres, then differentiate to produce transverse striations or elements. The homologous chromosomes, attached via their telomeres and their axial cores to the nuclear wall, come to lie roughly parallel about 3000 Å apart, possibly through movement of the telomeres. By mid-zygotene the first complete segments of the complex are formed: at a Y-shaped junction the two adjacent axial elements, now with their chromatin distributed outside the complex, are drawn together to become the lateral elements of the completed complex spaced 1000 to 1800 Å apart according to species. The transverse elements interdigitate and thus appear to form the central element. Pairing of the axial elements appears to be initiated at the telomeres in Locusta, but at many initiation sites in Lilium, and is complete by pachytene. By late pachytene the complex starts to disintegrate and has largely disappeared by diplotene. This sequence of events is remarkably similar in almost all studied species though details vary. The origin of the central element in Philaenus spumarius has been questioned by Maillet and Folliot (1965) who observed only faint transverse elements connecting the lateral elements with the 270 Å wide double central element, which possessed clear internal transverse elements.

A similar structure was observed by Gassner (1969). However, Coleman and Moses (1964) and Brockley and Bryan (1964) found other lateral elements to be distinctly bipartite, each part being associated with a 100 Å diameter DNA containing fibril. The observations of Maillet and Folliot and Gassner may merely be an extreme example of this. The attachment of telomeres to the nuclear wall appears to be general, e.g. Maillet and Folliot (1965), Woolam et al. (1966), Lu (1967), Moens (1969a), but the arrangement of these attachment sites may vary. Moens showed that all sites in Locusta were in the same region of the wall thus forming a loop with a half-twist in each chromosome and a bouquet-type arrangement. One of Maillet and Folliot's cross-sections at right angles to the long axis of the complexes shows them spaced around the nuclear wall and at a regular slight angle to it, Fig. 3.

FIGURE 3

SECTION AT RIGHT ANGLES TO THE AXES OF PHILAENUS SYNAPTONEMAL COMPLEXES (Maillet and Folliot, 1965)



From the number of complexes present they cannot correspond to Moens' loops, but might be the result of attachment of telomeres at opposite ends of the nucleus. The complex appears to be mostly protein, some of it basic (Sheridan and Barnett, 1969), with RNA in the lateral elements (Esponda and Stockert, 1971). The DNA is present in chromatin loops originating from the lateral elements and in at least one case also as two 100 Å fibrils in each part of the bipartite lateral elements (Brinkley and Bryan, 1964). Moens (1968) has suggested one micrograph showing two 25 Å fibres each splitting into two within the central element may represent the final DNA - DNA pairing. Somewhat similar fibres have been observed by Gassner (1969).

Whether the synaptonemal complex is responsible for crossing-over, or for regular disjunction, or both, is uncertain. Its absence from most achiasmate meioses, e.g. male Drosophila (Meyer, 1964), in which regular disjunction is found would argue for a role in crossing-over. Evidence from the c(3)G region of Drosophila supports a role in both crossing-over and disjunction, and is in direct contradiction with the conclusions of Grell (1967) as to the time of crossing-over (King, 1970): homozygous c(3)G female Drosophila show absence of the synaptonemal complex and irregular disjunction with no crossing-over,

while in the heterozygous state c(3)G and stubbloid, a homozygous lethal deficiency covering the same site, show limited synaptonemal complex development, terminated before pachytene, and slightly increased non-disjunction (Smith and King, 1968), but decreased crossing-over for stubbloid and increased for c(3)G (Hinton, 1966). In some species achiasmate meioses, induced or natural have been shown to involve a synaptonemal complex, e.g. Gassner (1969), Parchman and Roth (1971). In another achiasmate male possessing the complex, Panorpa nuptualis, Gassner (1967) has observed that while the distance between the lateral elements remains constant the bipartite structure becomes clearer with time, and suggests that the bipartite structure is due to homologous, rather than sister, chromatids. In Fig. 3 the sister chromatids would thus be closer than homologous chromatids. However, it is difficult to correlate this with the obvious pairing of axial cores.

The basis for the specificity of synaptonemal complex mediated synapsis of homologues is also uncertain. It seems unrealistic to propose that each transverse element has the ability to recognize only one element on the homologue, or that each segment of the axial core is specific to a particular DNA sequence. Yet the evidence of the point-by-point pairing of chromomeres at

pachytene shows a high degree of specificity. Comings and Okada (1970) attempt to explain the specificity by assuming the transverse elements are synthesized at times specific to the genes bound to the axial core at that point followed immediately by a non-specific pairing. This does not explain the observation of unpaired axial cores possessing transverse elements. von Wettestein (1971) suggests the lateral elements contain RNA and/or protein recognition molecules for the DNA (for Neotiella he estimates 6500 recognition sites per genome), and the central element consists of protein amenable to conformational change induced by the recognition molecules thus binding the lateral elements together. There is some evidence for components of the central element being formed in the nucleolus. A similar model was suggested by King (1970). The evidence from triploid chickens in which three lateral and two central elements are found (Comings and Okada, 1971), from DNA puffs in Acheta in which up to 400 copies of the complex may be stacked in concentric fashion (Jaworska and Lima-de-Faria, 1969), from Solanum - Lycopersicon hybrids in which complexes are formed despite a very low chiasma frequency and haploid tomatoes in which some segments of the complex are found (Menzel and Price, 1966), from haploid maize (Ting, 1971), and from observations by Moens (1968) on

an axial core that had folded back on itself after pachytene suggests that axial cores are non-specific structures, with regard to their binding both to chromatin and to other axial cores. Since three stages of homologue pairing probably occur: 3000 Å alignment of homologues, 1800 Å synaptonemal complex pairing and intimate DNA-DNA pairing, it would seem reasonable to suggest specificity is first attained by rough 3000 Å pairing by means unknown, but presumeably aided by the closeness of sites for attachment of homologous telomeres to the nuclear membrane, followed by a non-specific pairing of transverse elements which is only stabilised by the final DNA-DNA pairing. The nature of this last step may involve direct interaction between the DNAs, but King (1970) has suggested the third stage is only attained in regions undergoing crossing-over. Such a mechanism would be in accord with the synaptonemal complex being both an aid to, though not a prerequisite for, regular disjunction and a prerequisite for meiotic recombination. The occurrence of synaptonemal complexes in naturally achiasmate meioses may be an evolutionary relic.

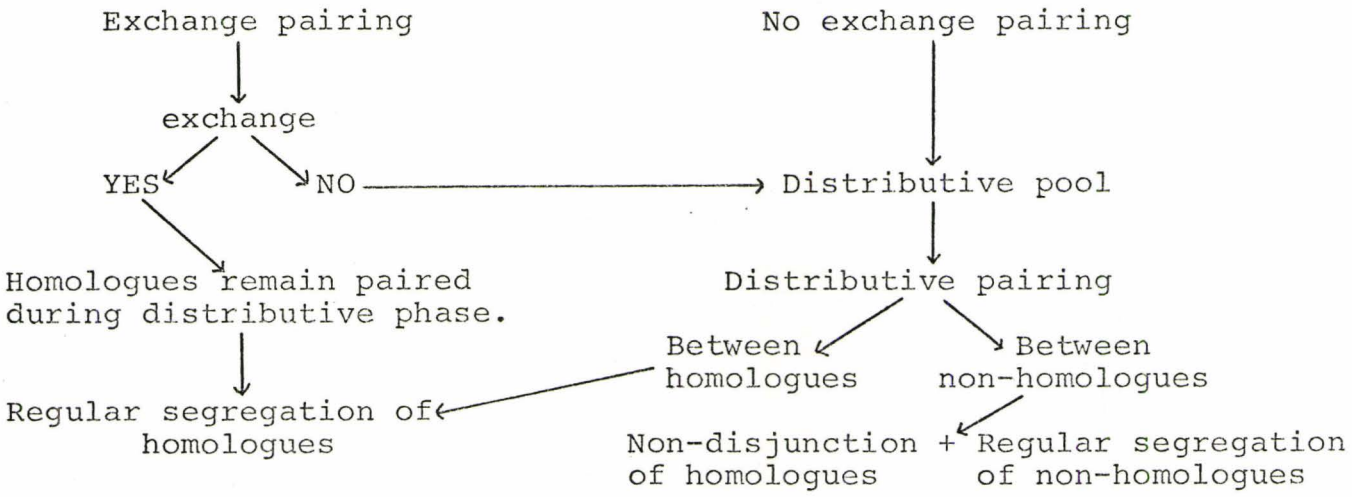
The concept of a pairing mechanism ensuring regular disjunction distinct from that leading to exchange has been arrived at independantly by the genetical

experiments of Grell (reviewed 1967, and more briefly 1969) with Drosophila melanogaster. Female Drosophila regularly show no crossing-over of the 4th chromosome, or between the X chromosomes and free X duplications. In one set of experiments Grell used females with a single 4th chromosome and varying sizes of free X duplications possessing varying amounts of eu- and heterochromatin. She was thus simultaneously able to: study pairing leading to exchange between the normal X chromosomes - by competition for this pairing, but not for the recombination itself, by the free duplication; study pairing leading to distribution - by the frequency of non-disjunction of the X duplication and the single 4th chromosome, and study the interaction between exchange pairing and distributive pairing. The results showed: that the degree of competition for exchange depended on the degree of homology between the free X duplication and the normal X chromosomes, reaching a maximum reduction in crossing-over of 68%; that the frequency of non-disjunction between the free X duplication and the single 4th chromosome depended on the size of the duplication and was independent of homology or eu- or heterochromatin, being lowest when the two chromosomes had similar sizes, and that participation of the free duplication in exchange pairing did not affect its ability to participate in distributive pairing. Further experiments supported these observations and

extended them in principle to any of the chromosomes of Drosophila, e.g. Grell (1970). Grell has interpreted these events as showing a distinct exchange pairing involving parasynapsis of homologous chromosomes, rather than local effective pairing (Pritchard, 1960), with exchange completed before distributive pairing is initiated between any chromosomes, homologous or otherwise, which have failed to undergo exchange pairing (Fig. 4).

FIGURE 4

MODEL FOR TWO PAIRINGS (modified from Grell, 1967)



Grell and Day (1970) have observed a size dependant pairing between non-homologous chromosomes in the oogonial cells of Drosophila; whilst this cannot be interpreted as distributive pairing it does lend support to the theory.

Grell also uses the ambiguous evidence of temperature shock and ^{3}H-thymidine labelling experiments, discussed earlier, to support the early exchange pairing model. Novitski (1964) has challenged Grell's interpretation of her results, but as she points out (1967) Novitski's model is essentially a restatement of her own.

Whilst the evidence for two types of pairing is unambiguous, the evidence that these occur at discrete times, and for actual exchange to preceed the distributive pairing is not. Nor does Grell's model correlate with what has been said earlier in this section about the role of the synaptonemal complex in crossing over, nor does it suggest a reason why free duplications taking part in exchange pairing fail to take part in recombination. The observation of three stages of exchange pairing, the second being a non-specific synaptonemal complex mediated pairing, suggests an alternative three stage explanation, superficially similar to the Novitski model, which unifies exchange and distributive pairing in chiasmate meioses:

1st stage of pairing: by means unknown, homologous chromosomes, including free duplications if present, align themselves roughly parallel about 3000 Å apart.

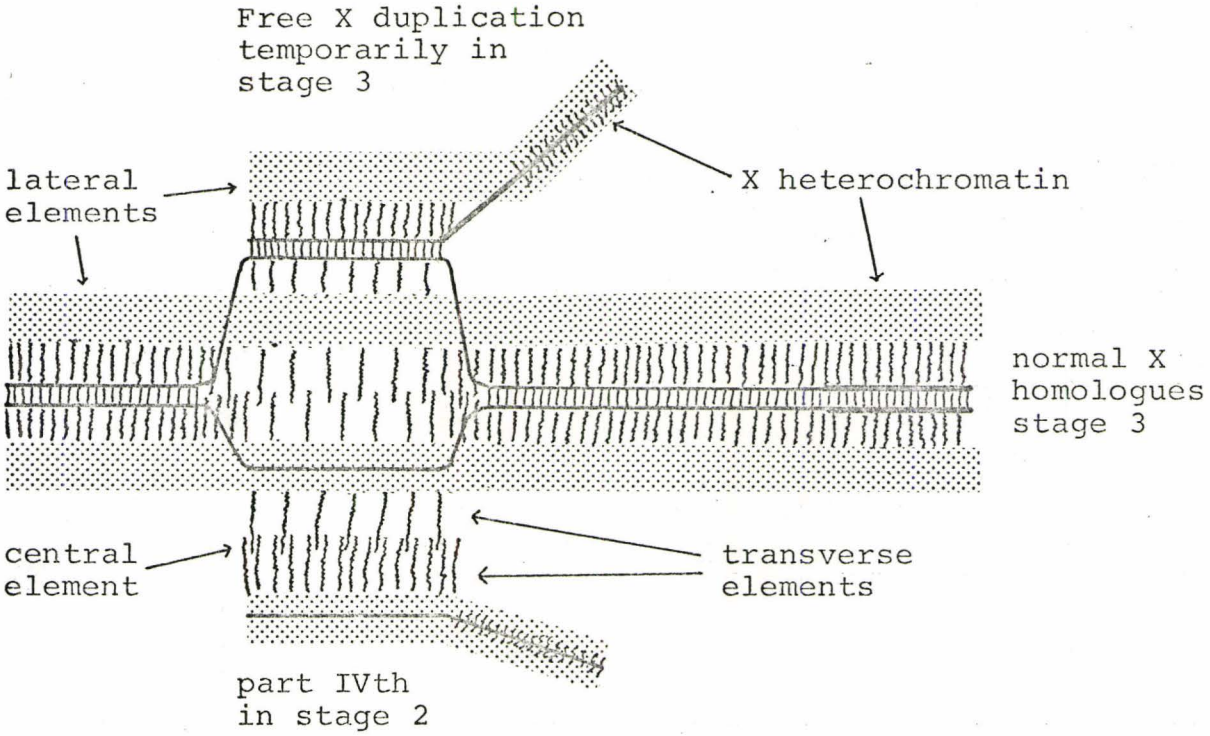
2nd and 3rd stages of pairing: the synaptonemal complex indiscriminately pulls adjacent chromosomes, of

whatever type - and including non-homologues by chance alignment, together, but only those synapsed regions capable of the 3rd stage of pairing, homologous DNA-DNA pairing, become stabilised. In the resulting equilibrium the majority of homologues will become stabilised at stage 3, and chromosomes lacking homologues will arrange themselves in stage 2 pairing with any available partners. A free X duplication would be expected to compete for stage 3 pairing with the normal X homologues at this time. If processes leading from stage 2 to stage 3 pairing then cease, but due to the continued presence of synaptonemal complex components stage 2 pairing is still possible, the strain imposed on the stage 3 pairing of a free X duplication by its non-homologous regions being attached to an axial core, but unable to participate in stage 3 pairing, would be expected to reverse the stage 3 pairing of its homologous regions and leave it free to compete with other non-homologues for size based non-specific stage 2 pairing. Since stage 2 to stage 3 transitions have ceased the gap left by the free X duplication will remain at stage 2, see Fig. 5. Exchange then takes place.

Whilst this three stage model is applicable to chiasmate meioses, its weakness is the need to postulate a further mechanism for distributive pairing in achiasmate

FIGURE 5

THE PRINCIPLES UNDERLYING THE PROPOSED MODEL FOR PAIRING



meioses lacking a synaptonemal complex. This objection disappears if stage 1 pairing, and rare transitions direct to stage 3 pairing, are found to be sufficient. Such a mechanism would accout for somatic crossing-over in male Drosophila and in homozygous c(3)G females (Le Clerc, 1946). The model also depends on stage 3 pairing not being caused by recombination as suggested by King (1970). Support for the model is mainly circumstantial, e.g. observations of inversion heterozygotes in Chironomidae which simultaneously show normal homologous

pairing and non-homologous pairing of the inverted section (Martin, 1967).

The site of initiation of cytologically observable pairing varies from species to species (Darlington, 1940), but is generally near the telomeres or centromeres. How and where other stages of pairing are initiated is unknown. Rough alignment of homologues followed by association of centromeres with pairing proceeding thence outwards has been suggested by Sandler et al. (1968) from studies on meiotic mutants in Drosophila. Studies with one of these mutants have already suggested a third process, non-exchange alignment, to be added to Grell's exchange and distributive pairings (Robbins, 1971) and, though details are as yet insufficient to support or contradict the model suggested here, further work may clarify the problem. The apparent absence of distributive pairing in maize led Weber (1969) to suggest it to be a special process evolved by Drosophila for the regular segregation of non-exchange 4th chromosomes.

One further frequently invoked control of recombination must be considered: heterochromatin.

> "A discussion on the relations between arrangement and activity of the chromosomal material should, of course, include a consideration of heterochromatin. The trouble here is that the study of heterochromatin is at a prescientific level. We have no alternative but to ignore it."
>
> (Pontecorvo, 1959)

Heterochromatin may have come a long way since 1959, but its function is still puzzling and often the subject of premature generalisations. Heterochromatin has often been claimed to be densely staining, transcriptionally inactive, late replicating and inactive in recombination, see Brown (1966) for review. Brown suggested a division of heterochromatin into facultative, for species in which homologues differ with respect to their heterochromatin, and constitutive, for species in which both homologues behave similarly, but the terms have come to be used for heterochromatin showing occasional transcriptional activity and heterochromatin showing none, a distinction which may have a fundamental molecular basis. Sieger et al. (1970) have shown, however, that regions functioning constitutively are not necessarily heteropycontic. Late replication, though often found (Lima-de-Faria and Jaworska, 1968), is not de rigeur: Comings and Mattoccia (1970) found the constitutive mouse satellite DNA in the heterochromatin to replicate at the same time as the euchromatin. Comings (1970) suggests that heterochromatin may show more intense replication, which on the occasions when it occurs late in S phase, may appear as clearly "late replicating". Hotta et al. (1966) have demonstrated replication during meiotic prophase in Lilium and though no evidence of localisation was found it showed a high GC content, and appeared to be necessary for the formation

of the synaptonemal complex (Roth and Ito, 1967).

Most of the genetic evidence for the function of heterochromatin has come from Drosophila melanogaster: Cooper (1959) has demonstrated the low frequency of meiotic recombination in the centric heterochromatin of the X chromosome, though it appears to show a high frequency of mitotic recombination (Kaplan, 1953), (Walen, 1964) and particularly in early embryogenesis (Brosseau, 1957). Mitomycin C, shown by Szybalski and Iyer (1964) to preferentially alkylate guanine bases, has been shown to induce a high frequency of chromosome aberrations in heterochromatin (Natarajan and Ahnstrom, 1969) and particularly in constitutive heterochromtin (Natarajan and Schmid, 1971). Schewe et al. (1971a) showed its induction of X-Y interchanges in Drosophila was independent of meiotic crossing-over since it affected males, females and female c(3)G homozygotes equally. In a second paper (1971b) they showed mitomycin C also increased meiotic crossing-over in the proximal heterochromatin on chromosomes 2 and 3, and suggested a mechanism whereby alkylation of one strand leads to a single strand break and crossing-over, whilst alkylation of two strands causes a double strand break and interchange. A corollary seems to be that Drosophila centric heterochromatin is rich in guanine and/or normally fails to recombine due to lack of

initiating single strand breaks, rather than due to poor pairing. The apparent increase in proximal recombination on the X chromosome caused by heterozygosity for autosomal inversions appears, however, to be due to increases in the proximal euchromatin (Roberts, 1965). The well known position effect caused by centric heterochromatin (Lewis, 1950) lends some slight support to the suggestion by King (1970) of a releasing factor for mobile recombinases, possibly RNA, synthesized by the proximal heterochromatin and diffusing into the adjacent euchromatin. However, it will be shown in section II that this model is unlikely. The localisation of many Drosophila meiotic mutants in proximal regions (Sandler et al., 1968) is strong evidence for a role of heterochromatin in meiosis: mei-S51, the system leading Robbins (1971) to postulate non-exchange alignment, is a double mutant of factors located close to the centromeres of the 2nd and 3rd chromosomes which increases non-homologous pairing and non-disjunction and decreases crossing-over; recovery disrupter, RD, is proximal on the X chromosome and induces fragmentation of the Y chromosome; the X chromosome sc^4 - sc^8 compound inversion is deficient for 80-90% of the proximal heterochromatin and causes failure of X-Y synapsis (Peacock, 1965); segregation distorter, SD, and some of its modifiers are located in the centric heterochromatin of the second chromosome. These last three examples belong

to the class of meiotic drive mutants (see review by Zimmering et al., 1970) of which the mechanism of SD, at least, can be explained by post-meiotic sperm dysfunction.

The mechanism by which heterochromatin carries out such diverse functions is unknown but a clue may come from studies of repeated gene sequences. Since early work on repetitive sequences, summarised by Britten and Kohne (1968), much evidence has suggested an association between the more highly repeated sequences, satellite bands from density gradient centrifugation of sheared DNA and heterochromatin. Heterochromatin, isolated by sonication and centrifugation from mouse (Yasmineh and Yunis, 1969, 1970), guinea pig (Yunis and Yasmineh, 1970) and calf (Yasmineh and Yunis, 1971), was shown to include a far greater proportion of satellite band DNA than euchromatin. Highly repeated gene sequences have been localised in centric heterochromatin by: autoradiography of Microtus agrestis chromosomes (Arrighi et al., 1970) and Drosophila melanogaster polytene chromosomes (Rae, 1970), (Botchan et al., 1971) to which labelled synthetic RNA, complementary to repeated sequences isolated by use of their rapid annealing properties, had been hybridised in situ; autoradiography of the polytene chromosomes of D. melanogaster (Botchan et al., 1971), D. melanogaster and D. virilis (Gall et al., 1971), D. hydei, D. neohydei and

D. pseudoneohydei (Hennig et al., 1970), Rhynchosciara hollaenderi (Eckhardt and Gall, 1971), and of the chromosomes of Plethodon cinereus cinereus (Macgregor and Kezer, 1971) to which labelled synthetic RNA, complementary to satellite DNA, had been hybridised; autoradiography of the polytene chromosomes of D. melanogaster (Jones and Robertson, 1970) and D. melanogaster and D. virilis (Gall et al., 1971) to which labelled synthetic RNA, complementary to the total nuclear DNA, had been hybridised under conditions where only repeated sequences would hybridise appreciably, and by autoradiography of mouse chromosomes to which labelled satellite DNA had been hybridised directly (Jones, 1970). In some polytene chromosomes the label was also found in non-centric heterochromatin, which may be due to the hybridisation of less repetitive sequences to distal polytene sites, while highly repetitive sequences were localised to centric regions which had not undergone polytenisation (Botchan et al., 1971). It has recently been found that Giemsa and quinacrine stains may be specific for repetitive sequences under some conditions (Rowley and Bodmer, 1971), (Yunis et al., 1971). The guinea pig α-satellite band DNA has been sequenced (Southern, 1970) and shown to be only 6 or 9 base pairs long. The mouse sequence is only 8 to 13 base pairs. Evidence has been found that these sequences

are in rapid evolution (Sutton and McCallum, 1971) as are other satellite sequences (Hennig and Walker, 1970), (Hennig et al., 1970). Highly repetitive DNA does not seem to be transcribed and as yet no role for it has been found, though various ones have been suggested, e.g. stabilising centromeres or chromosomes, protecting against non-disjunction, and pairing of sister chromatids (Walker, 1971). It may be that repeated sequences will provide a clue to the diverse functions of heterochromatin in meiosis.

With specific regard to Neurospora the following observations have been made: a large block of distinctive heterochromatin occurs in the centromere region of linkage group I of both N. crassa and N. sitophila (Singleton, 1953); the N. crassa synaptonemal complex (Gillies, 1971) is similar to that reported for Neotiella (von Wettstein, 1971) and possesses alternating thick and thin bands in the lateral elements, and occasional thickenings of the central element into dense nodes, smaller nodes, though possibly of a different type, were observed by Moens (1968); DNA from both N. crassa and N. sitophila shows about 30% of a low GC band. From the published data (Chakrabartty and Dutta, 1971), (Dutta and Chakrabartty, 1971) it is not possible to draw any conclusions conclusions about the presence of repeated gene sequences in either species, though it seems probable

that such sequences would have been detected in N. crassa if present. Percent dissimilarities in sequence between the two species are estimated as 3-7%.

Section II:

Models for the recombination control by rec-s

The results summarised in Chapter 3, section xv, will be assumed in this discussion. Since knowledge of meiotic events in Neurospora is limited no attempt will be made to correlate rec-s control directly with the diverse observations of the previous section, which will, instead, be used as a general guide to possible models. The models to be considered fall into two classes: recombinase models, postulating control of the recombination event itself, and pairing models, postulating control of pairing of homologues.

a) Recombinase models

King (1970) has suggested a model for recombination in Drosophila which accounts for the effects of chromosome aberrations and centric heterochromatin remarkably well. He postulates mobile enzyme complexes involved in recombination (recombinases) which travel inwards, generally from the telomeres, on a double "rail" of two non-sister chromatids. In homologous regions the chromatids may be pulled together and recombined. Inhomologies may prematurely detach the recombinase, leaving it free to attach elsewhere. Chiasma interference is explained by a

recovery time - the time taken to travel 7×10^6 nucleotides in Drosophila - before the recombinase is ready to function again. The action of centric heterochromatin is explained by postulating that the heterochromatin synthesises releasing RNA factors which diffuse along the chromosome, but apparently not between chromosomes, and detach approaching recombinases. Whilst consistent with data from Drosophila, King's model seems intuitively unlikely. A mobile complex spanning chromatids initially 1000 Å or more apart would require a considerable energy expenditure for only rare recombination events, a mechanism energetically very different from replication, transcription and translation where translocation of the enzyme complex, or nucleic acid, generally accompanies each functioning of the enzyme. The direction of diffusion of the releasing RNAs needs to be remarkably specific, unless non-homologous sections of chromosomes are kept well separated. The recovery time, which from King's estimates of the nucleotide distance and rate of travel is about 7×10^4 secs, or 19.4 hrs, seems extraordinary high.

King's model can be used to attempt to explain the control of recombination by rec-s in N. crassa by assuming that rec-s decreases production of the releasing RNAs. From the published estimate of 4.3×10^7 base pairs

per Neurospora genome (Horowitz and MacLeod, 1960) and an estimate of the relative length of the linkage group I pachytene chromosome, 0.25 of the total genome (Singleton, 1953), it is possible to estimate roughly the number of base pairs in linkage group I as 1.1×10^7. Assuming a N. crassa linkage group I total map length of 170 m.u. (Perkins, 1966b, Cameron, 1967), the region affected on each side of rec-s, if the centromere proximal region is included, is thus in the order of 5×10^5 base pairs. It seems unlikely that RNA molecules could diffuse such a large distance without affecting linkage group VI, unless the recombinases are specific for each linkage group and its releasing factors. King's model as it stands seems inadequate to explain the mechanism of action of rec-s.

The recombinase-on-a-rail model can be applied in several ways. It could be postulated that a product of rec-s causes an increase in activity of the recombinase, which travels outwards from an initiation site near rec-s with a tendency to decay or to fall off the chromosome. This again requires the recombinase to be linkage group specific.

It could be postulated that rec-s is a more efficient recognition site from which recombinases start their journeys. Such a mechanism has been suggested

(Angel et al., 1970) for the dominant cog$^+$ which is located in and increases recombination in the hist-3 to ad-3 region of linkage group I of N. crassa, a region also affected by rec-s. The increase in intragenic recombination at the hist-3 locus due to cog$^+$ is eliminated by the presence of the unlinked rec-w$^+$ in the cross, postulated to act as a repressor of the recombinase movement or function. No effect of cog on the arg-1 to hist-3 region was noticed. From a sentence by Catcheside and Austin (1971) on the probable number of independent rec genes so far found in N. crassa, it seems that Catcheside's group has circumstantial evidence showing rec-w$^+$ to have no effect at the hist-2 locus. If correct, this implies that recombinases moving proximally after attaching at cog$^+$ do not reach hist-2, a distance of less than 3 map units in N. crassa, and that King's hypothesis of mobile recombinases able to move large distances is untenable for N. crassa. Though the above argument rests on a number of unproven assumptions, in combination with the numerical implications of King's hypothesis it does not support any model for a rec-s action involving mobile recombinases.

The possibility that effects attributed to rec-s are due to a chance arrangement of rec-w and cog type genes must be considered. FGSC 246 is of mixed genetical

background which may include some Lindegren in the hist-2 to cr region and thus carry cog$^+$. Similarly rec-x, which is located near ad-5, controls recombination at the hist-2 locus and is probably allelic to rec-3 (Catcheside and Austin, 1971), may also be in FGSC 246 and descendants. Other strains backcrossed to OR probably carry the rec-x$^+$ allele of St. Lawrence strains. Whilst it is impossible to completely exclude the effects of these and other similar genes from this study, the regular segregation of rec-s in many different crosses makes such a chance arrangement unlikely. Moreover, Hargrave (Chapter 3, section XIV) has shown the rec-s effect in crosses of 30JA-5 to strains of Oak Ridge background, thus eliminating any rec genes introduced from FGSC 246. It is possible, however, that rec-w type genes contribute to some of the heterogeneity between crosses of similar type observed in this study.

The diverse interactions of the elements of the fine control systems in Schizophyllum commune under different conditions, e.g. temperature (Stamberg and Simchen, 1970), and the models of Schaap (1971), suggest a model based on numbers of recognition sites for a diffusible recombinase coded for by rec-s and rec-c. Let the number of recognition sites per unit length of DNA decrease similarly with distance from the centromere in rec-c and rec-s strains, and let rec-s synthesise a protein,

involved in recombination, that is more efficiently bound than the rec-c product (or rec-s produces more protein than rec-c). If the recognition sites are not saturated by the protein molecules, such a system might explain rec-s. Variations are possible taking dominance and different functions for the protein into account. The evidence from the mixed perithecia from the heterokaryon crosses is not against this model since it is uncertain what barriers might exist for the diffusion of the protein. The model, however, requires linkage group specificity for the protein, a special arrangement of recognition sites, and the curious chance location of rec-s in the region of highest density of these sites.

The linkage group specificity of rec-s is an important factor in considering models. The only region unlinked to rec-s so far studied is part of linkage group VI. It is therefore not possible at present to exclude the possibility that rec-s affects other linkage groups, nor will direct study of other linkage groups necessarily clarify the problem: if the large increase in part of linkage I were balanced by a small decrease over the rest of the genome the effect on other linkage groups might be too small to be detected. A test for the presence in only linkage group I of genetic sites specifically affected by rec-s is possible, however, using

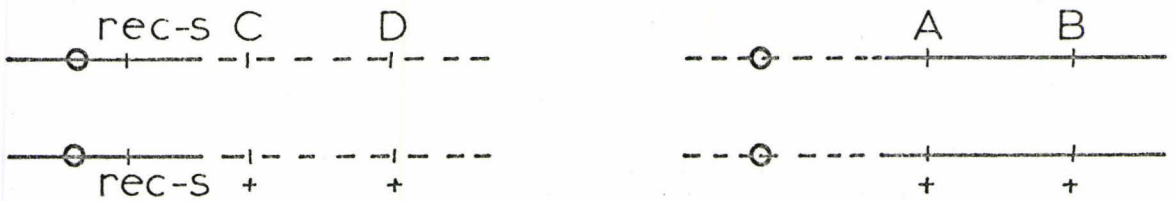
a cross homozygous for a reciprocal translocation with one break point just distal to rec-s. The aberration separating hist-3 from cog (Angel et al., 1970) may be suitable. A possible experimental design is illustrated below:

FIGURE 6

SUGGESTED CROSSES TESTING FOR SPECIFIC RECOGNITION SITES ON LINKAGE GROUP I

Control cross:

Translocation cross:



——— denotes linkage group I.

------- denotes another linkage group.

(The same crosses with rec-c would also be necessary.)

If rec-s produces a diffusible product acting on specific sites in AB, and the CD map distance in the control crosses is unaffected by the presence of rec-s in place of

rec-c, then the CD and AB map distances should be unchanged in the rec-s translocation cross. If rec-s non-specifically affects only regions closely linked to it, CD should be increased and AB decreased in the rec-s translocation cross compared to the rec-s control.

b) Pairing models

The magnitude of the region affected by rec-s, the distinctive specificity within that region, and the similarity of Scott-Emuakpor's N. crassa - N. sitophila comparisons to rec-c - rec-s comparisons in the more isogenic N. crassa background provide circumstantial evidence that rec-s is not comparable to the fine controls of Simchen and Stamberg (1969b), (see Chapter 1). Yet the lack of any gross effect on linkage group VI, and the specificity within linkage group I, argue against any coarse control by diffusible gene products, whether those products are synthesized by or recognised by rec-s. In the latter case recognition still necessitates a "non-diffusible" transmission of information from rec-s to distal parts of linkage group I. It is apparent that a coarse control with specificity is required. Such may be provided by control of chromosome pairing. The problem remains, however, of how a change at the rec-s site can be transmitted along the chromosome with diminishing intensity without returning to enzyme-on-a-rail type

hypotheses. Two possible solutions are offered.

The synaptic model.

This model was conceived as a theoretical solution to the above requirements. For clarity it is presented first in terms of a mechanical analogy, and then discussed in biological terms. Its name was chosen to clearly distinguish it from the desynaptic model, rather than to imply identity with cytologically observable synapsis.

Consider the process of suspension by springs of a long, uniform, flexible beam from a rigid horizontal support. The method of suspension is as follows: the beam is initially suspended under gravity by, say, 10 springs closely spaced around its centre point (Fig. 7a). Both beam and support have potential spring attachment sites evenly distributed along their lengths at intervals long enough that the curvature of the beam under its weight does not appreciably destroy the 1:1 opposition of homolgous sites. There is a limited number of springs available. Attachment of springs to opposite sites is a random, irreversible process such that the probability of attachment between a given pair of sites at a particular moment is a function of the separation of those sites, and of the number of unattached springs remaining at that moment. Consider the final result of such a process: the combination of decreasing probability of attachment

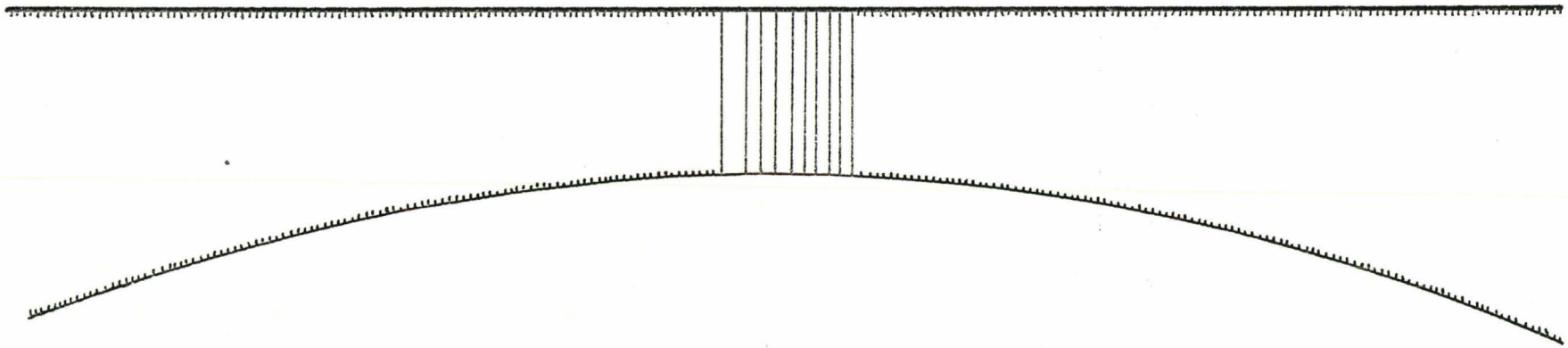
with distance from the centre, due to the curvature of the beam, and the decreasing probability of attachment as springs are used up will result in a tendency for springs to be densely distributed around the centre, and more thinly further out, Fig. 7b. Compare these results with those given by starting with 1000 closely spaced springs, Fig. 7c and d: due to the much closer initial separation of opposite sites the distribution around the centre is much denser and falls off more rapidly, and the separation of the beam and support near the centre is less and increases more rapidly, than in the case of 10 starting springs. Thus a change in the starting configuration can be transmitted along the beam.

Biological models for more efficient pairing in the rec-s region, resulting in higher recombination, follow from the principle illustrated by the mechanical model. Whilst details of such models are not evident, some suggestions can be made. Gravity is extremely unlikely to be a force affecting pairing, but on a limited time scale viscous drag opposing chromosome movement would have a similar effect. The rigid support is merely a convenient simplification for the mechanical model and is unnecessary for the biological model. Chromosomes may be expected to behave as flexible beams; there is evidence from studies with different sizes of tandem duplications in Drosophila

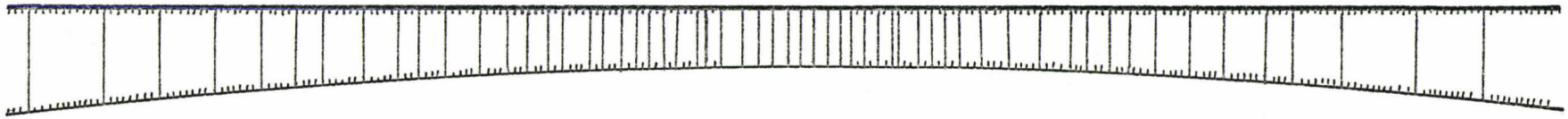
FIGURE 7

SKETCHES ILLUSTRATING THE RESULTS OF LOW AND HIGH NUMBER OF STARTING SPRINGS

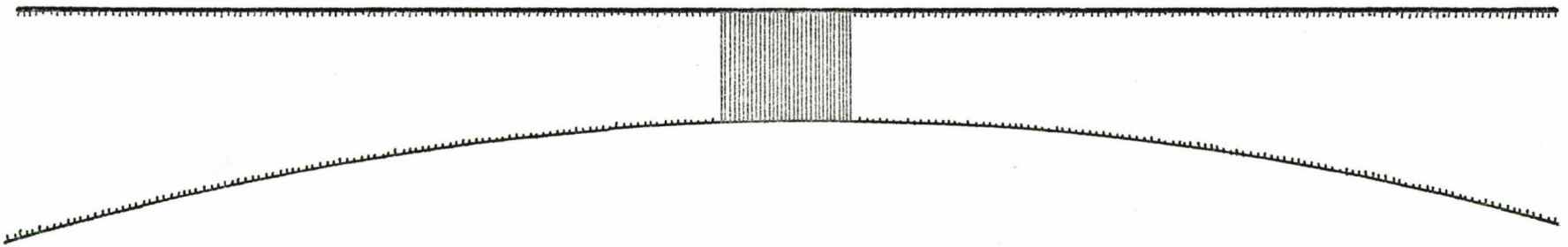
7a)



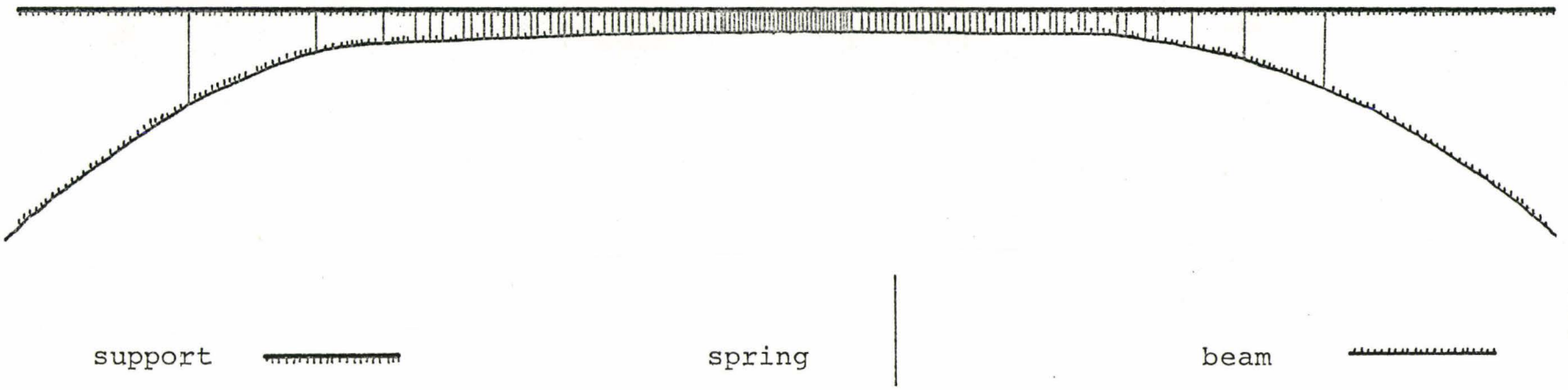
7b)



7c)



7d)



that suggests chromosomes have some structural rigidity over short distances (Roberts, 1966). A priori, it is unlikely that homologues are pushed together at prophase, therefore they must be pulled together, implying in the most general sense some contractile element, and/or pair by static chemical interactions. Rec-c and rec-s may be sites for binding of the initiating "springs", rec-s having a higher affinity for the springs, or a large number of sites, perhaps in the form of repeated gene sequences in the centric heterochromatin. The nature of the springs cannot be suggested without first knowing at which of the postulated stages of pairing (section I) such a model might act. Some component of the synaptonemal complex would be an obvious choice for a model acting at stage 2 pairing. Since no contractile elements have been reported to be involved in pairing the nature of the springs is of importance: from the model it is not necessary for the springs to be capable of expansion once contracted; they must, however, be capable of slowly contracting as the opposing forces of viscosity decrease.

Details of the extent and magnitude of the rec-s increase, of its effect on other linkage groups, and of the degree of dominance of rec-s are highly dependent on the many parameters of the model. Indeed, whilst it is

obvious that the model can explain the action of rec-s, its chief fault (or advantage - depending on point of view), next to the lack of independent supporting evidence, is that with the many possible minor modifications it would appear to explain any changes in recombination which show a monotonically decreasing pattern along a chromosome. The synaptic model is unable to explain the lack of rec-s effect immediately adjacent to the centromere, however, and requires a "post-synaptic" control for that region, perhaps by some feature of heterochromatin or local desynapsis.

The desynaptic model.

It has been shown by Lindsley and Novitski (1958) that the strength of centromere pull on an anaphase I bridge in Drosophila is a function of the centric heterochromatin. Kezer and Macgregor (1971) have shown the presence in Plethodon at diplotene of occasional strands still linking homologous centromeres together as they start to separate. These two observations suggest a model for rec-s: in rec-c x rec-c crosses separation of homologous centromeres starts before recombination has ceased, thus greatly inhibiting recombination in the immediate vicinity of the centromere, while only slightly inhibiting it at a distance. In crosses involving rec-s strains, separation of the centromeres will take place as in rec-c x rec-c crosses but the extent of desynapsis

is limited by a strong inter-homologue bond at the site of rec-s which is only broken later, when more time has elapsed for recombination. This desynaptic model has the merit, unlike the synaptic model, of explaining the unaffected centromere region and why that region appears greater left of the centromere than right of it. (No effect was found on ad-5 to centromere in this study but centromere to hist-2 was affected. Scott-Emuakpor showed no effect on either arg-3 to centromere or rg to centromere in N. sitophila as compared to N. crassa. Since no hist-2 - rg recombinants were detected in 800 N. crassa progeny (de Serres, 1958), these studies suggest rg is either left of and extremely close to the centromere, or right of the centromere and much closer to it than hist-2.) Under the opposing forces of the spindles' pull and the rec-s bond, desynapsis in rec-s crosses would attain the limiting state shown in Fig. 8a.

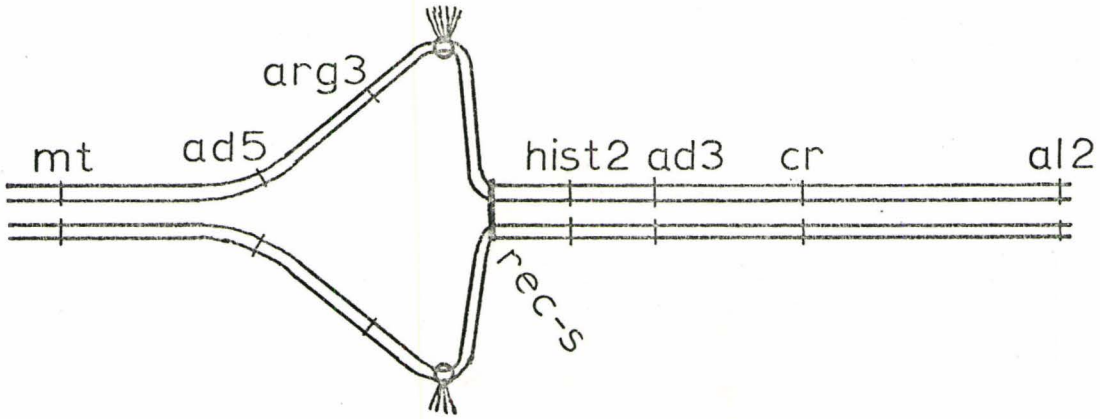
A necessary corollary to this model is that hist-2 must be distal to rec-s.

Desynaptic mutants are known in plants, and whilst they affect recombination (Moens, 1969b) they do not appear to show a decrease in effect with distance from the centromere. However, as Moens points out, the mutation may also cause a non-random recovery of progeny. Thompson (1964) suggested a similar mechanism to explain

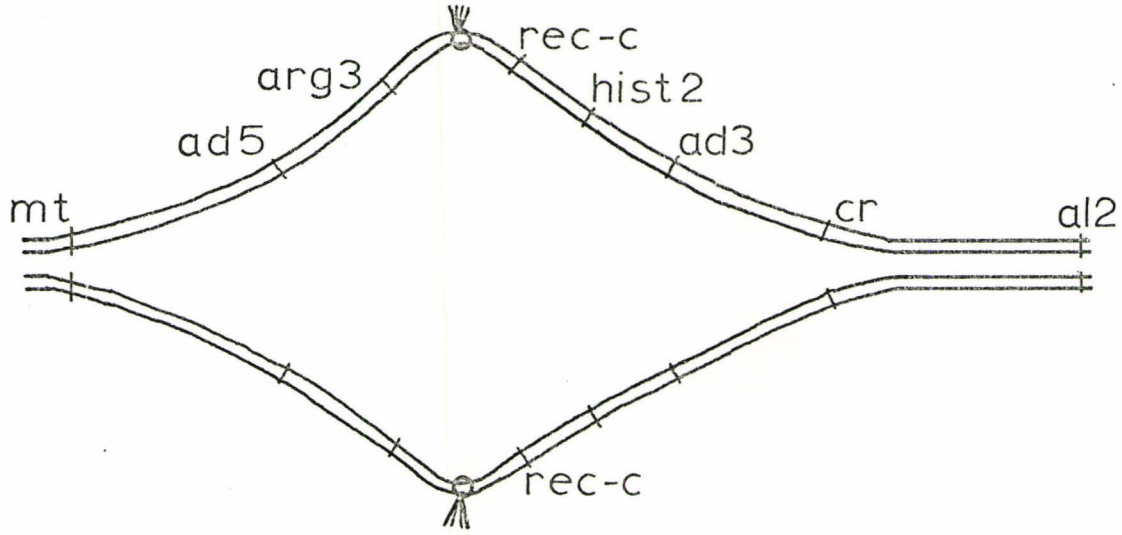
FIGURE 8

LIMITING DESYNAPTIC STATES FOR REC-S AND REC-C X REC-C CROSSES

8a) Rec-s crosses:



8b) Rec-c x rec-c crosses:



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

the reduction in crossing-over near the centromeres of Drosophila, but Lucchesi and Suzuki (1968) have shown the model to be inapplicable in that case.

Its simiplicity and the existence of precedents in other organisms makes the desynaptic model more attractive than the purely theoretical synaptic model. The existing data cannot distinguish between them. Fortunately, even if hist-2 is not found to be located left of rec-s, there are experiments which may distinguish them. The desynaptic model relies on the proximity of centromere and rec-s: if these can be separated by a chromosome aberration, markers remaining linked to rec-s should show a decrease in recombination, whereas on the synaptic model no change would be expected. Possible complications may occur in such an experiment from inter-chromosomal effects of aberrations, though none have been reported yet for Neurospora.

c) General comments

Other models explaining the mechanism of action of rec-s and rec-c may be possible, but they are not at present evident from the data.

The character of the difference between rec-s and rec-c remains unknown. It is unlikely to be a minor inversion or translocation itself since the rec-s effect is not limited to heterozygous crosses. It might, however,

be a position effect resulting from such an aberration. Deficiencies, duplications - whether minor tandem ones (e.g. Roberts, 1966), or multiply repeated sequences, point mutations, or a totally different DNA sequence are all possibilities.

It is considered that, whether or not rec-s itself provides any insight into the fundamental processes of recombination, the apparent removal of one factor limiting recombination in linkage group I may facilitate identification of other, previously non-limiting and therefore undetected, controls of recombination.

APPENDIX I

OLIVETTI UNDERWOOD PROGRAMMA 101 PROGRAMMES

USED IN THIS STUDY

Map distance and its standard error

Map distance is calculated as a percentage p from ascus data as: p = ½ x 100 x Total crossover asci/total asci scored n, from random spore data as: p = 100 x total recombinant spores/total spores scored n.

Standard error is calculated as $\sqrt{\frac{p\ (100 - p)}{n}}$.

AV	Touch V to start programme. Decimal wheel setting 5.
S	Enter n, touch S.
B↑	
S	Enter total recombinant spores or ½ total crossover asci, touch S.
↕	
B÷	
FX	
A◊	Prints map distance p.
C↕	
F↓	
C-	
CX	
B÷	
A√‾	

A ◇ Prints standard error.

B*

C*

V Returns to start of programme.

100 F↑ Constant.

G test for heterogeneity

The data to be tested for heterogeneity are tabulated as shown below.

		Row totals
	$a_{11} \cdots a_{1j} \cdots a_{1n}$ $\vdots \quad \vdots \quad \vdots$ $a_{i1} \cdots a_{ij} \cdots a_{in}$ $\vdots \quad \vdots \quad \vdots$ $a_{m1} \cdots a_{mj} \cdots a_{mn}$	$\sum_{j=1}^{n} a_{1j}$ $\sum_{j=1}^{n} a_{ij}$ $\sum_{j=1}^{n} a_{mj}$
Column totals	$\sum_{i=1}^{m} a_{i1}$ $\sum_{i=1}^{m} a_{ij}$ $\sum_{i=1}^{m} a_{in}$	$\sum_{i=1}^{m} \sum_{j=1}^{n} a_{ij}$ Grand total

The heterogeneity G value is calculated from:

$$G_H = 2 \left[\sum_{i=1}^{m} \sum_{j=1}^{n} a_{ij} \ln a_{ij} - \sum_{i=1}^{m} \left(\sum_{j=1}^{n} a_{ij} \ln \sum_{j=1}^{n} a_{ij} \right) \right.$$

$$\left. - \sum_{j=1}^{n} \left(\sum_{i=1}^{m} a_{ij} \ln \sum_{i=1}^{m} a_{ij} \right) + \sum_{i=1}^{m} \sum_{j=1}^{n} a_{ij} \ln \sum_{i=1}^{m} \sum_{j=1}^{n} a_{ij} \right]$$

(Sokal and Rohlf, 1969, p. 599)

Values for flnf have been taken from Rohlf and Sokal (1969), Table G for integral f, Table G^* for integral f + ½. Other values have been calculated as necessary.

The following programme provides a convenient method of storing several totals simultaneously, calculating G_H and providing a print-out, which aids the tracing of keying errors and the partitioning of the G_H value.

AW	Touch W to start programme. Decimal wheel setting 5.
S	Enter flnf for each a_{ij} in turn, touching S between entries.
B↓	Sub-totals may be obtained as required by keying B◊.
+	When all cell flnf values have been entered, touch Y.
↕	
W	
AY	
S	Enter flnf for each marginal row and column sum in turn.
C↓	Sub-totals may be obtained as required by keying C◊.
+	When all marginal flnf values have been entered, touch Z.
↕	
Y	
AZ	

S Enter flnf for grand total.

B↓

+

C-

E↕

E↓

E+

A◊ Prints G_H.

B*

C*

/◊

W Returns to start of programme.

The value of G_H is then compared with a χ^2 distribution with (m-1)(n-1) degrees of freedom (d.f.).

APPENDIX II

OBSERVATIONS ON THE HISTIDINE REQUIREMENTS OF ADENINE AUXOTROPHS

During preliminary work leading to this thesis a leaky histidine requirement was found in rec-s strains carrying the ad-5 mutant Y152M40. Since the absence of a histidine requirement was the criterion used for the selection of N. crassa hybrids carrying rec-s, this phenomenon was investigated further using biochemical tests as described in Chapter 2.

With the one exception of K4 x K110 #5, which seems to be a contaminant (Chapter 3, section V), no histidine requiring, adenine independent, strains were recovered from approximately 4000 progeny from crosses of Y152M40 in which no known histidine allele was segregating. Adenine requiring strains were frequently recovered which, on repeated retesting, showed a variation from absence of a histidine requirement to the normal leaky requirement. Backcrossing of such strains gave no clear evidence for a suppressor of the histidine requirement. It was concluded that the Y152M40 mutation itself was responsible for both the adenine and the variable histidine requirement.

Similar requirements were found in the wholly N. crassa ad-5 strains Y112M192 and Y175M253, thus eliminating any participation of rec-s, and to a lesser extent in the ad-1 mutant 3254. Ad-3A and ad-3B strains 2-17-186 and 2-17-114 showed no histidine requirement. Foley et al. (1965) reported that the growth of ad-4 mutants was also improved by histidine.

The probable adenine and histidine biosynthetic pathways and some of the known genetic blocks are shown in Fig. 9. The occurrence of three histidine requiring mutations in the adenine pathway after AICAR suggested the histidine requirement might arise from inhibition of the synthesis of IGP in the histidine pathway by an increase in the AICAR pool. An ad-3, ad-5 double mutant would therefore show a reduced histidine requirement compared to ad-5. This was not detected by the normal method of biochemical testing. Moreover, the double mutant showed a consistent slight reduction in the intensity of purple pigmentation of the medium by ad-3, suggesting ad-5 inhibits the pathway prior to ad-3 instead of after. A hist-2 (Y175M611), ad-3A double mutant showed no visible purple pigmentation (Hargrave, personal communication), whilst a hist-3 (Y175M614), ad-3A double mutant showed a slight reduction in pigmentation. Neither hist-2 nor hist-3 single mutants

require adenine.

Y175M614 did not complement with hist-3 (Y155M261) which has been shown by Ahmed (1968) to affect the last step in the histidine pathway. Y175M614 thus blocks at step 10 in the pathway. The step blocked by hist-2 is not known with certainty: hist-2 and hist-3 early alleles block before hist-6 and hist-7, all of which block before IGP. Hence hist-2 blocks either before or immediately after the two early hist-3 blocks.

It is known that utilisation of adenine in some organisms depends on its conversion to adenylic acid by either of two routes involving precursors common to both adenine and histidine pathways (Remy et al., Korn et al., 1955). No conclusions concerning the above phenomena can be made without quantitative studies. A suggestion may be made that any mutation, or combination of mutations, causing an increase in adenine requirement will result in a decreased PRPP pool and decreased synthesis of compounds early in both histidine and adenine pathways.

LEGEND FOR FIGURE 9

AICAR 5-Aminoimidazole-4-carboxamide ribotide.

CAIR 5-Amino-4-carboxyimidazole ribotide.

FAICAR 5-Formamidoimidazole-4-carboxamide ribotide.

IGP Imidazole glycerol phosphate.

PRATP Phosphoribosyl-ATP.

PRPP Phosphoribosylpyrophosphate.

SAICAR 5-Amino-4-imidazole-(N-succinylo-) carboxamide ribotide.

Details of pathways and mutants are taken from:

Moat and Friedman, 1960.

Ames et al., 1967.

Remy et al., 1955.

Korn et al., 1955.

Ahmed, 1968.

Bernstein, 1961.

Webber and Case, 1960.

FIGURE 9

PATHWAYS FOR SYNTHESIS OF ADENYLIC ACID AND HISTIDINE AND FOR UTILISATION OF ADENINE

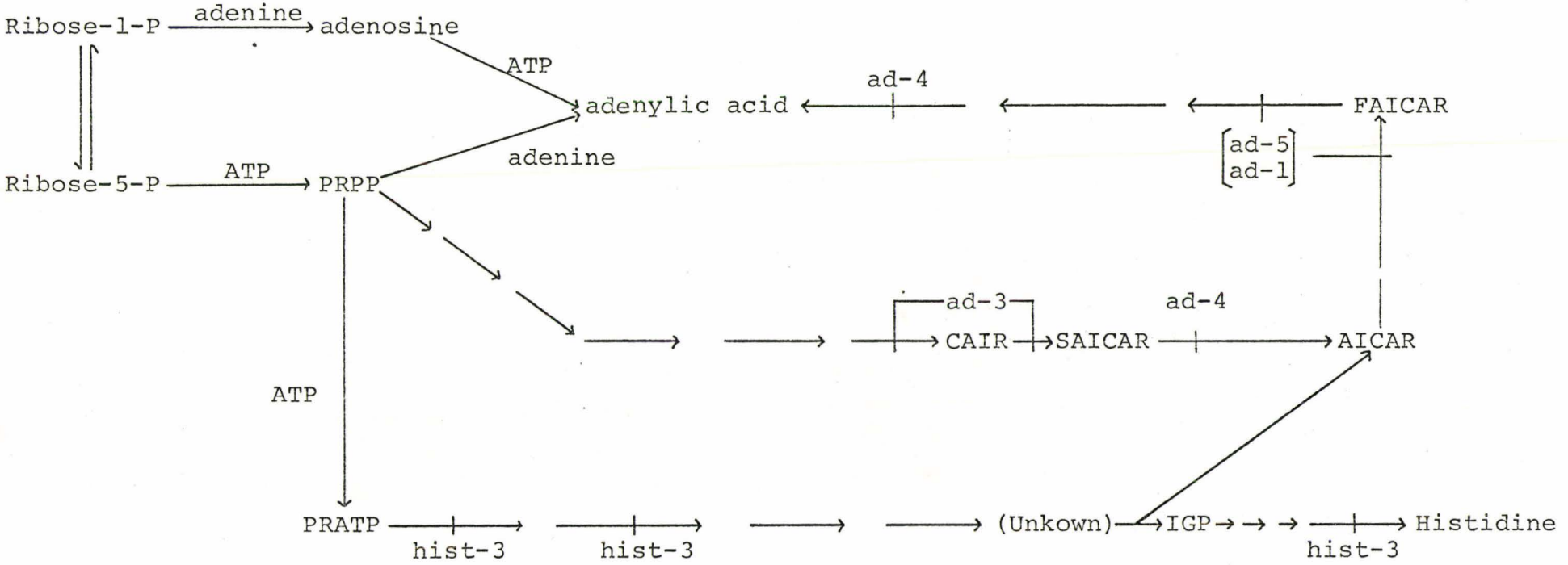


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FIGURE 10

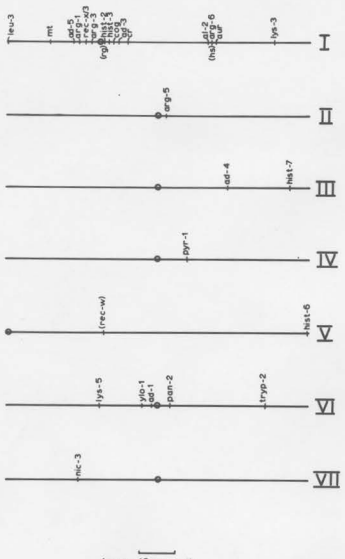
PEDIGREES OF STRAINS USED AND LINKAGE MAPS OF STRAINS REFERRED TO IN THIS STUDY

The linkage maps are diagrammatic only. Centromere positions relative to the telomeres are chosen for convenience only and do not portray their true positions.

The linkage group I centromere region has been expanded for clarity. Other map distances are approximate for rec-c x rec-c type crosses.

The positions of *hs*, *rec-w* and *rg* relative to their centromeres or other markers are uncertain.

$(JRS-8 \times FGSC\ 667)^3$ denotes three backcrosses of JRS-8 to FGSC 667.



FGSC 414 x FGSC 667
A, rec-s | a, hist-2, rec-c

CSB-8 x FGSC 668
a, rec-s | A, hist-2, rec-c

(JRS-8 x FGSC 667)^3
A, rec-s | a, hist-2, rec-c

30JA-5

30JA-5 x C1220 R_{229}
A, rec-s | a, rec-c, ad-3A
SC 180

30JA-5 x C162-461R
A, rec-s | a, hist-2, rec-c, ad-3B
SC 195

FGSC 987 x FGSC 246
A, rec-c | a, ad-5, hist-2, rec-c, cr

102-2 x FGSC 987
a, ad-5, hist-2, rec-c, cr | A, rec-c

202-1

30JA-5 x 202-1
A, rec-s | a, ad-5, hist-2, rec-c, cr
1 & CS4

987 x 202-1
A, rec-c | a, ad-5, hist-2, rec-c, cr
CC1

30JA-5 x 202-4
A, rec-s | a, ad-5, hist-2, rec-c, cr

P369

B27 x B56
a, ad-5, rec-s | A, rec-s, cr
SS2

14-5 x P369
a, rec-c, al-2, ad-1, pan-2(5) | A, hist-2, rec-c, cr

P369 x 14-5
A, hist-2, rec-c, cr | a, rec-c, al-2, ad-1, pan-2(5)

CC C1009

CC C973

C1009 R_{22} x C1009 R_{33}
A, rec-c, al-2 | a, rec-c, cr, ad-1, pan-2(5)
CC C1107

K4

K4 x ORa
A, ad-5, rec-s, cr | a, rec-c

ORa x K4
a, rec-c | A, ad-5, rec-s, cr
CS1

K110 x K4
a, rec-s | A, ad-5, rec-s, cr
SS1

(ORa x K4)A_{9-1} x C1009 R_{15}
a, ad-5, rec-s | A, hist-2, rec-c cr, al-2
SC C1085

#5 x C484 R_{17}
A, hist-2, rec-c, cr | a, rec-c, ylo-1, pan-2(3), tryp-2
CC C967

C967 A_{78-4}

C419 A_{1-7} x D11
A, ad-5, rec-s, cr | a, rec-c, arg-5
SC C651

C419 A_{1-8}

C1096 A_{1-1} x C1085 A_{35-7}
a, rec-c, ad-3A | A, ad-5, hist-2, rec-c, cr, al-2
CC C1219

C419 A_{1-8} x D11
A, ad-5, rec-s, cr | a, rec-c, arg-5
SC C661

C419 A_{1-8} x D36
A, ad-5, rec-s, cr | a, rec-c, nic-3
SC C663

D25 x C419 A_{1-8}
a, rec-c, pan-2(3) | A, ad-5, rec-s, cr
CS C703, 1078

C419 A_{1-8} x C481 R_{13}
A, ad-5, rec-s, cr | a, rec-c, pyr-1
SC C666

C973 R_6 x C967 A_{78-4}
a, hist-2, rec-c, cr, al-2, ad-1, pan-2(5) | A, rec-c, ylo-1, tryp-2
CC C1206 & het.

C703 A_{30-2} x C1164 R_{29}
a, cr, | A, rec-c, ad-3A, aur
C1129

C703 A_{26-1}

C703 A_{30-4} x C1164 R_{53}
A, cr, pan-2(3) | a, rec-c, ad-3A, aur
C1130

C860 R_{10} x C703 A_{26-1}
a, rec-c, lys-5 | A, rec-s, cr
CS C1182

C873 R_{20} x C703 A_{26-1}
leu-3, a, rec-c | A, rec-s, cr
CS C1183

30JA-5 x C703 A_{25-5}
A, rec-s | a, ad-5, rec-s, cr, pan-2(3)
SS C1198

C1100 R_{48} x C703 A_{3-7}
a, rec-c, aur | A, ad-5, rec-s, cr, pan-2(3)
CS C1199

C703 A_{22-1} x C967 A_{78-4}
a, rec-s, cr, pan-2(3) | A, rec-c, ylo-1, tryp-2
SC C1207, C1253, het