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Location of factors enhancing crossing over on linkage group I of Neurospora

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SUMMARY

An enhancement of cross-over frequencies previously reported by Newcombe & Threlkeld (1972) is shown to be due to two regions located on linkage group I, a few cross-over units to the right of the centromere. The distal region appears to be shorter than the proximal region, but equally effective in enhancing cross-over frequencies. The longer proximal region is readily divisable by cross-overs and probably spans several cross-over units.

1. INTRODUCTION

Recently Newcombe & Threlkeld (1972) reported on a series of experiments in which the centromere proximal region of linkage group I of *Neurospora sitophila* was transferred to *N. crassa*, and the similar region from *N. crassa* was transferred to *N. sitophila*. It was shown that the centromere proximal region from *N. sitophila* had a dominant effect in enhancing cross-over frequencies on linkage group I of *N. crassa*, and that the *N. crassa* centromere proximal region acted recessively in reducing crossing over on the linkage group in *N. sitophila*. It was unknown as to whether or not the effect originated from the centromere itself, from a gene closely linked to the centromere or from some other unspecified mechanism. The terms *Rec-s* and *rec-c* are used to describe the dominant and recessive genotypes originating from *N. sitophila* and *N. crassa* respectively. The terms do not necessarily infer the existence of conventional gene locations, but simply describe alternative genotypes related to genetic differences located on linkage group I effecting recombination.

This paper first describes work that demonstrates that *Rec-s* and *rec-c* may be separated from their respective centromeres, thus showing that the differential effects on recombination are not specifically a property of the centromeres. Further experimental data are presented to show that *Rec-s* may be subdivided into two regions lying between the locus *hist*-2 and the locus *ad*-3.

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2. MATERIALS AND METHODS

Methods used have been described elsewhere (e.g. Threlkeld, 1962), except for the prototroph analysis described below:

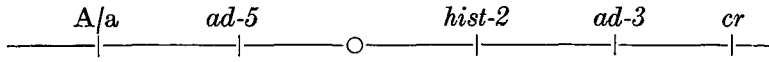
Spores from crosses were harvested in 1 ml aliquots of 0·1 % agar to each of which was added a further 4 ml of 0·1 % agar. A homogeneous suspension was formed using a vortex mixer. Quantities of the suspension containing approximately 1000 spores

Table 1. *Details of strains*

Isolate	Markers	Allele nos.	*Rec* type	Comments
ORA-1-1	*A*	—	*rec-c*	Isolates from cross of OR74A × ORa (Standard wild-type strains)
ORa-1-3	*a*	—	*rec-c*	
30JA-5	*A*	—	*rec-s*	See Newcombe & Threlkeld (1972)
202-1	*a ad-5 hist-2 cr*	Y152M40, Y152M14, B123	*rec-c*	
2-17-114A	*A ad-3B*	—	*rec-c*	Induced in OR74A by Griffiths using nitrous acid, and back-crossed once to ORa
2-17-114a	*a ad-3B*	—	*rec-c*	
PPr 8	*A cr*	B123	*rec-c*	Obtained from a cross *A hist-2 cr* × *a ad-5*
C443Rg	*a hist-2*	Y175M611	*rec-c*	See Ferraro (1971)
C162-461R	*a hist-2 ad-3B*	Y152M611	*rec-c*	Obtained from a cross of C443Rg × 2-17-114a
C162-9R	*A hist-2*	Y175M611	*rec-c*	

were added to 10 ml of appropriately supplemented moulten agar held at 60 °C and the spores were incubated at that temperature for 45 min, after which the agar was poured into Petri plates. The plates were incubated at 25 °C for approximately 48 h, at which time direct counts of prototroph colonies and percentage spore germination were made. Five replicas were made of each plate.

The strains used in this work are described in Table 1. Except where stated these strains are not known to possess the high frequency cross-over factor or factors under investigation (i.e. they may be described as *rec-c*). The linkage sequence of the markers included in the strains are as follows:



Strains 2-17-114A and 2-17-114a were kindly provided by Dr A. J. F. Griffiths, and we are indebted to Mr Michael Ferraro for strain C443Rg.

3. RESULTS AND DISCUSSION

Seventy-two asci were analysed from the cross 30JA5 × 202-1 (*A Rec-s* × *a ad-5 hist-2 cr rec-c*), two of which showed second division segregation for the *hist-2* marker (nos. 11 and 77), indicating a cross-over between *hist-2* and the centromere. In order to see if the cross-over had also occurred between *Rec-s*/*rec-c* and the centromere, at least one isolate from each spore pair (except for the non-germinated

pair 77-5, 6, in which case 77-3, 4, were both tested) of the two asci was crossed to an appropriate *rec-c* tester strain (one of ORa-1-3, PPr8, ORA-1-1, or 202-1), and recombination frequencies for linkage group I were determined for each cross. The results are shown in Table 2, and the genotypes of the ascospores from the two asci, complete for all markers studied together with the *Rec-s/rec-c* genotype are shown below:

11-1	A	*ad-5 hist-2 cr rec-c*	77-2	A	*hist-2 cr rec-c*
11-4	a	*ad-5 Rec-s*	77-3	a	*Rec-s*
11-6	a	*hist-2 cr rec-c*	77-4	a	*Rec-s*
11-7	A	*Rec-s*	77-5 (presumed)	A	*ad-5 hist-2 cr rec-c*
11-8	A	*Rec-s*	77-8	a	*ad-5 Rec-s*

It can be seen that the second division segregation pattern for *Rec-s/rec-c* corresponds to that for the markers on the right arm of the linkage group, and not to that for the mating type markers on the left arm. Results from the analysis of these asci

Table 2. *Results of crosses used to distinguish between* Rec-s *and* rec-c *genotypes for isolates of ascus 11 and ascus 77*

Isolates (ascus and spore no.)	Tester strain	No. of random spores analysed	Recombination frequencies (regions) *ad-5* —|— *hist-2* —|— *cr* —|	Genotype with respect to *Rec-s/rec-c*
11-1	ORa-1-3	94	← 6·4 ± 2·5 →	*rec-c*
11-4	PPr 8	91	← 32·2 ± 4·3 →	*Rec-s*
11-6	ORA-1-1	218	← 8·7 ± 1·9 →	*rec-c*
11-7	202-1	97	← 3·1 ± 1·8 → ← 30·9 ± 4·7 →	*Rec-s*
11-8	202-1	96	← 6·3 ± 2·5 → ← 36·5 ± 4·9 →	*Rec-s*
77-2	ORa-1-3	195	← 3·6 ± 1·7 →	*rec-c*
77-3	202-1	92	← 4·4 ± 2·1 → ← 44·6 ± 5·2 →	*Rec-s*
77-4	202-1	82	← 3·7 ± 2·1 → ← 40·2 ± 5·4 →	*Rec-s*
77-8	PPr 8	180	← 38·3 ± 3·6 →	*Rec-s*

suggest that the variation in cross-over frequencies is unlikely to be due to alternative states of the centromere, and is better looked for in a factor or factors on the right arm of the linkage group, probably distal to the *hist-2* marker. However, the possibility that spindle overlap has occurred and that *Rec-s/rec-c* have not been separated from the centromere cannot be ruled out.

The cross 30JA5 × C162-461R (*A Rec-s* × *a hist-2 ad-3B rec-c*) was made in the hope of gaining a more precise estimate of the location of the factor or factors associated with the *Rec-s/rec-c* alternatives. It was thought reasonable to speculate that *Rec-s* might lie between *hist-2*$^+$ and *ad-3B*$^+$ in strain 30JA5, for it has been shown that *Rec-s* segregates with the *hist-2* locus (present communication), but nevertheless is near the centromere (Newcombe & Threlkeld, 1972).

One hundred and ninety-two asci were isolated from the cross (91·8 % germination), of which 85 contained a cross-over between the *hist-2* and *ad-3B* markers

(second division segregation for *ad-3B* but not for *hist-2*, with symmetrical patterns, approximately equalling asymmetrical patterns), giving a high frequency of recombination in the region *hist-2* to *ad-3B* of approximately 22 % ($85/192 \times 1/2 \times 100$), as might be expected from a *Rec-s* × *rec-c* cross (Newcombe & Threlkeld, 1972). Isolates from each ascus exhibiting a cross-over between the *hist-2* and *ad-3B* markers were further crossed to known low cross-over tester strains, and the progeny from such crosses were analysed for *hist-2*$^+$ *ad-3B*$^+$ recombinants by prototroph analysis. Thus *a hist-2* isolates (from asci of the cross 30JA5 × C162-461R) were crossed to 2-17-114A; *A hist-2* isolates to 2-17-114a; *a ad-3B* isolates to C162-9R and *A ad-3B* isolates to C443Rg. A control cross of *rec-c* × *rec-c* (C443Rg × 2-17-114A) was also established and similarly analysed for prototrophs. The data are summarized in Table 3. These data include only asci where both recombinant classes germinated.

An inspection of the data reveals that the combined prototroph frequencies from the crosses of the two recombinants (*hist*$^+$ *ad* and *hist ad*$^+$) for each ascus are approximately equal, with each possessing a total recombination frequency of approximately 23 % for the region *hist-2 ad-3B* ($12{\cdot}5 \pm 1{\cdot}4$ % prototrophs). The control cross (*rec-c* × *rec-c*) shows a prototroph frequency of 0·58 %. The lowest frequency associated with a *hist-ad*$^+$ type recombinant is 0·42 % prototrophs (ascus 197), and with a *hist*$^+$ *ad* type recombinant it is 0·78 % (ascus 191), suggesting that the *Rec-s* genotype lies between the *hist-2* and the *ad-3* markers with possibly some extension of the genotype a little proximal to the *hist-2* marker. That only a few asci show a clear-cut segregation of high-frequency prototroph production from low, and that most of the asci show enhanced and intermediate frequencies of prototrophs for both recombinant classes indicates that the *Rec-s* genotype should be regarded as a relatively long region divisible by crossing-over, rather than a single conventional locus. Note that the higher intermediate frequencies are generally associated with the *hist ad*$^+$ recombinant, rather than with the *hist*$^+$ *ad* class.

The data recorded in Table 3 have been used to construct scattergrams, where each point represents information from a single ascus, with the data from one recombinant (e.g. *ad hist*$^+$) plotted against that from the other recombinant (*ad*$^+$ *hist*). The scattergrams are seen in Fig. 1; in the first, direct use is made of the prototroph frequencies, and the second utilizes the arcsin transformation of that data. It becomes clear that the data reflect at least two phenomena, and perhaps two regions, one of which may be regarded as a locus, and the other as a relatively long region. Thus the percentage prototroph frequencies associated with the *hist* recombinant between 0·42 and 1·28 and their paired *ad* recombinant frequencies of 9·85–11·04 fall into one class plotted as the point $ad = 19{\cdot}25 \pm 1{\cdot}13$, $hist = 5{\cdot}31 \pm 0{\cdot}67$ (arcsin transformation), or $ad = 11{\cdot}13 \pm 1{\cdot}28$, $hist = 0{\cdot}93 \pm 0{\cdot}22$ for the untransformed data. A regression line analysis of the remaining data (arcsin) gives a highly significant correlation between the paired *ad* and *hist* frequencies, and a regression line of $y = 21{\cdot}43 - 0{\cdot}62x$, for the transformed data, where $y = ad\text{-}3$ isolates and $x = hist\text{-}2$ isolates.

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Table 3. *Recombination data of asci from cross* $30JA5 \times C162\text{-}461R$

Ascus and spore numbers	Genotype	Average % prototrophs	Total % prototrophs
3-4	A + ad-3B	3·78	
-5	a hist-2 +	9·37	13·15
5-7	A + ad-3B	4·55	
-1	a hist-2 +	5·85	10·40
9-3	A + ad-3B	3·76	
-7	A hist-2 +	8·63	12·69
13-7	A + ad-3B	4·94	
-3	a hist-2 +	8·74	13·68
14-1	A + ad-3B	3·47	
-7	a hist-2 +	7·94	11·41
18-6	A + ad-3B	2·53	
-1	a hist-2 +	7·56	10·09
22-7	A + ad-3B	10·28	
-4	A hist-2 +	0·84	11·12
26-7	A + ad-3B	5·41	
-3	a hist-2 +	5·74	11·15
28-7	A + ad-3B	12·88	
-3	A hist-2 +	0·84	13·52
33-5	A + ad-3B	0·95	
-1	a hist-2 +	13·31	14·26
35-1	A + ad-3B	4·01	
-7	a hist-2 +	9·40	13·41
37-1	A + ad-3B	4·95	
-6	a hist-2 +	9·97	14·92
39-3	a + ad-3B	6·55	
-5	a hist-2 +	8·65	15·20
46-7	A + ad-3B	10·94	
-1	a hist-2 +	0·82	11·76
49-1	A + ad-3B	4·40	
-7	a hist-2 +	8·92	13·32
52-5	A + ad-3B	11·04	
-7	A hist-2 +	1·28	12·32
53-7	A + ad-3B	13·22	
-4	A hist-2 +	0·80	14·02
54-3	A + ad-3B	4·34	
-7	a hist-2 +	6·81	11·15
57-1	A + ad-3B	4·05	
-7	a hist-2 +	8·61	12·66
58-3	A + ad-3B	5·13	
-5	a hist-2 +	9·00	14·13
62-4	A + ad-3B	13·08	
-5	A hist-2 +	0·83	13·91
69-7	A + ad-3B	4·48	
-1	a hist-2 +	7·49	11·97

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Table 3 (*cont.*)

Ascus and spore numbers	Genotypes	Average % prototrophs	Total % prototrophs
72-5	A + ad-3B	2·82	
-3	a hist-2+	7·24	10·06
73-1	A + ad-3B	11·45	
-5	a hist-2+	0·96	12·41
75-3	A + ad-3B	10·26	
-7	A hist-2+	0·83	11·09
76-5	A + ad-3B	2·21	
-3	a hist-2+	13·86	16·07
78-7	A + ad-3B	9·25	
-1	a hist-2+	1·08	10·33
80-3	A + ad-3B	4·03	
-5	a hist-2+	7·39	11·42
81-3	A + ad-3B	3·71	
-7	a hist-2+	8·79	12·50
85-3	A + ad-3B	4·41	
-5	A hist-2+	9·57	13·98
90-7	a + ad-3B	11·08	
-3	A hist-2+	1·19	12·27
96-7	A + ad-3B	13·77	
-1	a hist-2+	0·89	14·66
98-1	A + ad-3B	1·60	
-5	a hist-2+	12·32	13·92
100-3	A + ad-3B	5·27	
-5	a hist-2+	6·84	12·11
102-1	A + ad-3B	2·99	
-5	a hist-2+	11·07	14·06
105-5	A + ad-3B	10·99	
-1	a hist-2+	1·28	12·27
109-3	A + ad-3B	4·47	
-7	a hist-2+	6·30	10·77
116-5	A + ad-3B	3·92	
-4	a hist-2+	8·00	11·92
118-7	A + ad-3B	10·26	
-3	a hist-2+	0·68	10·94
120-1	a + ad-3B	4·43	
-6	A hist-2+	9·17	13·60
121-2	A + ad-3B	3·70	
-5	a hist-2+	11·94	15·64
130-1	A + ad-3B	2·97	
-3	a hist-2+	10·04	13·01
134-1	A + ad-3B	4·77	
-5	A hist-2+	7·84	12·64
135-1	A + ad-3B	4·52	
-7	A hist-2+	7·59	12·11
152-5	A + ad-3B	4·05	
-3	A hist-2+	8·71	12·76

Table 3 (*cont.*)

Ascus and spore numbers	Genotype	Average % prototrophs	Total % prototrophs
155-5	A + ad-3B	10·26	
-3	A hist-2 +	1·27	11·43
161-5	a + ad-3B	4·71	
-1	A hist-2 +	7·40	12.11
166-5	A + ad-3B	5·64	
-1	a hist-2 +	7·27	12·91
170-1	A + ad-3B	5·29	
-5	a hist-2 +	6·24	11·53
175-3	a + ad-3B	1·31	
-7	a hist-2 +	12·05	13·36
177-1	A + ad-3B	3·19	
-5	a hist-2 +	10·57	13·76
179-5	A + ad-3B	9·62	
-3	a hist-2 +	0·91	10·53
180-7	A + ad-3B	4·54	
-2	a hist-2 +	6·30	10·84
181-5	A + ad-3B	2·03	
-1	a hist-2 +	10·38	12·41
182-4	A + ad-3B	11·61	
-5	a hist-2 +	1·04	12·65
183-1	A + ad-3B	1·54	
-7	a hist-2 +	10·62	12·16
188-1	A + ad-3B	3·42	
-5	a hist-2 +	10·68	14·10
191-7	A + ad-3B	0·78	
-3	a hist-2 +	10·54	11·32
194-7	A + ad-3B	1·46	
-3	a hist-2 +	10·13	11·59
195-5	A + ad-3B	5·02	
-3	a hist-2 +	6·66	11·68
197-6	A + ad-3B	9·85	
-2	a hist-2 +	0·42	10·27
200-3	A + ad-3B	6·28	
-5	a hist-2 +	5·67	11·95
202-3	A + ad-3B	5·07	
-6	a hist-2 +	6·64	11·71

The overall view of the various aspects of the data derived from the cross 30JA5 × C162-461R and described above, may be seen in the form of a model, the diagram of which, expressed in the genotypes of the two parental strains, is seen in Fig. 2.

Rec-s$_1$ and *Rec-s*$_2$ represent parts of the genome responsible for the enhancement of cross-over frequencies. The operation of the model is as follows: a cross-over in the region *Rec-s*$_1$ results in *hist*$^+$ *ad* and *hist ad*$^+$ recombinants, each of which in

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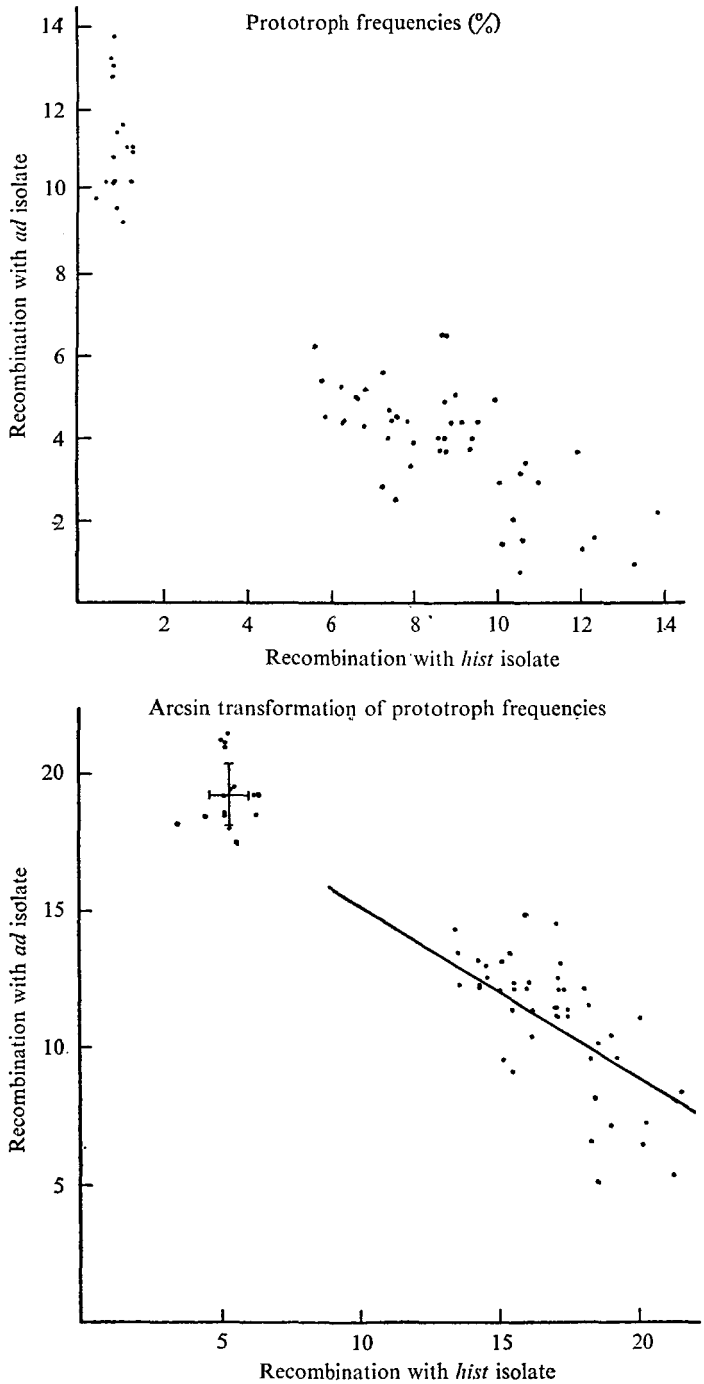

Fig. 1. Recombination frequencies associated with the *hist* recombinant and with the *ad* recombinant isolates of each ascus described in Table 3, plotted as paired data on scattergrams.

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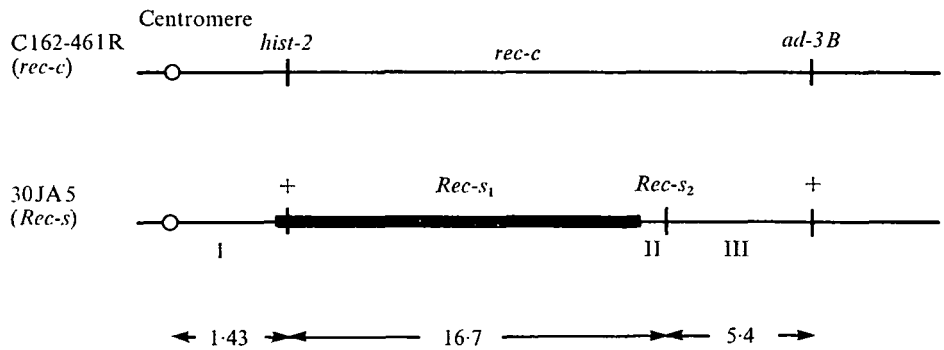

Fig. 2. A model of the *Rec-s* genotype based on the proposed genotypes of the strains C162-461R and 30JA5.

appropriate tester crosses will give rise to enhanced prototroph production, with a greater enhancement being associated with the *hist ad*$^+$ recombinant class, for a single cross-over in the region *Rec-s*$_1$, will always result in *Rec-s*$_2$ segregating with that recombinant. Thus cross-overs in the region *Rec-s*$_1$ would result in the production of a class of recombinants with the potential for a range of prototroph frequencies, and with a correlation between paired data such that the association of a higher protroph frequency with one recombinant would be accompanied by a lower frequency for the other recombinant, as indicated by the scattergrams.

Cross-overs in region III should result in a distinct class of asci showing a low (approximating the control) frequency of prototrophs to be associated with the *hist ad*$^+$ recombinant, and a high frequency associated with the *hist*$^+$ *ad* recombinant. Cross-overs in region II should result in a discontinuity of the data, so that the gradual increase in prototroph frequencies associated with the *ad* recombinant class as cross-overs in *Rec-s*$_1$ fall at more distal sites, comes to a halt with cross-overs falling in region II, and further enhancement is not reached until cross-overs fall in region III. The discontinuity is seen from the scattergrams, and from the fact that no prototroph values between 6·55 and 9·25 % were recorded for *ad* recombinants. Similarly, a discontinuity exists for the *hist* recombinants, and no prototroph values between 1·28 and 5·67 % were recorded for this class. Thus for the *hist* recombinants a cross-over in region III would result in protroph frequencies of less than 1·28 %, and a cross-over in region II would result in prototroph frequencies in excess of 5·67 %. These data together indicate that *Rec-s*$_2$ exists as a locus that was not divisible, within the limits of the experiments described, by cross-overs.

Some estimate of the lengths of the various regions shown in the model is attempted below. Based on the data from the first cross described (30JA5 × 202-1) in which two asci of a total of 70 showed second division segregation for *hist*, region I approximates 1·43 cross-over units. Of a total of 192 asci from the cross 30JA5 × C162-461R, 64 asci (including those not listed in Table 3, where only one recombinant germinated) fell into the class of intermediate enhancement of prototrophs for recombinants, the class represented by the repression line in Figure 1. This suggests that *Rec-s*$_1$ plus region II covers a distance of 16·7 cross-over units. Although the scattergrams

show a greater clustering of points in the area of higher prototroph frequencies for the *ad* recombinants, it is not possible to identify clearly a class of asci resulting from a cross-over in region II, suggesting that region II might be relatively short. It is in fact possible that one non-homogeneous region exists in place of *Rec-s*$_1$ and *Rec-s*$_2$. Region III appears to be approximately 5·4 cross-over units long, for there are 21 asci (including those in which only one recombinant spore germinated) of the total of 192 that appear to originate from a cross-over in that region; these asci fall into the class represented by the point *ad* = 19·25, *hist* = 5·31 on the scattergram.

It should be noted that all the cross-over values that have been determined for the model are valid only when the *Rec*-s genotype is present, and a word of caution must be introduced. The simple relationship between map distances and cross-over frequencies, implied in the above argument, may be far from reality and the above model may be a gross distortion of that reality. Nevertheless, the model is thought to be useful if only for pointing the way for future experiments.

Obviously the mechanism by which *Rec-s*$_1$ and *Rec-s*$_2$ achieve an enhancement of cross-over frequencies is of considerable interest; however, few clues are present in the existing data as to what these mechanisms may be; the clues that do exist reside in the knowledge of the dominant nature of the phenomena, the length of the region occupied by *Rec-s*$_1$, and the fact that the enhancement is expressed over relatively long distances of linkage group I. An additional clue comes from the work of Michael Ferraro (1971), in which he demonstrated that although *Rec-s* behaves as a dominant in crosses, no such influence could be detected in *Rec-s* + *rec-c* heterokaryons crossed to *rec-c* strains. Further work is being specifically directed at recombination between alleles of loci close to *Rec-s*$_1$ and *Rec-s*$_2$, and to other aspects of the differences between *Rec-s*$_1$ and *Rec-s*$_2$.

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