THE CONTROL OF RECOMBINATION AT THE HISTIDINE-1 LOCUS OF NEUROSPORA CRASSA

by

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This thesis describes the results of my own work, performed under the supervision of Professor D.G. Catcheside.

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SUMMARY

Prototrophic recombinants were obtained from repulsion phase crosses of pairs of 36 different auxotrophic alleles at the histidine-1 locus of Neurospora crassa. Examinations of prototroph frequencies, and the distributions of the four classes of prototrophs specified by segregation of flanking markers which were closely linked to his-1, were made in both rec-1$^{(+)}$ x rec-1^{+} and rec-1 x rec-1 genetic backgrounds. One intensively studied allele combination (his-1^{K83} x his-1^{K625}) revealed that a range of prototroph frequencies, due to additional factors (unidentified), could be found within each of the two types of rec-1 background. Other less intensively studied allele pairs exhibited similar variations in prototroph frequency. The differences between rec-1 x rec-1 and rec-1$^{(+)}$ x rec-1^{+} prototroph frequencies remained large enough to allow easy discrimination between the two backgrounds.

Determination of a fine structure map of the sites of his-1 allelic differences was generally accomplished by using the prototroph frequency data in conjunction with significant differences between the numbers in

the two classes of prototrophs recombinant for flanking markers. Significant differences between the numbers of the two proximal flanking markers (*am-1* and *am-1*$^{+}$) were also used to order the sites of *his-1* allelic difference in *rec-1*$^{(+)}$ x *rec-1*$^{+}$ backgrounds while significant differences between the numbers of the two distal alleles (*inos* and *inos*$^{+}$) were used when the allelic crosses were *rec-1* x *rec-1*. The proximal flanking markers provided ambiguous orders for *rec-1* x *rec-1* crosses while the distal flanking markers provided ambiguous orders for *rec-1*$^{(+)}$ x *rec-1*$^{+}$ crosses.

A comparison of the distribution of flanking markers among prototrophs of *his-1*^{K83} x *his-1*^{K625} *rec-1* x *rec-1* and *rec-1* x *rec-1*$^{+}$ crosses showed that there was a marked change in the proportions of the two parental classes, from 1.51:1 to 0.55:1, but none in the proportions of the two recombinant classes (1.88:1). The change in proportions was inseparable from the *rec-1* locus.

The ratios formed by the numbers in the two recombinant classes of prototrophs were found to be

significantly different for different allele pairs. With the possible exception of crosses involving three alleles which differed in origin from the rest, the differences could not be directly associated with the rec-1 constitution, nor could they be associated with proximity of the sites of allelic difference or any other common factor.

The ratios formed by the numbers in the two classes of prototrophs with parental associations of flanking markers were also found to be different for different allele pairs. Compared to the his-1^{K83} x his-1^{K625} results, the ratios were influenced in the same directions, but to differing extents, by the rec-1 and rec-1^{+} alleles. The degree of separation of the sites of allelic difference could not be directly associated with any change in ratio.

The results are explained by assuming that the rec-1 genes influence the point of origin, with respect to the his-1 locus, of hybrid DNA. Calculations made on this basis show that the rec-1$^{(+)}$ x rec-1^{+} results can be explained by hybrid DNA originating distally. The results from all but eight of the rec-1 x rec-1

allele combinations can be explained by hybrid DNA originating proximally. The remaining eight rec-1 x rec-1 allele combinations which were studied require the presence of a proportion of hybridity covering the distal his-1 mutant site only, the remainder of the hybridity covering both sites.

Inequality between the amount of full non-sister chromatid exchange and no exchange was found to have no effect in the calculation of the proportions of the prototrophs which resulted from the different types of hybrid DNA.

The rec-1^{+} gene is assumed to produce a regulator which blocks, or otherwise affects, proximally originating hybrid DNA at the his-1 locus, or the occurrence of recombination which produces such hybrid DNA. The difference between flanking marker distributions among prototrophs from crosses involving different allele pairs are suggested to be a consequence of the specific alleles causing altered chromatid pairing characteristics.

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CHAPTER I

INTRODUCTION

A repulsion phase cross between individuals containing different allelic auxotrophs will generally yield prototrophic progeny at a very low frequency. Analysis of interallelic crosses often shows that about 50% of the prototrophic progeny have undergone non-sister chromatid exchange in the region of the locus being studied. Therefore, unless the selection of these prototrophs has also selected atypical exchange events, crossing-over has occurred in the immediate vicinity of prototroph production approximately half the time.

The associations of flanking markers found among prototrophs from allelic crosses were originally ascribed to the occurrence of multiple cross-over events within short regions (Giles, 1951). This explanation was followed by another, the copy-choice hypothesis, which suggested that daughter chromatids were formed by copying the parental chromatids so as to leave the latter chromatids intact (Lederberg, 1955; Freese, 1957). Close association of homologous chromosomes could result

in switching of the templates. Unsynchronised synthesis of the daughter chromatids could result in 3:1 ratios due to utilisation of the same template over short distances.

A number of experimental ovservations, all of which have been confirmed using several different experimental systems, were found which contradicted the copy-choice hypothesis:

i) Meselson and Stahl (1958) found that DNA replication in Escherichia coli was semi-conservative.

ii) Olive (1959) found post-meiotic 5:3 and 3:5 ratios for ascospore colour in Sordaria fimicola asci from a hyaline by normal ascospore colour cross.

iii) Bole-Gowda et. al. (1962) found that pooled results from a number of studies showed that the pair of chromatids involved in cross-overs were chosen nearly at random. Only the daughter chromatids are expected to be involved in successive cross-overs if the copy choice hypothesis is applicable.

iv) Rossen and Westergaard (1966) showed that

the DNA of Neottiella rutilans replicates before nuclear fusion.

A number of variants of the copy-choice hypothesis have been suggested, none of which accommodate all of the objections.

Recombination by breakage and rejoining of DNA molecules in procaryotes has been demonstrated (Meselson and Weigle, 1961; Kellenberger et. al., 1961). Evidence that such events occur in eucaryotes was obtained by Taylor (1965) when he labelled male grasshopper chromosomes radioactively and then followed the meiotic event of spermatogenesis.

The study of recombination in bacteriophages (reviewed by Meselson, 1967) indicates that the point of exchange between chromosome fragments is a region of complementary polynucleotide chains of different parentage. Mismatched base pairs in this region of heteroduplex, or hybrid, DNA could be corrected by either postmeiotic segregation or enzymic excision and repair processes, resulting in prototroph production. Many different hypotheses deal with the sequential

events involved in hybrid DNA initiation, duration, termination and the genetic implications of the whole process.

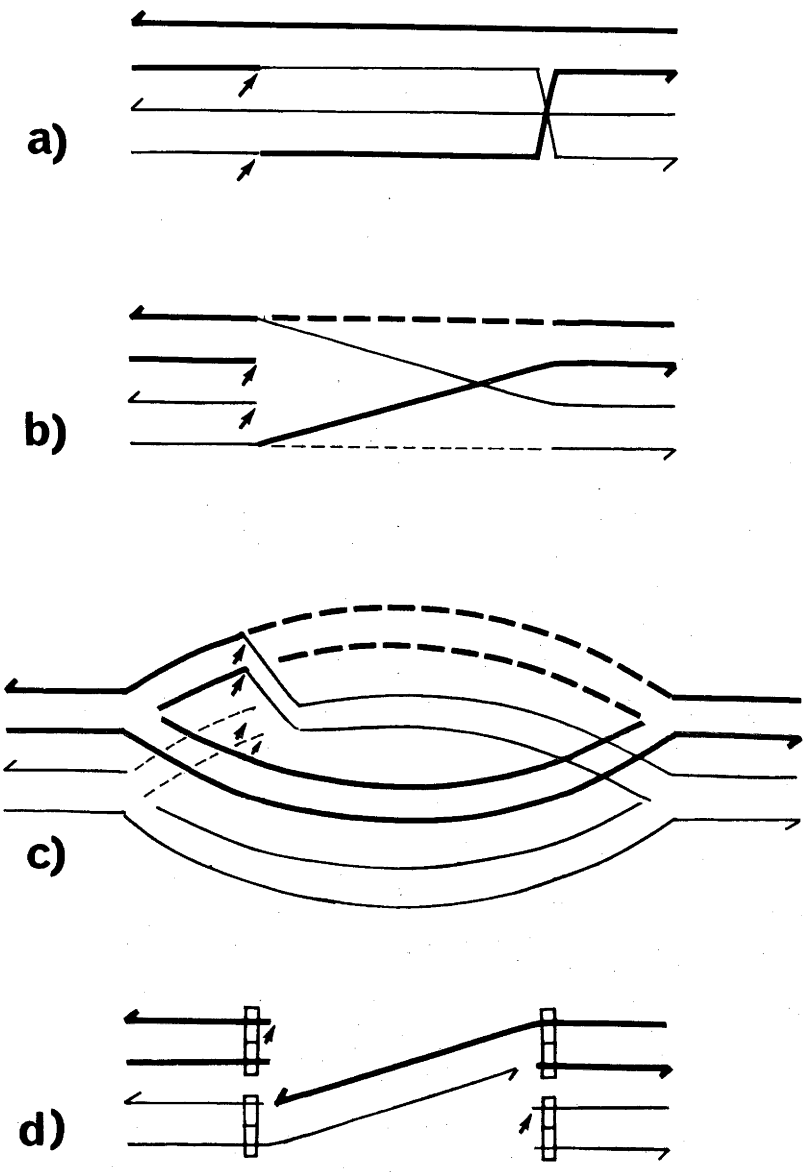
Holliday (1964, 1968) supposes that a specific sequence of bases, a recombinator, is the specific substrate for an endonuclease which initiates recombination by causing the first single strand breaks at exactly the same point in strands of the same polarity in non-sister chromatids. The chromatids then unravel to form single strands which are free to anneal or coil up with the unbroken complementary strands of the opposite homologous chromatids (fig.1a). Continuity of the DNA strands is assumed to be accomplished by breakage and reunion terminating the DNA hybridity. The pair of chromatid strands in which the breakage occurs determines whether or not the event results in a cross-over between non-sister chromatids.

Whitehouse (1963, 1967) suggests that the initial break is caused in strands of opposite polarity by the failure of the phosphodiester backbones of newly synthesized nucleotide chains of DNA to join across the

Figure 1. Illustrations of four different hypotheses of recombination. The lines represent the single DNA strands belonging to two of the four chromatids present during meiotic recombination. The heavy and light lines distinguish between the strands from the chromatids of the two parents. Polarity of the DNA strands is shown by the arrow heads. The short arrows (↗) indicate the locations of the initial breaks in the DNA strands. The dotted lines illustrate DNA strands which will be degraded. Single strand dissociation and reassociation following the breaks left the chromatid configurations shown. Ensuing events, leading to continuity of the chromatids, depend on the hypothesis. a) (Holliday, 1964). Breaks in two DNA strands of the same polarity at the location of the single strand exchange will either restore the parental chromatids (except for the hybrid DNA section) or lead to non-sister chromatid exchange, depending on the pair of strands broken. b) (Whitehouse, 1963). Duplication of the strands involved in the exchange, over the length of the exchange, will give a cross-over between non-sister chromatids.

Fig. 1 (cont.)

Duplication of all, or a portion of, the strands about to be excised, followed by dissociation of the new strands and their annealing to the cross-over strands (resulting in DNA hybridity), with further duplication making up any deficiencies, will also lead to chromatid continuity and non-sister chromatid exchange. c) (Stahl, 1969). A second exchange between either pair of non-sister arcs, with subsequent removal of the non-essential duplex regions, will lead to continuity which may or may not be associated with non-sister chromatid exchange. Hybrid DNA is formed over short regions during the breakage and rejoining process. d) (Taylor, 1967). Failure of duplication of the DNA between the links allowed the formation of hybrid DNA regions and therefore correction of mis-matched base pairs. Continuity of the chromatids occurs after full replication of the regions. Hybrid DNA can also be formed (by an unspecified process) in neighbouring regions.


a)
b)
c)
d)

operator of a gene. The strands are then free to uncoil and separate over the greater part of the polaron (fig. 1b). Synthesis of nucleotide chains occurs, beginning at the site of the break, using the unbroken DNA strands as templates. The newly synthesized chains, which need not be of equal length on each chromatid, can dissociate from their templates and anneal with the complementary broken chains from the other molecule. The unpaired non-crossover chains are broken down and any remaining gaps in the recombined chromatids are filled in. The net result is a cross-over region which, if it contains a base difference contributed by the parents, may cause DNA hybridity in neither, either or both of the chromatids involved, depending on the length of the newly synthesised chains before they anneal with their complementary, originally broken chains. Recombination between flanking regions is requisite. A second cross-over is required to give a conversion event without outside marker recombination. According to experimental data, there must be a high probability of this second cross-over event occurring together with the first.

The hypothesis of Stahl (1969) is unlike those of Holliday and Whitehouse because it assumes that the breakage and reunion of DNA duplexes, which form short hybrid DNA regions, is not reciprocal and that mismatched base pairs are not subjected to enzymic correction. Meiotic products with postmeiotic segregation characteristics would therefore arise when one or the other of two allelic differences were included in a hybrid DNA section. The other types of atypical segregation products are made possible by assuming that the exchanges (at least two) take place within overlapping loops (fixed pairing regions) formed by reduplicated sections within non-sister chromatids (fig. 1c). The hypothesis involves a very complex sequence of events requiring a large number of assumptions.

Taylor (1967) suggests that certain regions of the chromosomes (replicons) have one double stranded chromatid and one single stranded chomatid at the pairing stage due to incomplete replication in the pre-meiotic nuclei. Provided the single strands in non-sister chromatids are of opposite polarity, they may then

separate at opposite ends and anneal (fig. 1d) giving opportunity for enzymic correction of mismatched base pairs. Such events would then be followed by replication of the region, producing 6:2 type conversion events at sites differing by one base pair. A 5:3 segregation is thought to be able to arise in neighbouring, fully replicated, areas which are brought into close proximity by the preceding events. This would allow the formation of heteroduplex DNA and therefore post-meiotic segregation.

The length of the hybrid DNA section depends on the hypothesis involved but is generally conceded to be as great as a thousand nucleotide pairs, which is as long or longer than an average gene.

In addition, a number of extra assumptions can be applied to each of the hypotheses described above and to numerous variations which have not been described. Each of the hypotheses has been made flexible enough to accommodate almost any data which can be derived from a repulsion phase cross of two allelic mutants with closely linked flanking markers.

The major uncertainties are concerned with a number of variables. The first is the location of the initial breakage point and the polarity of the strands involved. The second is the length and nature of the hybrid DNA. The third is the method of termination of the hybridity. Another important consideration is the method and direction of the enzymic correction of mismatched base pairs, e.g. Emerson (1966) showed that the rate and direction (i.e. to mutant or wild-type) of repair of the two mismatched base pairs in a region of hybrid DNA were different when he analysed data on the w-62 locus of Ascobolus.

The necessity for retaining flexibility in the hypotheses on the mechanism of hybrid DNA formation has been promoted by the results obtained from tetrad analysis. Crosses which result in two heterozygous sites within a locus give wild type recombinants which apparently arise either by crossing-over between the sites giving reciprocal recombinants or by conversion yielding non-reciprocal recombinants.

The proportion of reciprocal to non-reciprocal recombinants has been found to vary, depending on the

organism, the locus and the alleles used. Lissouba et. al. (1963) found that 17 out of the 21 asci with prototrophs, found after crossing mutants 1130 and 55 at the series 19 locus of Ascobolus immersus, were the result of reciprocal exchange between mutants. Fogel and Hurst (1967) found that 101 out of 1,053 asci containing prototrophs, among crosses of hi-1 alleles of Saccharomyces cerevisiae, were due to reciprocal recombination. Stadler and Towe (1963) found no evidence of reciprocal recombination among 67 asci with prototrophs in cys allelic crosses in Neurospora crassa.

The proportions of the different ascus segregation types found among the asci containing prototrophic recombinant progeny have also been shown to vary considerably. Emerson and Yu-Sun (1967) found 2+:6w, 1+:7w, 3+:5w and 4+:4w aberrant asci among the progeny of crosses of closely linked white spore mutants in Ascobolus immersus. The 2+:6w asci were always in the majority (up to 100% of the abnormal asci from some crosses); the 1+:7w asci reached as high as 42% of the abnormal asci; while the 3+:5w and 4+:4w asci were very rare (less than 3% of the abnormal asci).

Crosses of alleles which yield prototrophs have also been examined in a less exact way, utilising only the random products of meiosis. The most informative data obtained in this manner comes from the analysis of randomly selected prototrophs from crosses of auxotrophic alleles flanked by closely linked markers. According to the expectations of the hypotheses based on the hybrid DNA theory and the tetrad analysis data, all four possible associations of flanking markers occur among the prototrophs. The relative proportions of the four classes of prototrophs vary considerably in the published reports. The reasons for this variation can be ascribed to the nature of the origin, extent and termination of the hybrid DNA which is assumed to affect the locus involved.

Catcheside (1966b, 1968) has proposed mathematical methods of calculating the relative contributions of the different extents of hybrid DNA to the various classes of prototrophs from an allelic cross with closely linked flanking markers. Basically, the interpretation of the hybrid DNA theory on which the calculations are based is that of Holliday (1964), because the extra, unknown parameters in more complex

hypotheses (e.g. Whitehouse 1967) exceed the degrees of freedom, making the formulae impossible to solve. Essential to the calculations is the assumption that the hybrid DNA process is symmetrical and not biased to strands of a given polarity. This simplification allows the derivation of formulae whose parameters are few enough to calculate. An advantageous feature of this analysis is that relative efficiency of repair and direction of repair of mismatched bases has no effect on the relative proportions of the different combinations of flanking markers among the prototrophs.

It is relatively safe to assume that the molecular events involved in recombination are accomplished by enzymic reactions. The structures and functions of the enzymes concerned are determined by the genetic constitution of the organism. Information pertaining to the process of recombination can therefore be derived from a genetic study of the control of altered recombination characteristics.

In many instances the amount of recombination has been shown to be reduced by genetic factors. This

reduction is generally accompanied by degrees of sterility and is therefore difficult to examine genetically.

Increased recombination due to mutants lacking, or having impaired, enzyme function has been described as due to at least three loci in phage T4 (Bernstein, 1968). The enzymes involved were polynucleotide ligase, DNA polymerase and deoxycytidylate hydroxymethylase. Bernstein suggests that the defective polynucleotide ligase fails to connect newly synthesized pieces of DNA to the older portion of growing strands and thus leads to increased chromosome breakage and recombination. In this instance the host is thought to provide the ligase required for the rejoining step of recombination. The defective DNA polymerase is thought to increase recombination by introducing structural errors, perhaps nicks in the deoxyribose - phosphate backbone, which increase the probability of breakage and rejoining. The dCMP hydroxymethylase is involved in the synthesis of 5 hydroxymethyldeoxycytidlate (dHMP). The limited availability of dHMP, caused by the defective enzyme, is believed to cause misincorporation of other bases into DNA, thus increasing the likelihood of breakage and rejoining.

Increased recombination is a technically desirable characteristic to study, especially when examining allelic recombination in eucaryotes. A verified increase in recombination found at a particular locus made Neurospora crassa the organism of choice for the study of recombination contained in this thesis.

Allelic recombination at the histidine-1 locus of N. crassa is under the control of a genetically inherited factor which, in the homozygous recessive state (rec-1 x rec-1) increases the yield of prototrophs about 15 fold over that found in the presence of the dominant allele rec-1^{+} (Jessop and Catcheside, 1965). Catcheside (1968) and Catcheside and Austin (1969) have found that there is no alteration in prototroph frequency due to rec-1 x rec-1 being substituted for rec-$1^{(+)}$ x rec-1^{+} for any of eight loci, including the five his loci which could be tested. This apparent lack of effect of rec-1 on any locus other than his-1 probably means that the rec-1^{+} gene does not specify any of the enzymes Bernstein has described as influencing recombination frequency, because such enzymes should have a general effect on the whole genome.

Catcheside (1966a) and Whitehouse (1966) independently suggested that rec-1$^+$ produces a regulator which could possibly be implicated in both recombination at the his-1 locus and transcription of the his-1 gene into messenger RNA. However, evidence against the rec genes having the latter function has been provided. D.E.A. Catcheside (1968) showed that rec-3 (which affects am-1 as rec-1 affects his-1) has no effect upon the repressibility of the enzyme specified by the am-1 gene.

The rec-1 factor also seems to affect the distribution of flanking markers among his-1 prototrophs, but the degree and nature of the change have not been well defined. A change in the segregation of flanking markers can also change the apparent relative order of the sites of allelic difference within a locus and therefore needs to be considered in any analysis of data based on the distribution of flanking markers among prototrophs.

There were therefore two primary objectives to this study, each of which is dependent on the other. The first objective was to obtain an extended, more

comprehensive, fine structure map of the his-1 locus. The second was to try to determine the nature of the action of the product of the rec-1 gene, possibly in terms of hybrid DNA formation.

CHAPTER II

MATERIALS AND METHODS

The present study utilizes a system involving 36 allelic auxotrophic mutants of the histidine-1 locus of Neurospora crassa. The flanking markers incorporated into the stocks were amination-1, four units proximal, and inositol, six units distal from his-1. The rec-1 gene and its dominant allele rec-1^{+} were described by Jessop and Catcheside (1965), and are distal and loosely linked to his-1 with about 20 to 25 per cent recombination. Catcheside and Austin (1969) located rec-1 between adenine-7 and asparagine.

The introduction of the colonial temperature sensitive mutant cot-1 into all of the stocks greatly reduced the labour and difficulty of the experiments. The backgrounds of all the alleles used in the study are listed in table I. These alleles were subsequently introduced to either an Emerson a or Emerson A background to varying degrees during the various stock building exercises, i.e. insertion of flanking markers, changing the rec-1 constitution, changing the mating type and improving fertility. All stocks were given

TABLE I

Backgrounds of alleles used in this study

Locus	Allele (isolation no.)	Original Genetic Background	Mutagen	Reference
am-1	32213 (*am-1*^{1})	Lindegren	uv	Fincham (1950)
	K314 (*am-1*^{6})	Emerson a	uv	Catcheside (1960)
inos	37401	Lindegren	uv	
cot-1	C102	Lindegren x Emerson	uv	Mitchell and Mitchell (1952)
rec-1	*rec-1*	Emerson A	-	Jessop and
	rec-1^{+}	Emerson a	-	Catcheside (1965)
his-1	K75 K83 K90 K93	Emerson a	uv	Catcheside (1960)
	K140 K141 K148 K240	"	"	"
	K245 K270 K274 K518	"	"	"
	K616 K617 K619 K620	"	"	"
	K624 K625 K626 K627	"	"	"
	K629 K631 K632 K633	"	"	"
	K634 K644 K646 K647	"	"	"
	K649 K651 K652 K744	"	"	"
	Y155-M302 Y175-M460	74a and 5.5A or 3.1a	X-ray	Weber and Case (1960)
	Y189-M89			
	C84	7A x 8a	uv	Haas et.al. (1952)

numbers prefixed by the letter "S" while all crosses were given numbers prefixed by the letter "O".

Only the allele numbers of the his-1 mutants will be given in the following text, e.g. his-1^{K83} will be written as K83.

The basic medium for growing the stocks was Vogels (Vogel, 1955), suitably supplemented. Colony size was controlled by varying the sugar content, e.g. VM = 2% sucrose, SS = 0.5% sorbose + 0.1% sucrose, SGF = 0.5% sorbose + 0.0125% glucose + 0.025% fructose. Crosses were made by placing conidia of both parents in 16 x 150 mm. test-tubes containing folded filter paper slips resting in 5 mls. of liquid Westergaard-Mitchel crossing medium (Westergaard and Mitchell, 1947). All media were supplemented with appropriate growth factors at the following rates: L-histidine at 300 mg./l. (200 mg./l in the presence of alanine), L-alanine at 450 mg./l. and inositol at 20 mg./l. Glycine was added at the rate of 1250 mg./l. to scoring plates lacking alanine because glycine helps to inhibit the leaky growth characteristic of am-1 auxotrophs.

From 2 to 200 test tubes were used for each cross, depending on the likely prototroph yield and fertility of the stocks involved. The method used to analyse these crosses for prototroph frequency was basically that used by Catcheside (1966a). The ascospores were rinsed from 1 to 5 crossing tubes with distilled water, filtered through gauze, and allowed to settle to the bottom of a boiling tube. Most of the water and floating conidia were then decanted and the retained ascospores were rinsed into screw-capped bottles (A bottles) containing 15 mls. of layer agar (Vogel's minimal with 1% Difco agar) at 56°C. The A bottles were then placed in a 56°C water bath for 50 minutes to break the ascospore dormancy and to kill the remaining conidia. B and C bottles, each usually containing 19.5 mls. of layer agar at 56°C, were used to obtain a 1,600 fold dilution of the ascospores in the A bottle by putting ½ ml. of the A solution in the B bottle and then putting ½ ml. of the B solution in the C bottle. The bottles were shaken vigorously on a Vortex-Genie (Scientific Industries Inc., Springfield, Mass., U.S.A.) to ensure even distribution of the ascospores during dilution. Three ml. samples of C were spread on two C plates (SGF + alanine + histidine

+ inositol) to obtain estimates of the number of viable ascospores per ml. in the A bottle. Three ml. samples of A were spread on five A plates (SGF + alanine + inositol) to obtain the prototrophs provided by recombination at the his-1 locus. The A plates had been previously warmed in a 56°C incubator for about 30 minutes to slow setting of the layer agar so that the ascospores would all settle on to the top of the SGF medium. The A and C plates were then placed at 25°C for about 16 hours to give a convenient colony size, and then at 34°C for approximately 24 hours. This high temperature exposure of cot-1 stocks causes the hyphal branches to cease terminal growth in favour of a large number of lateral branches, giving the established colony a compact "colonial" character.

The number of colonies on the two C plates was multiplied by 4,000 after counting to give the total number of viable ascospores on the five A plates (some of the earlier crosses were only diluted 800 fold, requiring a multiplication factor of 2,000). The prototrophic colonies on the A plates were then counted and the ones which had not fused together were tested to determine their genetic constitution with regard to

the two flanking markers. Two bits, each free from ascospores, were picked from the tip of a single hyphal branch (thickened by short branches due to the cot-1 gene acting at 34°C) and placed, respectively, on SS plus alanine and SS plus inositol plus glycine plates.

The scored segregating progeny were placed in groups ranging in constitution from a limited number of prototrophs from a single plating (i.e. five A plates) to the total of the prototrophs from several platings, depending on prototroph numbers and/or fertility. This grouping, so that the smallest class always had an expectation greater than five, allowed contingency χ^2 tests to be used to determine the heterogeneity of the data. The prototroph frequency was tested for heterogeneity, both between replicates of crosses and between crosses of different stocks of the same alleles, by using a contingency χ^2 test utilizing the A and C counts.

The standard error of the prototroph frequency was calculated from the formula

$$\delta = \sqrt{\frac{1}{\Sigma\left(\frac{1}{\delta^2}\right)}}$$

where δ^2 is the variance of the prototroph frequency for each plating calculated from the formula

$$\delta^2 = \left(\frac{KA}{C}\right)^2 \left(\frac{1}{A} + \frac{1}{C}\right)$$

where $\frac{KA}{C}$ is prototroph frequency per 10^5 viable ascospores (K = 25 with a 1,600 fold dilution or K = 50 with an 800 fold dilution of the stock ascospore solution in the A bottle) while the letters A and C represent the total number of colonies found on the A and C plates respectively. Standard errors for the prototroph frequencies from unreplicated crosses were calculated using the square root of the formula for the variance, i.e.

$$\delta = \frac{KA}{C} \sqrt{\frac{1}{A} + \frac{1}{C}}$$

CHAPTER III

RESULTS

A. FINE STRUCTURE OF THE *HIS-1* LOCUS.

1. *Prototroph frequency variation between crosses involving the same alleles in different stocks.*

Jessop and Catcheside (1965) showed that the prototroph frequency from crosses of *his-1* alleles was about 15 fold greater in *rec-1* x *rec-1* backgrounds than in *rec-1* x *rec-1*$^{+}$ backgrounds.

The preliminary analysis of a number of crosses involving the same pair of *his-1* alleles in different stocks revealed that the *rec-1* x *rec-1* (and possibly the *rec-1*$^{(+)}$ x *rec-1*$^{+}$) prototroph frequency could vary considerably. A detailed examination of this variability would be of value since prototroph frequency is one of the main tools used to obtain fine structure maps.

Due to the technical difficulty of handling a large number of *rec-1*$^{(+)}$ x *rec-1*$^{+}$ crosses, the study was begun using 38 *rec-1* progeny from cross number 0174 (*am-1*^{6} K83 *rec-1*$^{+}$a x K625 *inos* *rec-1* A). Of the

38 progeny, 30 had the parental association of chromosome V markers (i.e. were K625 inos rec-1 in genotype), while 8 were recombinant between inos and rec-1, i.e were am-1^{6} K83 rec-1 in genotype. The prototroph frequencies shown in table II, summarized in fig. 2a, were obtained when these progeny were crossed to other stocks (as shown in table III) to determine their rec-1 constitution. Since each of the progeny is represented by only one plating, the range of 32.3 to 53.2 prototrophs per 10^{5} viable ascospores did not seem extremely unusual. The higher prototroph frequencies, however, were unexpected, especially the 94.3 figure obtained from cross 0325 (stock number S158 crossed to one of its parents, S79).

Cross 0325 was also of a low fertility, yielding only a calculated 280,000 viable ascospores from two crossing tubes compared to a range of 1,776,000 to 3,244,000 from the seven remaining crosses of stocks recombinant for rec-1. The parental type rec-1 stocks also gave much larger populations, ranging from a calculated 592,000 to 1,924,000 viable ascospores from each of the single crossing tubes used in the platings. 0325 was repeated and the walls of the

TABLE II

Prototroph frequencies for 0174 *rec-1* progeny x *rec-1* testing stocks.

Cross Number	Genotype	Stocks	Number of Colonies A	Number of Colonies C	Prototrophs /10^5	Standard Error
0322	*am-1*^{6} K83 a x K625 *inos* A	S155 x S79	860	497	43.3	2.44
0325	"	S158 x "	264	70	94.3	12.68
0328	"	S161 x "	721	444	40.6	2.45
0334	"	S167 x "	892	479	46.6	2.64
0337	*am-1*^{6} K83 A x K625 *inos* a	S170 x S84	1,968	708	69.5	3.04
0341	"	S174 x "	1,445	811	44.5	1.95
0345	"	S178 x "	1,229	728	42.2	1.97
0346	"	S179 x "	2,055	708	72.5	3.16
0352	*am-1*^{6} K83 a x K625 *inos* A	S6 x S185	590	400	36.9	2.39
0353	"	" x S186	535	380	35.2	2.36
0354	"	" x S187	511	267	47.8	3.61
0356	"	" x S189	532	320	41.6	2.94
0357	"	" x S190	456	353	32.3	2.29
0358	"	" x S191	308	148	52.0	5.20
0360	"	" x S193	413	256	40.3	3.21
0361	"	" x S194	420	261	40.2	3.17
0362	"	" x S195	463	227	51.0	4.13
0363	"	" x S196	588	341	43.1	2.93
0365	*am-1*^{6} K83 A x K625 *inos* a	S7 x S198	294	169	43.5	4.20
0366	"	" x S199	543	322	42.2	2.96
0367	"	" x S200	930	429	54.2	3.16
0368	"	" x S201	509	331	38.4	2.71
0369	"	" x S202	669	437	38.3	2.35
0370	"	" x S203	583	366	39.8	2.66
0371	"	" x S204	711	276	64.5	4.57
0372	"	" x S205	621	292	53.2	3.77
0373	"	" x S206	687	328	52.4	3.51
0375	"	" x S208	683	460	37.1	2.34
0376	"	" x S209	657	344	47.7	3.18
0378	"	" x S211	615	348	44.2	2.96
0379	"	" x S212	696	408	42.6	2.66
0380	"	" x S213	809	481	42.0	2.42
0381	"	" x S214	635	387	41.0	2.63
0382	"	" x S215	696	382	45.5	2.90
0384	"	" x S217	479	281	42.6	3.20
0385	"	" x S218	748	400	46.8	2.90
0386	"	" x S219	751	441	42.6	2.55
0387	"	" x S220	801	470	42.6	2.48

Figure 2. Prototroph frequency variability among K83 rec-1 x K625 rec-1 crosses of different stocks. (a) Progeny from cross number 0174 (S2 x S79) were crossed to S6 (circles), S7 (dots), S79 (cross-hatched) and S80 (diagonal lines). (b) Progeny from cross 0174 crossed to their siblings, S158 (circles) and S170 (dots). Both of these stocks had previously given relatively high prototroph frequencies. (c) Progeny from a cross of S158 x S145 (0471: am-1^6 K83 rec-1 x K83 inos rec-1) crossed to S79. The results from the am-1^6 K83 progeny are denoted by circles while the results from the K83 inos progeny are denoted by dots.

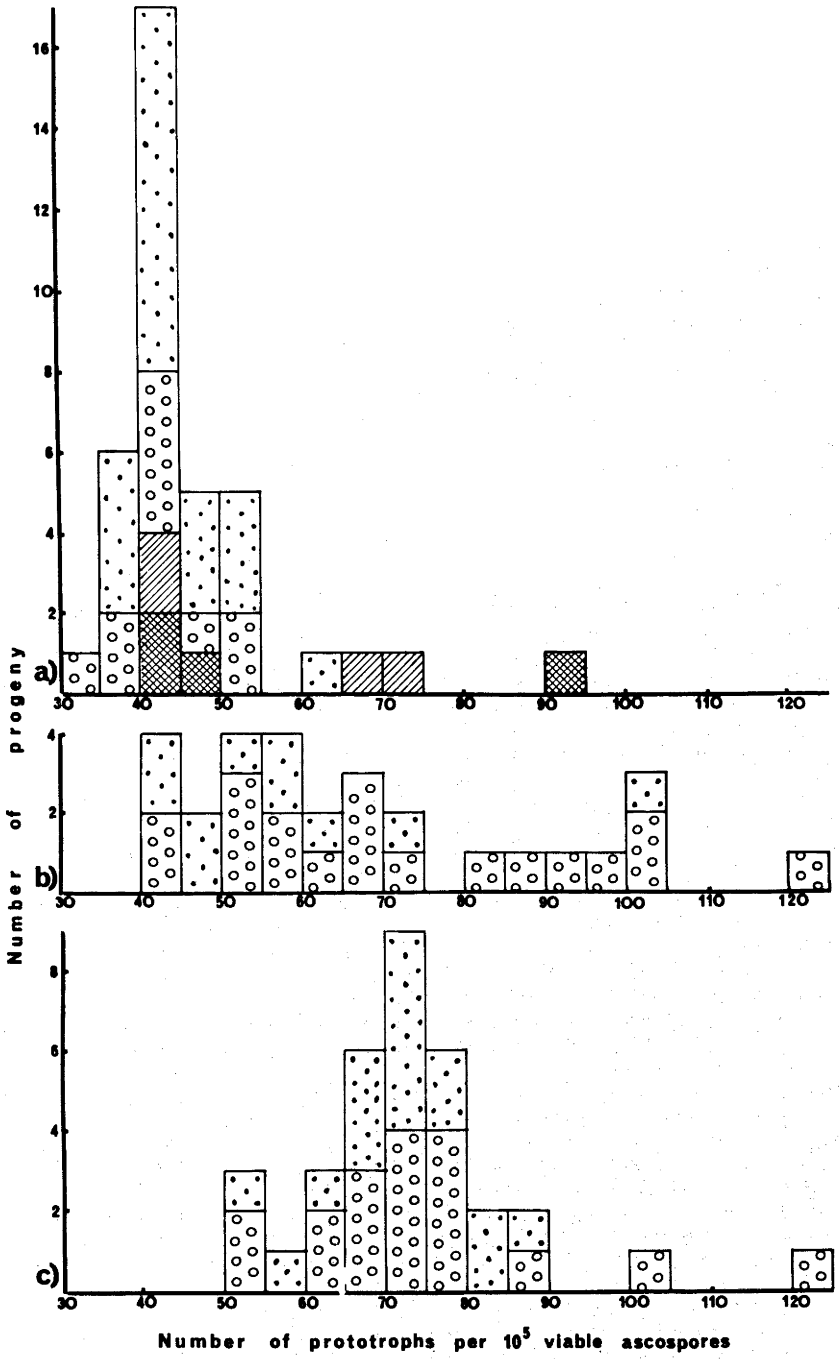

Number of progeny
a)
b)
c)
30
40
50
60
70
80
90
100
110
120
Number of prototrophs per 10^5 viable ascospores

TABLE III

Crosses used to determine the rec-1 genotypes of the 0174 progeny.

Progeny		Testing Line
Number Tested	Genotype	
13	am-1^{6} K83 rec-1^{+} A x	K625 inos rec-1 a (S84)
12	am-1^{6} K83 rec-1^{+} a x	K625 inos rec-1 A (S79)
4	K625 inos rec-1^{+} A x	am-1^{6} K83 rec-1 a (S 7)
2	K625 inos rec-1^{+} a x	am-1^{6} K83 rec-1 A (S 6)
20	K625 inos rec-1 A x	am-1^{6} K83 rec-1 a (S 7)
10	K625 inos rec-1 a x	am-1^{6} K83 rec-1 A (S 6)
4	am-1^{6} K83 rec-1 A x	K625 inos rec-1 a (S84)
4	am-1^{6} K83 rec-1 a x	K625 inos rec-1 A (S79)

crossing tubes were examined, with negative results, for an abnormally high level of abortive (colourless) ascospores. The cross was plated in two replicates of two crossing tubes each, giving prototroph frequencies of 109.2 and 98.0/10^5 respectively. The fertility was similar to that noted in 0325 originally (272,000 and 356,000 viable ascospores were calculated to have been plated in the replicates). No ungerminated spores were found when the C plates were examined microscopically.

Table IV shows the prototroph frequencies obtained when S158 was crossed to several other stocks. The lack of prototrophs from 0487 indicates that the mutant <u>his-1</u> allele in S158 is K83. 0488 shows that the stock (S170) giving the 69.5 prototrophs per 10^5 viable ascospores in fig. 2a also contains K83. The prototroph frequencies quoted for these two crosses are the upper 95% confidence limits as determined using Steven's table (Stevens, 1942). The increase in prototroph frequency from S158 crossed to a K625 <u>rec-1</u>$^+$ stock is very little, if any, as shown by 0467. The cross with the K624 stock shows no increase in prototroph frequency over that noted for other K83

TABLE IV

Prototroph frequencies of crosses involving
S158 (*am-1*^{6} K83 *rec-1* a)

Cross Number	Parent of A mating type	Number of Platings	Prototrophs (A)	Total viable ascospores (,000)	Prototrophs/10^5	Standard Error
0487	*am-1*^{6} K83 *rec-1* A (S6)	2	0	2,028	(0.48)	
0488	*am-1*^{6} K83 *rec-1* A (S170)	2	0	328	(0.915)	
0467	K625 *inos* *rec-1*$^{+}$ A (S80)	2	34	1,004	3.386	± 0.580
0472	K624 *inos* *rec-1* A (S122)	1	120	1,124	10.67	± 1.025
0470	Y189-M89 *inos* *rec-1* A (S69)	1	468	1,404	33.33	± 1.779
0491	K625 *inos* *rec-1* A (S81)	2	2,178	4.004	54.40	± 1.428

x K624 crosses. Replicated crosses of K83 x Y189-M89 have given prototroph frequencies of 17.47 ± 0.696 and 23.16 ± 0.722 so the value of 33.33 ± 1.779 for 0470 is a significant increase. The cross involving S158 x S81 (a sibling of S79) shows that the very high yielding potential of S79 is not present in S81.

The prototroph frequencies in fig. 2a indicate that stocks S6 and S7 may carry a dominant for a relatively low rec-1 prototroph frequency while stocks S79 and S84 allow expression of higher prototroph frequency. If this assumption is correct, crossing known high prototroph frequency yielding stocks to all of the K625 inos rec-1 progeny from cross 0174 which were previously crossed to S6 and S7 should allow detection of segregation for high and low prototroph frequencies. The nature of this segregation would depend on the genetic nature and linkage of the factor(s) controlling the relatively high prototroph yields. The only known high prototroph frequency stocks containing K83 were among the progeny of 0174, so two of these, of opposite mating type, were selected to be crossed to their K625 inos rec-1 sibling stocks.

S158, which yielded 94.3 prototrophs per 10^5 ascospores when crossed to S79, and S170, which yielded 69.5 prototrophs per 10^5 when crossed to S84 , were the stocks selected for testing. The results of the 0174 F1 crosses were shown in fig. 2b. The prototroph frequency for each cross was obtained by averaging the results from two platings, each of which was derived from one crossing tube (table V). The characteristics of the distribution of the frequencies show that the high yielding potential is segregating among the progeny of 0174 and that very high frequency is recessive, probably involving more than one genetic factor.

As a control, two of the *am-1*^{6} *K83 rec-1* progeny of 0174 which gave prototroph frequencies of less than $50/10^5$ when crossed to S79 and S84 (S155=$43.3/10^5$; S174=$44.5/10^5$), were crossed to some of their K625 *inos rec-1* siblings. The results, derived from two platings of each of these crosses (table V) are compared to the results obtained when the same K625 stocks were crossed to S6 and S7, and S158 and S170, in table VI. S155 and S174 repress the relatively high prototroph

TABLE V

Prototroph frequencies for 0174 F_1 crosses.

Cross Number	Stocks	Colony counts A	Colony counts C	Heterogeneity between replicates χ^2	Heterogeneity between replicates P	Replicate values Prototrophs /10^5	Replicate values Standard Error	Cross values Prototrophs /10^5	Cross values Standard Error
0851	S158 x S198	190	79			60.1	8.05		
		195	105	2.056	0.20-0.10	46.4	5.62	52.3	4.61
0852	S158 x S199	367	158			58.1	5.52		
		612	300	1.203	0.30-0.25	51.0	3.59	53.4	3.01
0853	S158 x S200	484	126			96.0	9.60		
		338	92	0.083	0.80-0.75	91.8	10.80	94.3	7.18
0854	S158 x S201	47	19			61.8	16.81		
		73	25	0.216	0.70-0.50	73.0	16.92	68.2	11.92
0855	S158 x S202	419	144			72.7	7.03		
		544	139	4.801	0.05-0.025	97.8	9.30	85.1	5.61
0856	S158 x S203	53	19			69.7	18.65		
		76	38	1.001	0.50-0.30	50.0	9.93	56.6	8.77
0857	S158 x S204	507	93			136.2	15.37		
		382	90	2.357	0.20-0.10	106.1	12.43	121.4	9.67
0858	S158 x S205	71	14			126.8	37.08		
		144	39	0.857	0.50-0.30	92.3	16.66	101.4	15.20
0859	S158 x S206	518	200			64.7	5.39		
		201	84	0.263	0.70-0.50	59.8	7.77	63.3	4.43
0860	S158 x S208	392	231			42.4	3.52		
		357	199	0.210	0.70-0.50	44.8	3.97	43.5	2.63
0861	S158 x S209	83	23			90.2	21.26		
		85	18	0.591	0.50-0.30	118.0	30.63	102.4	17.46
0862	S158 x S211	444	241			46.0	3.68		
		280	165	0.421	0.70-0.50	42.4	4.16	44.6	2.76
0863	S158 x S212	100	39			64.1	12.10		
		456	169	0.059	0.90-0.80	67.4	6.07	66.8	5.43
0864	S158 x S213	72	33			54.5	11.47		
		76	31	0.152	0.70-0.50	61.3	13.06	57.8	8.62
0865	S158 x S214	134	39			85.9	15.63		
		151	36	0.590	0.50-0.30	104.9	19.45	95.0	12.18
0866	S158 x S215	64	23			69.6	16.9		
		178	41	2.241	0.20-0.10	108.5	18.8	94.5	12.57
0867	S158 x S217	184	72			63.9	8.88		
		91	31	0.307	0.70-0.50	73.4	15.26	66.7	7.68
0868	S158 x S218	85	17			125.0	33.21		
		89	37	5.029	0.025-0.02	60.1	11.76	80.6	11.09
0869	S158 x S219	214	105			51.0	6.07		
		338	149	0.481	0.50-0.30	56.7	5.58	54.3	4.11
0870	S158 x S220	98	44			55.7	10.10		
		296	92	2.884	0.10-0.05	80.4	9.60	72.4	6.96

cont/

TABLE V cont.

Cross Number	Stocks	Colony counts A	Colony counts C	Heterogeneity between replicates χ^2	Heterogeneity between replicates P	Replicate values Prototrophs /10^5	Replicate values Standard Error	Cross values Prototrophs /10^5	Cross values Standard Error
0871	S170 x S185	184	113			40.7	4.86		
		240	126	0.932	0.50-0.30	47.6	5.24	44.4	3.56
0872	S170 x S186	148	73			50.7	7.25		
		214	110	0.050	0.90-0.80	48.6	5.70	49.4	4.48
0873	S170 x S187	351	160			54.8	5.23		
		323	141	0.097	0.80-0.75	57.3	5.78	56.0	3.88
0874	S170 x S189	163	52			78.4	12.48		
		77	29	0.379	0.70-0.50	66.4	14.46	74.1	9.45
0875	S170 x S190	355	151			58.8	5.71		
		233	123	2.137	0.20-0.10	47.4	5.28	53.6	3.88
0876	S170 x S191	205	137			37.4	4.13		
		230	105	5.596	0.02-0.01	54.8	6.45	44.9	3.48
0877	S170 x S193	162	109			37.2	4.60		
		213	93	6.103	0.02-0.01	57.2	7.12	46.4	3.86
0878	S170 x S194	94	39			60.2	11.48		
		159	60	0.152	0.70-0.50	66.2	10.04	63.9	7.56
0879	S170 x S195	152	48			79.2	13.4		
		253	53	3.378	0.10-0.05	119.3	18.03	100.2	10.60
0880	S170 x S196	316	163			48.5	4.67		
		457	183	3.789	0.10-0.05	62.4	5.46	55.8	3.55
0881	S155 x S198	461	243			47.4	3.76		
		366	209	0.464	0.50-0.30	43.8	3.80	45.7	2.67
0882	S155 x S199	419	157			66.7	6.24		
		643	294	2.894	0.10-0.05	54.7	3.85	58.9	3.28
0883	S155 x S200	588	287			51.2	3.69		
		538	223	2.319	0.20-0.10	60.3	4.80	55.2	2.92
0884	S155 x S201	243	129			47.1	5.13		
		376	183	0.377	0.70-0.50	51.4	4.63	49.6	3.47
0885	S155 x S202	435	242			44.9	3.60		
		498	254	0.610	0.50-0.30	49.0	3.78	47.0	2.61
0886	S174 x S185	612	361			42.4	2.81		
		147	98	0.700	0.50-0.30	37.5	4.89	41.3	2.44
0887	S174 x S186	107	79			33.9	5.02		
		392	285	0.008	0.95-0.90	34.4	2.68	34.3	2.36
0888	S174 x S187	362	196			46.2	4.09		
		304	150	0.488	0.50-0.30	50.7	5.06	48.1	3.18
0889	S174 x S189	356	194			45.9	4.09		
		466	238	0.294	0.70-0.50	48.9	3.90	47.6	2.82
0890	S174 x S190	452	229			49.3	4.00		
		228	124	0.264	0.70-0.50	46.0	5.13	48.2	3.16

TABLE VI

Comparison of prototroph frequencies between progeny of 0174 crossed to the low prototroph yielding testing stocks and high and low yielding siblings.

0174 Progeny	High prototroph frequency siblings	Low prototroph frequency siblings	Low prototroph frequency testing stocks
Stock No.	S170	S174	S6
S185	44.4 ± 3.56	41.3 ± 2.44	36.9 ± 2.39
S186	49.4 ± 4.48	34.3 ± 2.36	35.2 ± 2.36
S187	56.0 ± 3.38	47.3 ± 3.18	47.8 ± 3.61
S189	74.1 ± 9.45	47.6 ± 2.82	41.6 ± 2.89
S190	53.6 ± 3.88	48.2 ± 3.16	32.3 ± 2.29
	S158	S155	S7
S198	52.3 ± 4.61	45.7 ± 2.67	43.5 ± 4.92
S199	53.4 ± 3.01	58.4 ± 3.28	42.1 ± 2.97
S200	93.1 ± 7.18	55.2 ± 2.92	54.8 ± 3.21
S201	68.2 ± 11.92	49.6 ± 3.44	38.4 ± 2.71
S202	85.1 ± 5.61	47.0 ± 2.61	38.3 ± 2.35

yields which were obtained with some of the stocks when crossed to S158 and S170. It should be noted, however, that while four of the crosses of S155 and S174 give similar results to those crosses involving S6 and S7, four give significantly higher prototroph frequencies. This would imply that a portion of the high yielding potential had been inherited by some of the 0174 progeny tested, although not too much reliance should be placed on these comparisons due to the low degree of replication and due to the small differences.

A further experiment involved a cross between two relatively high yielding K83 rec-1 stocks which were suitably marked with am-1 and inos allelic differences. The cross was number 0471: am-1^{6} K83 rec-1 a (S158) x K83 inos rec-1 A (S145). Thirty four progeny from 0471 were crossed to S79 and two replicates of each cross were plated to obtain the estimates of prototroph frequencies shown in table VII and summarized in fig. 2c. Out-crosses and F1 crosses of the progeny from the original cross in this experiment (0174) yielded prototroph frequencies which were often below $50/10^{5}$ (fig. 2, a and b). Cross 0471, however, did

TABLE VII

Prototroph frequencies for 0471 isolates x S79

Cross Number	Colony counts A	C	Heterogeneity between replicates χ^2	P	Replicate values Prototrophs /10^5	Standard Error	Cross values Prototrophs /10^5	Standard Error
0715*	1,347	458					73.5	3.98
0716*	1,313	508					64.6	3.38
0717	169	52			81.2	12.88		
	162	62	1.005	0.50-0.30	65.3	9.76	72.6	7.78
0718	389	133			73.1	7.34		
	433	108	4.610	0.05-0.025	100.2	10.78	85.3	6.07
0719	237	60			98.8	14.3		
	209	50	0.070	0.80-0.75	104.5	16.4	101.4	10.78
0720	818	290			70.5	4.82		
	987	330	0.394	0.70-0.50	74.8	4.75	72.8	3.38
0721	889	299			74.3	4.87		
	719	224	0.568	0.50-0.30	80.2	6.14	76.9	3.86
0722	251	83			75.6	9.57		
	272	115	2.130	0.20-0.10	59.1	6.58	66.0	5.42
0723	273	107			63.8	7.28		
	240	97	0.034	0.90-0.80	61.8	7.44	62.9	5.20
0724	523	173			75.6	6.63		
	731	239	0.010	0.95-0.90	76.5	5.70	76.1	4.32
0725	185	102			45.3	5.59		
	194	76	3.496	0.10-0.05	63.8	8.64	53.2	4.69
0726	364	134			67.9	6.86		
	342	122	0.046	0.90-0.80	70.1	7.39	68.9	5.03
0727✓	254	52					122.1	18.59
0728✓	753	264					71.3	5.10
0729	335	63			132.9	18.25		
	145	40	2.913	0.20-0.10	90.6	16.18	116.5	12.11
0730	757	227			83.4	6.31		
	765	254	0.946	0.50.0.30	75.3	5.45	79.1	4.12
0731	307	107			71.7	8.05		
	181	78	1.458	0.25-0.20	58.0	7.86	65.9	5.62
0732	311	117			66.4	7.21		
	334	91	4.060	0.05-0.25	91.8	10.85	77.5	6.00
0733	241	112			53.8	6.15		
	309	129	0.045	0.90-0.80	55.6	5.68	54.8	4.17

cont/

TABLE VII cont.

Cross Number	Colony Counts A	C	Heterogeneity between Replicates χ^2	P	Replicate values Prototrophs /10^5	Standard Error	Cross Values Prototrophs /10^5	Standard Error
0791	372	172			54.1	4.98		
	269	121	0.037	0.90-0.80	55.6	6.08	54.7	3.86
0792	832	280			74.3	5.13		
	571	163	2.142	0.20-0.10	87.6	7.78	79.2	4.28
0793	174	52			83.6	13.22		
	143	58	1.902	0.20-0.10	61.6	9.60	72.0	7.76
0794	609	206			73.9	5.96		
	462	122	3.646	0.10-0.05	94.7	9.64	81.6	5.07
0795	518	172			75.3	6.62		
	402	138	0.063	0.90-0.80	72.8	7.18	74.2	4.87
0796	499	153			81.5	7.53		
	564	226	4.873	0.05-0.025	62.4	4.91	70.1	4.11
0797	311	104			74.8	8.47		
	427	179	2.465	0.20-0.10	59.6	5.31	65.2	4.50
0798	469	173			67.8	6.03		
	721	240	0.783	0.50-0.30	75.1	5.60	72.0	4.10
0799	103	23			112.0	25.82		
	94	32	1.884	0.20-0.10	73.4	15.03	89.5	12.99
0800	571	224			63.7	5.02		
	692	264	0.068	0.80-0.75	65.6	4.24	64.7	3.45
0801	666	206			80.8	6.44		
	324	109	0.379	0.70-0.50	74.3	8.23	78.6	5.07
0802	478	184			64.9	5.63		
	685	238	0.796	0.50-0.30	72.0	5.41	68.9	3.90
0803	128	42			76.7	13.55		
	105	41	0.462	0.50-0.30	64.0	11.79	70.2	8.89
0804	162	41			98.9	17.27		
	113	41	2.044	0.20-0.10	68.9	12.56	83.8	10.16
0805	245	104			58.9	6.89		
	180	79	0.035	0.90-0.80	57.0	7.69	58.1	5.13
0806	189	56			84.4	12.84		
	233	103	4.334	0.05-0.025	56.6	6.69	66.4	5.93

* Results from 1 plating, 2 tubes.
✓ Results from 1 plating, 1 tube.

not give progeny with prototroph yields below $53.2/10^5$ when crossed to S79. The very high prototroph frequencies shown in fig. 2c (i.e. $>100/10^5$) come from stocks which have the S158 am-1^6 marker and are all associated with comparatively low fertility, approximately the same as that shown by cross number 0325 (S158 x S79). A possible explanation is that an accumulation of recessive genes is responsible for the very high prototroph frequency and that such an accumulation has a deleterious effect on fertility. This would be very difficult to examine critically due to the complexities of the inheritance of fertility and the difficulty involved in handling stocks which are relatively infertile.

The increased yield of prototrophs discussed above is not limited to K83 x K625 crosses. Cross number 0470 (K83 x Y189 - M89, table IV) indicated an increase over previously obtained values for that allele combination. A definitely verified increase in prototroph frequency for another allele pair is shown in table VIII. The comparison is between two different K83 stocks (S7 and S158) crossed to one K140 stock (S303); replication was achieved by plating 8 crossing

TABLE VIII

Prototroph frequencies obtained from crosses between two K83 stocks (S7 and S158) and one K140 stock (S303)

Cross and Replicate (Plating) Number	A plate count	C plate count	Prototrophs /10^5 ascospores	Standard Error
S7 x S303				
0829.1	717	284	63.1	4.42
.2	1,166	351	83.0	5.06
.3	909	370	61.4	3.79
.4	845	345	61.2	3.91
.5	965	415	58.1	3.41
.6	882	353	62.5	3.93
.7	1,002	361	69.4	4.26
.8	885	333	66.4	4.27
Totals	7,371	2,812	65.5	1.43
S158 x S303				
0830.1	815	245	83.2	6.06
.2	739	224	82.5	6.29
.3	686	203	84.5	6.75
.4	755	228	81.8	6.26
.5	452	154	73.4	6.85
.6	812	250	81.2	5.87
.7	810	243	83.3	6.10
.8	557	173	80.5	7.00
Totals	5,626	1,720	81.8	2.25

tubes individually for each cross. A contingency χ^2 calculated using the A and C plate counts from 0829 gives a value of $\chi^2_7 = 23.40$, P = 0.005 - 0.001, most of which is due to replicate number 2 (removal of replicate 2 gives $\chi^2_6 = 5.429$, P = 0.70 - 0.50). The flanking marker data collected for replicate 2 indicates that there was no contamination or reversion in the tube, therefore the deviation must have originated during the dilutions involved in the plating process. However, the extensive replication tends to negate the effect of replicate 2 on the total prototroph frequency. A contingency χ^2 similarly calculated for 0830 gives $\chi^2_7 = 1.718$, P = 0.975 to 0.95; a most homogeneous sampling. The relatively low prototroph yield associated with S7, compared to that associated with S158, is similar to that noted in the series of K83 x K625 crosses discussed previously. A rec-1 x rec-1 cross of K625 and K140 yielded only 0.920 $\pm$ 0.075 prototrophs per 10^5 viable ascospores so K625 and K140 are probably very closely linked.

As will be shown in a later section, the distribution of flanking markers among the prototrophs

TABLE IX

Heterogeneity within and between *am-1*^{6} K83 *rec-1* x K140 *inos rec-1* cross results. The data for each cross are comprised of four replicates of 96 prototrophs.

Cross Number	Flanking marker constitution of prototrophic progeny							
	am-1^{6} +	+ *inos*	+ +	*am-1*^{6} *inos*	Total	D.F.	χ^2	P
0829	128	86	105	65	384	9	10.69	0.30-0.25
0830	115	92	106	71	384	9	7.894	0.70-0.50
Total	243	178	211	136	768	3	1.166	0.80-0.75

TABLE X

Prototroph frequencies for *am-1*^{6} Y175-M460 *rec-1*$^{+}$ siblings x S142 and S145

Cross Number	Stocks	Colony counts A	Colony counts C	Heterogeneity between replicates χ^2	Heterogeneity between replicates P	Replicate values Prototrophs /10^5	Replicate values Standard Error	Cross values Prototrophs /10^5	Cross values Standard Error
0734	0602.1 x S142	22	153			3.59	0.819		
		16	104	0.037	0.90-0.80	3.85	1.033	3.70	0.642
0735	0602.2 x "	35	198			4.42	0.810		
		35	238	0.511	0.50-0.30	3.68	0.666	4.01	0.514
0736	0602.3 x "	17	112			3.79	0.988		
		26	175	0.004	0.95-0.90	3.71	0.781	3.74	0.612
0737	0602.4 x "	32	190			4.21	0.804		
		40	217	0.123	0.75-0.70	4.61	0.793	4.42	0.565
0738	0602.5 x "	55	298			4.61	0.677		
		37	265	1.494	0.25-0.20	3.49	0.612	4.08	0.454
0739	0602.6 x "	22	183			3.00	0.678		
		26	194	0.125	0.75-0.70	3.35	0.700	3.18	0.487
0741	0602.8 x "	25	203			3.08	0.652		
		22	193	0.062	0.90-0.80	2.85	0.641	2.97	0.457
0742	0602.9 x "	23	102			5.64	1.301		
		22	151	1.828	0.20-0.10	3.64	0.831	4.45	0.700
0743	0602.10 x "	38	221			4.30	0.755		
		30	176	0.001	0.95-0.90	4.26	0.842	4.28	0.562
0744	0602.11 x S145	79	283			6.98	0.888		
		98	397	0.523	0.50-0.30	6.171	0.696	6.51	0.548
0745	0602.12 x "	95	296			8.02	0.946		
		63	340	9.346	0.005-0.001	4.63	0.635	6.21	0.527
0746	0602.13 x "	50	244			5.12	0.795		
		59	278	0.028	0.90-0.80	5.30	0.760	5.22	0.550

χ^2 test for the 9 low value crosses (prototroph frequency = $3.88 \pm 0.180/10^5$) gave a $\chi^2_8 = 7.450$, $P = 0.50 - 0.30$ and the χ^2 test for the 3 high value crosses (prototroph frequency = $6.04 \pm 0.318/10^5$) gave a $\chi^2_2 = 2.800$, $P = 0.25 - 0.20$. This clearly allows the data to be broken into a low yielding group and a high yielding group. Low yields in these crosses are associated with a reduction in fertility which averages 39% (the low value crosses averaged 187 prototrophs on the two C plates per plating, while the high value crosses averaged 306). Flanking marker segregation among the 946 prototrophs analysed was homogeneous between all the crosses (contingency $\chi^2_{33} = 35.51$, $P = 0.50 - 0.30$).

A large change in prototroph frequency is associated with the rec-1$^+$ factor. Detailed examination of several crosses between the same pair of alleles has shown that this large change is apparently overlaid with small ones. The small changes are of a magnitude which renders them difficult to study, primarily because of the large amount of replication required.

The preceeding results and their description emphasise the need for caution when considering prototroph frequencies, especially when they are being used to define the fine structure of a locus. Much more needs to be learned of the nature of the control of fluctuations within rec-1 x rec-1 and rec-1$^{(+)}$ x rec-1^{+} crosses between the same allelic pairs, but involving different stocks. Until the nature of the control is defined, a critical analysis of prototroph frequency cannot be made by averaging values from crosses between the same alleles when different stocks are used, unless the values can definitely be shown to be identical. Comparisons of different alleles crossed are also difficult unless there is assurance of homogeneity of background.

2. The linear relationships between groups of alleles.

Arranging the fine structure map of the his-1 alleles used in this study can be simplified by first considering the fact that a number of allele pairs, believed to have arisen independently, produced no prototrophs when they were crossed. Six groups of his-1 alleles are involved, such that crosses between

any pair in a group produce no prototrophs, except rare ones which could be back mutations of one of the parents.

The alleles within each of the groups belong to the same complementation groups (Catcheside, 1960; Jessop and Catcheside, 1965; D.G. Catcheside, personal communication), with interesting exceptions. The site of difference of K626, which belongs to complementation group D, is apparently located at the same site as the differences of the alleles K617, K620, K627 and K518, all from complementation group A. K624 of complementation group B apparently has a site of difference in common with the A group allele K629 and Y155-M302 (which has been assigned to a new group, G). The complementation of Y155-M302 and the alleles belonging to the other his-1 complementation groups was determined by allelic crosses. The occurrence of pseudo-wild type colonies (Pittenger, 1954) was used to distinguish between complementing and non-complementing combinations. Case and Giles (1960) stated that the presence or absence of pseudo-wilds in critical crosses is an unequivocal and regularly reproducable means of determing allelic complementation. The placing of

Y155-M302 in group G was made because this allele forms easily recognisable pseudo-wild type colonies in crosses with members of complementation groups C, E and F. Pseudo-wilds were not found in crosses of Y155-M302 to members of complementation groups A, D and, probably due to the location of its site of difference, B. A heterocaryon test of Y155-M302 and the K624 B group allele also gave negative results. The stocks used in this test, however, did not possess the additional nutritional mutants which could be used to force the heterocaryon. A circular complementation map is required to illustrate the relationships involved (fig. 3).

The crosses of alleles with differences at the same sites (table XI), with the exceptions of cross 0632 and cross 0843, are rec-1 x rec-1. They would therefore yield the maximum number of prototrophs possible for the allele pair being tested, depending on the presence of rec-1 modifiers in the stocks. The lowest yield of prototrophs yet obtained from a rec-1 x rec-1 cross between his-1 alleles, other than those in table XI, was found in cross 0912

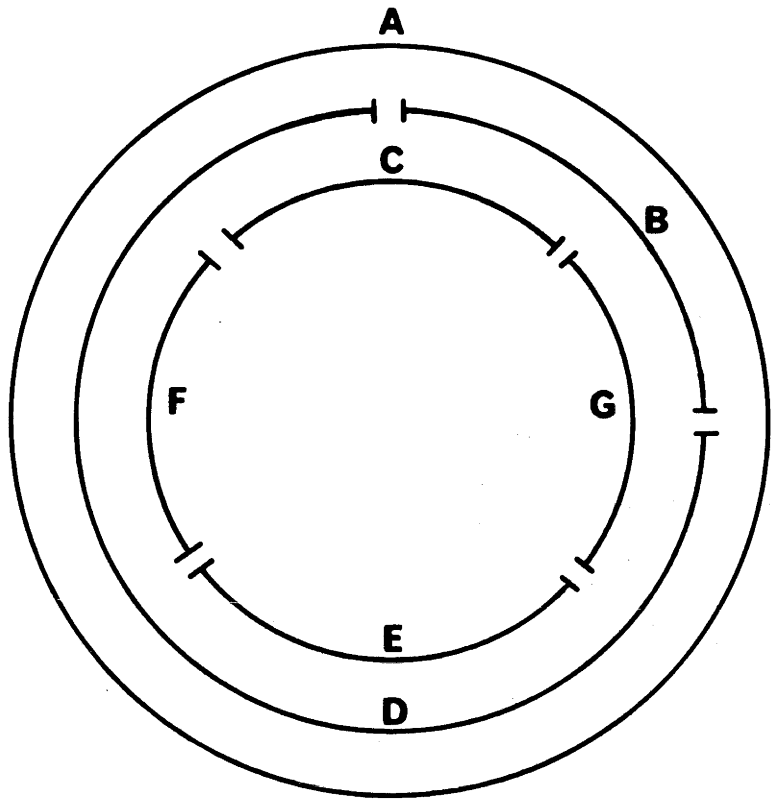


Figure 3. Complementation map of his-1.

TABLE XI

Data on alleles whose sites of difference map at similar locations
(all crosses are rec-1 x rec-1 except those with a *)

Group	Cross #	Alleles	Stocks	Complementation group(s)	Number of Platings	Number of Prototrophs	Estimated total viable ascospores (,000)	95% upper confidence limit prototrophs /10^5 ✓
I	0809	K83 x K148	S6 x S310	A	4	0	2,672	0.112
	0816-B	K83 x K274	S7 x S321	A	4	0	8,836	0.034
	0837	K83 x K644	S6 x S361	A	4	0	6,788	0.044
	0843*	K83 x K649	S7 x S353	A	4	0	13,052	0.023
	0893	K83 x K633	S6 x S337	A	4	0	1,852	0.162
	0938	K274 x K148	S321 x S310	A	4	0	1,308	0.229
	0941	K274 x K644	S321 x S361	A	4	3	8,000	
II	0632*	K617 x K620	S260 x S15	A	4	0	5,908	0.051
	0633	K617 x K620	S261 x S15	A	4	0	1,168	0.257
	0605	K617 x K627	S231 x S58	A	4	0	2,312	0.130
	0631	K617 x K627	S232 x S60	A	4	2	6,716	
	0613	K620 x K627	S15 x S60	A	3	0	6,296	0.048
	0753	K617 x K626	S260 x S292	AxD	4	0	7,620	0.039
	0844	K617 x K626	S304 x S292	AxD	4	0	5,376	0.056
	0819	K617 x K518	S231 x S323	A	4	0	2,268	0.132
	0941	K626 x K627	S293 x S59	A	4	0	3,736	0.080
	0942	K626 x K518	S293 x S323	A	4	0	4,872	0.062
	0934	K626 x K620	S293 x S15	A	4	0	1,076	0.279
III	0916	K629 x K624	S63 x S119	AxB	4	0	3,128	0.096
	0953	Y155-M302 x K624	S289 x S118	GxB	2	0	1,068	0.028
	0957	Y155-M302 x K629	S289 x S63	GxA	2	0	200	1.500
IV	0821	Y189-M89 x K90	S70 x S308	E	4	0	8,516	0.035
V	0316	K141 x K625	S73 x S848	F	2	0	912	0.329
	0675	K141 x K625	S73 x S251	F	4	0	5,288	0.057
VI	0918	K632 x K140	S336 x S303	A	4	0	1,512	0.198
	0920	K632 x K631	S336 x S334	A	4	0	1,988	0.151

✓ Based on Stevens tables (Stevens, 1942)

(K625 x K632) where 15 prototrophs grew out of an estimated total viable ascospore population of 8,084,000, for a prototroph frequency of 0.186 per 10^5 viable ascospores. Using Steven's table (Stevens, 1942) the 95% confidence limits of $0.186/10^5$ are found to be 0.106 and 0.304. The lower limit of 0.106 is above the upper limits of all crosses with $>2.8 \times 10^6$ ascospores (table XI). The crosses with smaller populations exceed this figure.

There are two crosses which do possess prototrophs which could be explained on the basis of back mutations, i.e. in each case the prototrophs had the flanking marker constitution of one of the parents. The prototroph frequencies and their 95% confidence limits were 0.038 (0.008 to 0.110) and 0.030 (0.0036 to 0.108) for the K274 x K644 and K617 x K627 crosses, respectively.

A summary of the prototroph frequencies observed when the members of different groups of alleles were inter-crossed is given in table XII. A more extensive analysis of these data can be found in table XIII where

TABLE XIII

Estimate of prototroph frequencies, per 10^5 viable ascospores, in his-1 allelic crosses.

his-1 alleles crossed	rec-1 x rec-1			rec-1(+) x rec-1+		
	Number of platings	Prototrophs /10^5	Standard Error	Number of platings	Prototrophs /10^5	Standard Error
K83 x K627	16	5.29	0.171			
K83 x K626	4	(6.21)*	0.399			
K83 x K620	8	6.95	0.418			
K83 x K617	4	4.68	0.764	39	0.367	0.020
	5	and 6.86	0.334			
K83 x K518	4	3.62	0.227			
K148 x K617	4	4.24	0.420			
K274 x K617	4	7.12	0.643			
K644 x K617	4	4.84	0.550			
K649 x K617				4	0.388	0.061
K83 x C84	4	(3.19)	0.171	38	(0.140)	0.008
K83 x K744	5	10.6	0.572			
K83 x K90	8	15.8	0.545	25	0.499	0.029
K83 x Y189-M89	10	17.5	0.696	37	0.810	0.031
	10	and 23.2	0.722	12	and 1.06	0.040
K83 x K647	9	(12.8)	0.429			
K83 x K270				4	0.967	0.117
K83 x K75	4	12.1	0.603			
K83 x K93	4	12.4	0.426			
K83 x K629	10	10.8	0.356			
K83 x K624	18	9.54	0.266	36	0.485#	0.022
K83 x Y155-M302	4	11.1	0.435	10	0.415	0.036
K83 x K651	9	31.9	1.212	17	1.42	0.067
K83 x K634	4	(30.6)	1.110			
K83 x Y175-M460	4	61.2	2.51	12	3.88	0.180
				6	and 6.04	0.318
K83 x K616				5	2.90	0.171
K83 x K619				4	3.62	0.220
K83 x K646				4	2.32	0.207
K83 x K631	4	41.0	1.94			
K83 x K632	4	46.5	3.19			
K83 x K140	7	63.0	1.50	6	5.86	0.150
	8	and 81.8	2.251			
K83 x K141	4	43.7	1.07	50	2.15	0.052
	4	and 50.0	1.79			
K83 x K625	2	from(34.3)	2.36	15	from2.27	0.078
	2	to (121.4)	9.67	10	to 3.89	0.134
K633 x K625	4	40.8	1.79			
K649 x K625	4	44.8	2.40	2	3.58	0.447
K83 x K652	4	52.7	4.92			
K83 x K240	8	76.5	2.74	6	(4.98)	0.212
K83 x K245				5	(6.55)	0.356
K83 x C84	24	1.18	0.051			
K620 x K744	4	3.15	0.213			

cont/

TABLE XIII cont.

his-1 alleles crossed	rec-1 x rec-1 Number of platings	Prototrophs /10^5	Standard Error	rec-1(+) x rec-1+ Number of platings	Prototrophs /10^5	Standard Error
K617 x K744	2	3.76	0.223			
	4	and 8.09	0.890			
K626 x K744	4	6.52	0.550			
K617 x Y189-M89	5	21.5	0.947	29	0.876	0.042
K617 x K90	4	22.5	1.940			
K627 x K90	3	16.6	0.993	12	0.500	0.053
K626 x Y189-M89	4	(20.4)	0.616	12	0.904	0.050
K617 x K270				4	0.628	0.098
K617 x K75	4	9.20	0.527			
K617 x K93	4	15.9	0.844			
K626 x Y155-M302	2	8.37	1.051			
K617 x K629	4	8.18	0.408			
K620 x K629	4	11.0	0.645			
K617 x K624	4	17.6	1.406			
K617 x K619				4	3.70	0.200
K617 x K140	4	(12.6)	5.91	7	8.31	0.316
K518 x K625	4	(51.2)	3.01			
K617 x K625	3	73.8	2.93	30	from3.64	0.114
				2	to 6.07	1.328
K626 x K625	5	73.2	1.82	1	from2.56	0.442
				1	to 6.60	0.698
K617 x K240	4	12.1	5.79			
K617 x K245				4	6.82	0.347
C84 x K744	4	(1.14)	0.107			
C84 x K90	4	8.71	0.360	12	0.361	0.032
C84 x K625	1	from53.0	4.67	10	2.90	0.107
	1	to 84.3	6.97			
K744 x K629	4	(8.26)	0.517			
K744 x K624	2	9.89	1.211			
K90 x K647	4	0.462	0.092			
K90 x K629	12	6.23	0.281			
K93 x K629	3	0.813	0.478			
K270 x K525				8	2.78	0.182
K629 x K625	4	36.6	1.414			
K651 x K634	4	6.95	0.230			
K651 x K140	4	(56.6)	2.42			
K651 x K625	6	21.1	0.526	22	(1.16)	0.048
K634 x Y175-M460	4	2.96	0.154			
K634 x K625	4	8.56	0.323			
Y175-M460 x K140	5	9.41	0.364	6	1.00	0.069
Y175-M460 x K240	4	6.80	0.297			
K619 x K625				4	0.054	0.031
K140 x K625	4	0.920	0.075			
K631 x K625	4	0.340	0.059			
K632 x K625	4	0.186	0.047			
K140 x K652	4	4.17	0.562			
K631 x K652	4	0.478	0.303			
K140 x K240	4	7.39	0.550			
K631 x K240	4	0.855	0.200			
K625 x K652	4	0.363	0.056			
K625 x K240	4	0.352	0.050			
K625 x K245				4	0.136	0.031

*( ) = homogeneity between replicates at the 5% to 1% probability levels.
#____ = homogeneity between replicates only below the 1% probability level.

the prototroph frequency and its standard error is listed for each allelic cross and the heterogeneity of the data contributing to each value is indicated. A range of prototroph frequencies is exhibited by the inter-group crosses but, as discussed previously, this range can also be encountered in a cross of the same two his-1 alleles in different stocks (e.g. the I x V prototroph frequency range in the rec-1 x rec-1 data in table XII is from K83 x K625 crosses). The inconsistencies in the prototroph frequencies therefore do not necessarily indicate that the allele pairs which give no prototrophs when crossed have differences located at different sites. Weighted means for each inter-group cross could help eliminate the effects of minor variations. However, the parents of the crosses were not chosen at random and the number of crosses in each class is generally small.

The range of prototroph frequencies also resulted in an overlap or merging of the prototroph frequencies when some of the groups were crossed to members of another group, e.g. the highest prototroph frequency from a II x III rec-1 x rec-1 cross was larger than the lowest II x IV rec-1 x rec-1 prototroph frequency.

However, in each such instance, prototrophs were produced when the overlapping groups were crossed together (e.g. the III x IV rec-1 x rec-1 cross gave 6.23 prototrophs per 10^5 viable ascospores). The overlap was therefore probably due to modification of the major effects of the rec-1 alleles such as that previously discussed.

3. The contributions of prototroph frequency and flanking marker distribution to a fine structure map of the his-1 locus

Most of the crosses analysed in this study were accomplished using a number of crossing tubes which were then harvested to give replicates of one to several tubes each, the number of replicates and tubes depending on the fertility of the cross and the rec-1 constitution. This replication was used as a method of increasing the relative accuracy and reliability of the estimates of prototroph frequency.

The prototroph frequency data obtained for the his-1 locus, during the course of this study, are summarised in table XIII. Crosses between the same

alleles, but using different parental stocks, were grouped only if contingency χ^2 tests suggested homogeneity at least at the 5% probability level. Crosses which could not be grouped with this degree of certainty are represented by two values if only two crosses were involved or by a range of values if more than two crosses were involved. Heterogeneity between the replicates of crosses of the same stocks is signified by brackets for the 5% to 1% probability range and by underlining for the replicates which were below the 1% level. Many of the heterogeneous crosses were not repeated because the distribution of the flanking markers among the prototrophic progeny did not obviously indicate contamination. Also, the prototroph frequencies were not essential in these cases because enough other crosses had been made, using the same alleles crossed to other alleles, to yield the information required.

A number of points of comparison of various aspects of rec-1 control of prototroph frequency can be derived from the data in table XIII:

i) A range of prototroph frequencies was often

encountered when a single pair of alleles was crossed using different stocks. The rec-1 x rec-1 prototroph frequency varied by a factor of up to 3.53 (34.4 to 121.4 prototrophs per 10^5 viable ascospores in the well studied K83 x K625 crosses).

ii) The rec-1$^{(+)}$ x rec-1^{+} prototroph frequencies for the same allele pair in different stocks varied from 2.27 to 3.89 prototrophs per 10^5, a factor of 1.71, in the highly replicated and homogenous K83 x K625 crosses. The only instance of a larger range of values in this category was found in the unreplicated K626 x K625 crosses which gave a less reliable range of 2.56 to 6.60 prototrophs per 10^5, a factor of 2.58.

iii) A comparison of the data quoted in i) and ii) above reveals that the rec-1/rec-1^{+} ratio of prototroph frequencies varies from 34.4/3.89 = 8.84 to 121.4/2.27 = 53.5 for the K83 x K625 crosses. No other allele pair tested in this study gave a rec-1/rec-1^{+} ratio outside this range. This is to be expected since no other allele pair was examined using as

many different stocks in as many crosses. The rec-1/rec-1$^+$ ratios given are not very meaningful because the prototroph frequencies used in their calculation were not from homogeneous backgrounds. The ratios do, however, demonstrate the prototroph frequency variability which can be exhibited by a specific allele pair.

iv) Despite the wide range of the rec-1/rec-1$^+$ ratio, the differences between the prototroph frequencies of the two types of cross are such that there is no difficulty in separating them into either rec-1 x rec-1 or rec-1$^{(+)}$ x rec-1$^+$ categories.

The variation in prototroph frequency makes the task of constructing a fine structure map of the his-1 locus solely by prototroph frequency very difficult. Therefore, an attempt to order the sites of allelic difference was made using the three criteria discussed by Catcheside (1966b). Criterion I depends on a significant difference between the numbers in the two classes of prototrophs recombinant for flanking markers. The more frequent class is assumed to have the proximal marker (am-1 or am-1$^+$) which went into the cross

associated with the more distal *his-1* allele difference and the distal marker (*inos* or *inos*$^+$) which was associated with the more proximal *his-1* allele difference. Criterion II depends on a significant difference between the numbers of the proximal flanking alleles among the prototrophs. The more frequent proximal allele is assumed to have entered the cross associated with the more distal *his-1* allele difference. Criterion III depends on a significant difference between the numbers of the distal flanking alleles among the prototrophs. The more frequent distal allele is assumed to have entered the cross associated with the more proximal *his-1* allele difference. The three criteria are not independent.

The results are shown in table XIV for the *rec-1* x *rec-1* data, in table XV for the *rec-1*$^{(+)}$ x *rec-1*$^+$ data and in table XVI for the K83 x K625 data. The data are either from replicated crosses of the same stocks or from crosses of different stocks which gave results statistically the same at the 5% level of probability. The homogeneity of the four prototrophic classes between replicates was determined with a

TABLE XIV

Ordering sites of allelic difference using prototrophs from rec-1 x rec-1 crosses

Parents		Prototrophic progeny					Proximal allele difference by criterion		
am-1	inos	am-1 +	+ inos	+ +	am-1 inos	Total	I	II	III
K83	K627	308	322	296	143	1,069	K83	K83	K83
K626	K83	106	67	42	55	270		K83	
K620	K83	105	83	68	64	320			
K617	K83	158	104	75	127	464	K83	K83	
K83	K617	109	134	83	85	411			
K83	K518	67	91	63	42	263	K83	K83	
K617	K148	26	19	17	30	92		K148	
K617	K274	43	51	21	41	156	K274		K274
K617	K644	26	16	14	31	87	K644	K644	
K83	C84	109	90	98	61	358	K83		K83
K744	K83	159	100	72	110	441	K83	K83	
K83	K90	343	280	309	237	1,169	K83		K83
K83	Y189-M89	791	432	601	335	2,159#	K83	Y189-M89	K83
K83	K647	316	92	287	90	785	K83		K83
K83	K75	151	149	143	106	549	K83		
K83	K93	193	200	222	149	764	K83	K83	K83
K83	K629	366	289	322	156	1,133	K83	K83	K83
K83	K624	587	388	414	280	1,669	K83		K83
K83	Y155-M302	223	105	157	89	574	K83	Y155-M302	K83
K83	K651	504	390	392	294	1,580*	K83		K83
K83	K634	115	75	130	64	384	K83		K83
Y175-M460	K83	132	260	121	255	768	K83		K83
K83	K631	125	64	135	60	384	K83		K83
K83	K632	98	72	109	57	336	K83		K83
K83	K140	243	178	211	136	768	K83		K83
K83	K141	910	564	873	477	2,824	K83		K83
K625	K633	170	294	92	212	768	K633	K633	
K625	K649	92	136	59	97	384	K649		K649
K83	K652	120	77	92	33	322	K83		K83
K83	K240	326	369	262	194	1,151	K83	K83	
K617	C84	137	130	93	97	451			
K620	K744	71	79	51	32	233	K620		

cont/

TABLE XIV cont.

Parents		Prototrophic progeny					Proximal allele difference by criterion		
am-1	inos	am-1 +	+ inos	+ +	am-1 inos	Total	I	II	III
K617	K744	119	119	86	75	399			
K626	K744	51	42	34	26	153*			
K617	Y189-M89	271	99	235	115	720	K617		K617
K617	K90	87	49	56	38	230			K617
K90	K627	79	138	69	122	408	K627		K627
K626	Y189-M89	290	118	238	122	768	K626	Y189-M89	K626
K617	K75	131	95	97	57	380	K617		K617
K617	K93	174	159	133	73	539	K617		K617
K626	Y155-M302	33	13	26	7	79	K626		K626
K617	K629	182	84	137	64	467	K617		K617
K620	K629	175	75	97	58	405*	K620	K629	K620
K617	K624	123	49	53	40	265		K624	K617
K617	K140	350	165	146	107	768	K617	K140	K617
K625	K518	52	165	59	108	384	K518	K625	K518
K625	K617	38	85	33	52	208	K617		K617
K617	K625	240	82	163	91	576	K617	K625	K617
K626	K625	455	125	196	184	960*		K625	K626
K617	K240	212	125	171	116	624	K617	K240	K617
K744	C84	30	31	12	21	94			
K90	C84	98	213	87	181	579	C84		C84
K625	C84	157	572	176	358	1,263	C84	K625	C84
K744	K624	41	17	24	10	92	K744		K744
K744	K629	123	52	111	35	321	K744		K744
K90	K647	11	4	10	0	25	K90		K90
K90	K629	373	142	144	162	821		K629	K90
K625	K629	139	278	114	237	768	K629		K629
K651	K634	120	62	152	50	384	K651	K651	K651
K651	K140	333	131	192	112	768	K651	K140	K651
K651	K625	362	154	277	130	923*	K651	K625	K651
Y175-M460	K634	65	108	65	144	382	K634		K634
K625	K634	75	143	48	118	384	K634		K634
Y175-M460	K140	145	113	113	68	439	Y175-M460		Y175-M460
Y175-M460	K240	115	92	119	58	384	Y175-M460		Y175-M460
K625	K140	40	52	37	28	151‡			
K625	K631	6	4	8	14	32			
K625	K632	4	5	2	4	15			
K625	K652	5	12	3	5	25			

* = 5%-1% probability of homogeneity between replicates contributing towards the total.

‡ = <1% probability of homogeneity between replicates contributing towards the total.

Note: No contingency χ^2 tests were done on the populations of 92 and less.

TABLE XV

Ordering sites of allelic difference by assessment of rec-1(+) x rec-1+ prototrophic progeny.

Parents		Prototrophic progeny					Proximal allele difference by criterion		
		am-1	+	+	am-1				
am-1	inos	+	inos	+	inos	Total	I	II	III
K617	K83	71	57	44	55	227			
K617	K649	7	8	3	12	30	K649		
K83	C84	65	73	61	26	225	K83	K83	
K83	K90	55	81	54	60	250*			K90
K83	Y189-M89	415	322	380	179	1,296	K83	K83	K83
K83	K270	23	15	12	11	61			
K83	Y155-M3Q2	29	25	53	11	118	K83	K83	K83
K83	K624	95	150	116	69	430	K83	K83	
K83	K651	144	187	154	99	584	K83	K83	
Y175-M460	K83	207	252	126	361	946	K83	K83	K83
K83	K619	65	66	76	78	285			
K616	K83	84	51	32	96	263	K83	K83	
K83	K646	26	28	55	20	129	K83	K83	K83
K83	K140	170	324	166	132	792	K83	K83	K140
K83	K141	327	614	394	274	1,609*	K83	K83	K141
K625	K649	29	6	8	22	65	K649	K649	
K83	K240	79	246	167	109	601	K83	K83	K240
K83	K245	58	161	111	62	392	K83	K83	K245
K617	K270	11	5	14	7	37			K617
K617	Y189-M89	104	83	175	55	417	K617	K617	K617
K90	K627	24	19	13	25	81			
K626	Y189-M89	103	33	107	63	306#	K626		K626
K617	K619	92	70	112	54	328*	K617	K617	K617
K617	K140	170	266	190	121	747	K617	K617	
K617	K625	245	327	342	155	1,069	K617	K617	K617
K626	K625	154	212	205	113	684	K626	K626	
K617	K245	97	146	121	67	431	K617	K617	
K80	C84	23	26	12	40	101	C84	C84	C84
K625	C84	242	176	117	249	784	C84	C84	C84
K625	K270	102	33	26	46	207	K270	K270	K625
K651	K625	112	165	183	96	556	K651	K651	
Y175-M460	K140	34	60	63	39	196	Y175-M460	Y175-M460	
K625	K245	2	5	0	6	13	K245		K245

* #: see table XIV

Note: No contingency χ^2 tests were done on the populations of 101 and lower.

TABLE XVI

The relative order of the sites of difference of the K83 and K625 alleles.

K83 Parent (PD)	K625 Parent (pd)	Prototrophs /10^5 viable ascospores	Prototrophic progeny					Proximal allele difference by criterion		
			PD	pd	pD	Pd	Total	I	II	III
rec-1 x *rec-1*										
am-1 +	+ *inos*	45.4	1,344	884	1,227	700	4,155	K83		K83
+ *inos*	*am-1* +	43.1	679	439	566	274	1,958*	K83		K83
am-1 inos	+ +	37.2	185	136	168	87	576	K83		K83
+ +	*am-1 inos*	37.4	184	126	189	77	576	K83	K83	K83
am-1 +	+ *inos*		2,436	1,450	2,083	1,327	7,296*	K83	*K625*	K83
rec-1 x *rec-1*$^+$										
am-1 +	+ *inos*	2.72	569	975	698	370	2,612	K83	K83	
+ *inos*	*am-1* +	4.05	116	287	220	115	738	K83	K83	
am-1 inos	+ +	3.38	130	248	159	92	629	K83	K83	*K625*
+ +	*am-1 inos*	3.89	183	317	254	142	896	K83	K83	
am-1 +	+ *inos*		902	1,524	1,043	677	4,146#	K83	K83	*K625*

* # See table XIV

contingency χ^2 test. Heterogeneity between the replicates contributing to the individual cross totals in tables XIV, XV and XVI is denoted by a * for the 5% to 1% probability range and by a # for those below the 1% level. The orders derived from the data using the three criteria are presented only if the two classes of prototrophs involved are significantly different at the 5% level.

The analysis of the position of the K83 site of difference relative to the K625 site of difference is shown in table XVI where the extensive data obtained from this allele combination, in other aspects of this study, are summarized. The first four sets of data in each rec-1 category are from the study on the effect of flanking marker position on flanking marker segregation among the prototrophs. The last set of data in each rec-1 category is from the study on the effect of rec-1 segregation on flanking marker segregation among prototrophs. Although these latter data were heterogeneous, they were grouped because no individual cross varied from the general trends exhibited by all allele pairs when the three ordering criteria were applied.

The orders of the allelic differences which are underlined in tables XIV, XV and XVI differ from the orders postulated after a consideration of all factors. The more proximal allele difference in cases where two of the three criteria disagree and the third is insignificant was determined by examining a combination of prototroph frequencies and orders based on other allele combinations. One example of discrepancy is the K83 x Y155 - M302 rec-1 x rec-1 combination in which criterion II differs from I and III. The prototroph frequency for Y155-M302 x K83 suggests that if it is distal to K83, the Y155-M302 site of allelic difference could be located close to those of K629 and K624. Crosses of Y155-M302 with K629 and K624 give no prototrophs so criterion II, in this case, is in disagreement with all the other available ordering criteria. Another example is K617 x K624 where criterion I gives an insignificant difference while criteria II and III disagree. Since K617 x K629 showed that the K617 site of difference was proximal to the K629 site of difference and since K629 x K624 gave no prototrophs (table XI), K617 is assumed to possess the more proximal site of difference. The order derived from criterion II is therefore considered to be wrong in this instance.

Criterion II, in the table XIV rec-1 x rec-1 crosses, disagrees with the other ordering methods in 14 out of 25 instances where the differences between the two proximal flanking marker classes are significant at the 5% probability level. One of the cases of disagreement is contributed by the group III K629 allele crossed to the group IV K90 allele. The reliability of the data for this allele combination has been assured by the fact that 12 replicates were related. Eight of the replicates were from two crosses of the same two stocks derived from single conidial isolates on two occasions (each giving rise to four replicates), while the remaining four replicates came from a cross of single conidial isolates of two different stocks. The contingency $\chi 2$ using the four prototroph classes in each of the three crosses gave a value of 5.623, P at six degrees of freedom = 0.50 - 0.30. If the K90 site of difference were placed distally to the K629 site of difference, as the prototroph frequencies from I x III and I x IV crosses would seem to indicate (table XII), the disagreement in ordering would come from criterion III rather than criterion II. That would make this particular cross give results which would be unlike

those from the other rec-1 x rec-1 crosses and more like those from the rec-1 x rec-1^{+} crosses. Placing the K90 site proximally to the K629 site is not totally unjustified on the basis of prototroph frequencies since the ranges of frequencies found for group II x III and group II x IV crosses did merge (table XII). This means that the prototroph frequencies from the I x III and I x IV crosses could be misleading (due to modifiers acting on rec-1?). On these grounds the K90 site is placed proximally to the K629 site, and therefore the group IV site is placed proximally to the group III site of allelic differences.

Criterion II, besides giving orders of sites of allelic difference which disagree with those obtained from the other ordering means, could not be used in 44 instances in the table XIV rec-1 x rec-1 crosses, compared with 16 instances for criterion I and 19 for criterion III. In total, criterion II gave an order which was not ambiguous in only 11 of the 69 allele combinations in table XIV. Six of these cases were from group I x group II crosses.

The crosses of the alleles whose sites of allelic difference are apparently in close proximity frequently render criterion I non-committal, i.e. there are insignificant differences between the classes being examined. The problem is aggravated by the fact that, without unreasonable expenditure of effort, only small numbers of prototrophs can be collected from such crosses.

In the rec-1$^{(+)}$ x rec-1^{+} crosses, contrary to the rec-1 x rec-1 crosses, the ordering criterion which frequently disagrees with the majority of the ordering means available is criterion III. As with the rec-1 x rec-1 crosses the criterion exhibiting a number of cases of disagreement also exhibits the largest proportion of insignificant differences between the two classes being used for its determination.

Criterion I either consistently agrees with the order derived from a combination of criterion II and/or III and the prototroph frequency data, or is non-committal, for all the allele combinations in tables XIV, XV and XVI.

The linear order of the sites of differences of the groups of alleles is assumed to be I, II, IV, III and VI or VI and V, from proximal to distal respectively, in relation to the centromere. This order is derived from a consideration of the prototroph frequencies and the distribution of the two prototrophic classes recombinant for the parental flanking markers. The small prototroph numbers obtained from VI and V inter-crosses do not allow the sites of these two groups to be placed in position relative to each other on the basis of flanking markers. The group IV site is placed proximal to the group III site of allelic differences on the basis of the relative proportions of the two distal flanking markers among the prototrophs. The only other possible ambiguity in linear order is found in I x II crosses; however, every instance of a significant difference between the various classes of flanking markers used to order sites of allelic difference supports the group I site being placed proximal to the group II site (table XIV).

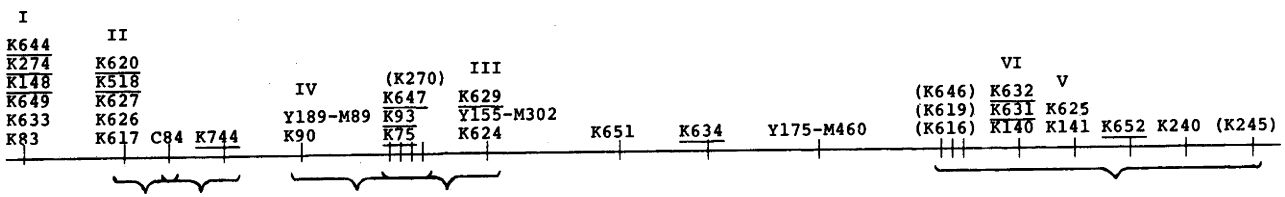
If the range of prototroph frequencies is considered, there is relative agreement between the fine structure

maps which can be obtained using either the data from the rec-1 x rec-1 inter-group crosses or that from the rec-1$^{(+)}$ x rec-1^{+} inter-group crosses.

A fine structure map for the his-1 locus is shown in fig 4. The order of the sites of allelic difference is primarily derived from criteria I and III for rec-1 x rec-1 crosses and criteria I and II for rec-1$^{(+)}$ x rec-1^{+} crosses because there is no instance in which these criteria give an order not possible on the basis of a comparison of prototroph frequencies. The orders within the brackets below the map are arbitrary since the three criteria, applied to these allele combinations, did not yield significant differences in any of the comparisons. Since the prototroph frequencies within either rec-1 or rec-1^{+} crosses have been shown to vary considerably, the map could not be drawn to an exact scale. It is merely an attempt to show approximate separation and relative position of the sites of allelic difference studied. Thirty of the 36 sites of his-1 allelic difference examined can be placed at 15 relatively definite sites. Locating the remaining six sites of allelic

FIGURE 4

A genetic map of the his-1 gene based on prototroph frequencies from rec-1 x rec-1 and rec-1$^{(+)}$ x rec-1^{+} crosses and on flanking marker segregation among the prototrophs (see text). The relative positions of the sites of allelic difference are merely approximate and not to scale. The brackets below the map enclose allele sites which have not been ordered in relation to each other. The sites of difference of the alleles whose numbers are neither underlined nor bracketed were mapped using data from both rec-1 x rec-1 and rec-1$^{(+)}$x rec-1^{+} crosses; the sites of difference of the underlined alleles were mapped using only rec-1 x rec-1 crosses, and the sites of difference of the bracketed alleles were mapped using only rec-1$^{(+)}$ x rec-1^{+} crosses. The roman numerals denote the allele groups described in the text.



difference (those of K75, K270, K646, K616, K619 and K245) at unique sites requires crosses of these alleles to alleles with neighbouring sites of difference. This could not be easily accomplished using the stocks which contained these six alleles, primarily because five of the alleles were in rec-1^{+} stocks and the remaining allele was in stocks which were infertile in such crosses.

Jessop and Catcheside (1965) constructed similar genetic maps of the his-1 locus by using 12 of the 36 alleles whose sites of allelic difference are mapped in fig. 4, and two alleles which were not used in this study. The orders and relative positions of the sites of allelic difference which they proposed are virtually the same as those mapped in fig. 4, with the exceptions of sites which are apparently in close proximity and allelic differences which have been herein mapped in groups at single sites. In addition, utilization of a larger number of A complementation group alleles has shown that the sites of these non-complementing alleles are spread over the whole map. All of the sites of the A group

alleles studied by Jessop and Catcheside were localized at the proximal end of the his-1 locus.

B. FLANKING MARKER DISTRIBUTION AMONG HIS-1 PROTOTROPHS.

1. The reliability of the data.

The high concentrations of ascospores placed on the A plates brought the growing prototrophic colonies into contact with a large number of viable histidine auxotrophs. The only crosses analysed were between non-complementing allele pairs, however, there was a possibility of fusion between the prototrophs and auxotrophs which could affect the flanking marker constitution of the prototrophs. To test this possibility, A plates containing a number of prototrophs which had been analysed for flanking marker constitution, as described in the materials and methods section, were replaced in the 34°C incubator for three additional days and then re-analysed. The cross involved was rec-1 x rec-1^{+} and therefore required a high density of ascospores on the A plates to provide large populations of prototrophs. An average of 252,000 viable ascospores were calculated to be present on each A plate.

Initially, 144 prototrophs from six platings (30 A plates) were analysed. When these colonies (except for two which had fused) were re-tested, one prototroph which had been previously scored as am-1 inos was found to be am-1 +. Since the SS + alanine and SS + inositol testing plates from the first scoring had been discarded, it was impossible to check for a mistake in the former reading. The experiment was therefore repeated using 179 prototrophs from another six platings which, incidentally, had exactly the same calculated viable ascospore concentration. A total of 173 of these prototrophs were re-tested after three additional days at 34°C. None of the 173 colonies had changed in phenotype. It can therefore be concluded that, on plates lacking histidine, fusions between his-1 prototrophs and his-1 auxotrophs, if they occur, would not significantly affect the flanking markeranalysis.

D.R. Smyth (personal communication) found that there was no significant change in flanking marker distribution among am-1 prototrophs when ascospore concentration was varied from 0.9×10^5 to 22.58×10^5

per five plate plating. Since more viable ascospores come into intimate contact as their concentration increases, this also suggests that if any fusions do occur between prototrophs and auxotrophs they do not affect flanking marker analysis. High ascospore concentrations can therefore be safely used.

2. The effect of rec-1 on flanking marker distribution among his-1 prototrophs.

a) The influence of flanking markers.

The initial crosses analysed in this study suggested that the substitution of rec-1^{+} for rec-1 altered the distribution of flanking markers among his-1 prototrophs. Parental stocks which contained either the K83 or the K625 allele at the his-1 locus were chosen as tools to examine this phenomenon. These were used because a relatively high yield of prototrophs could be obtained from this allele combination. Also, examination of the segregation of flanking markers among the prototrophs from a single allele pair, in depth, before studying the his-1 locus as a whole, allows a study of variables affecting the experimental system. One such variable would be any viability differences associated with

the flanking markers. It is necessary to know if the presence of any of the allelic differences used for flanking markers, or any factors linked to these differences, cause reduced viability among his-1 prototrophic ascospores under the existing experimental conditions.

Prototrophic progeny were examined from crosses with all possible parental associations of the genetic factors flanking the his-1 locus to detect and eliminate the effects of possible viability differences associated with the flanking markers. The data, and their statistical analysis, from the series of crosses of the type P K83 D x p K625 d (where P and p, D and d, designate the proximal and distal flanking markers respectively) are summarized in tables XVII and XVIII. Homogeneity of the data, within crosses and between crosses, was analysed by a contingency χ^2 test between the four classes of flanking markers among the prototrophic progeny and the N replicates. The probability values shown in table XVII indicate that the methods used, and the standards of analysis, were reliable. All the replicates of individual crosses may

TABLE XVIII

Heterogeneity χ^2 values obtained between crosses containing the same flanking marker associations

Cross Numbers	D.F.	χ^2	P
08 + 0175, 0177, 0216	6	5.709	0.50-0.30
0411 + 0906, 0427 + 0907	3	8.127	0.05-0.025
0174, 0176, 0215	6	9.860	0.20-0.10
0417 - 0422	15	22.457	0.10-0.05

therefore be grouped. The probability values shown in table XVIII indicate that all the crosses of different parental stocks having the same flanking marker associations may be grouped. There is no evidence, in the stocks used, of genetic differences affecting the distributions. The grouping of all crosses with like associations of flanking markers was shown in table XVI (the first four sets of data in each rec-1 class).

Contingency χ^2 tests between the crosses having different flanking marker associations show marginally acceptable homogeneity; for rec-1 x rec-1 $\chi^2_9 = 15.89$, P = just greater than 0.05, for rec-1 x rec-1$^+$ $\chi^2_9 = 17.47$, P = just below 0.05.

Viability differences between the various combinations of the flanking markers could lead to the observed heterogeneity. Calculations of the expectations for the four classes of prototrophs on the basis of equal viability can be used in χ^2 tests to determine if such differences are affecting the data. The rec-1 x rec-1 crosses are analysed in this manner in table XIX. The 7,265 prototrophs from

TABLE XIX

Analysis of the distribution of flanking markers among prototrophs to determine the effects of any possible viability differences associated with the flanking markers.

	Distribution of flanking markers among prototrophs				
Cross nos.	PD	pd	pD	Pd	Total
08 + 0175, 0177, 0216	$\frac{\text{am-1}}{1,344}$ +	+ $\frac{\text{inos}}{884}$	+ + 1,227	$\frac{\text{am-1 inos}}{700}$	4,155
0411+0906, 0427+0907	+ $\frac{\text{inos}}{679}$	$\frac{\text{am-1}}{439}$ +	$\frac{\text{am-1 inos}}{566}$	+ + 274	1,958
0679	$\frac{\text{am-1 inos}}{185}$	+ + 136	+ $\frac{\text{inos}}{168}$	$\frac{\text{am-1}}{87}$ +	576
0677	+ + 184	$\frac{\text{am-1 inos}}{126}$	$\frac{\text{am-1}}{189}$ +	+ $\frac{\text{inos}}{77}$	576
Total	2,392	1,585	2,150	1,138	7,265

The numbers expected in each class, on the basis of equal viability, can be calculated by multiplying the appropriate row and column totals and dividing the resulting figure by the grand total:

1,368.0	906.5	1,229.6	650.8
644.7	427.2	579.4	306.7
189.6	125.7	170.5	90.2
189.6	125.7	170.5	90.2

The χ^2 values are calculated after summing the appropriate values in each flanking marker class:

	Expected	Observed	χ^2
$\frac{\text{am-1}}{}$ +	2,055.9	2,059	0.005
+ $\frac{\text{inos}}{}$	1,811.9	1,808	0.008
+ +	1,851.6	1,821	0.514
$\frac{\text{am-1}}{}$ inos	1,545.5	1,577	0.629
		$\Sigma\chi^2$ =	1.156

the rec-1 x rec-1 crosses give a $\chi^2_3 = 1.156$, P = 0.80 - 0.75 while the 4,875 prototrophs from the rec-1 x rec-1$^+$ crosses give a $\chi^2_3 = 3.823$, P = 0.30 - 0.25. The evidence therefore indicates that the small differences between the crosses having flanking markers arranged in different combinations are not due to viability differences between the various combinations of flanking markers.

Presumably there are chance differences, between the stocks, which have small effects upon the relative viabilities in the several crosses. Indeed, a large part of the heterogeneity is due to the + K83 inos x am-1^1 K625 + crosses; omission of these gives $\chi^2_6 = 7.566$ (P = 0.25 - 0.20) for the rec-1 x rec-1 crosses and $\chi^2_6 = 5.006$ (P = 0.5 - 0.7) for the rec-1 x rec-1$^+$ crosses. The effect is probably not directly associated with the am-1^1 gene because merely removing crosses 0411 and 0906 (same parents but crossed on different occasions) gives $\chi^2_9 = 11.68$ (P = 0.25 - 0.20) in the rec-1 x rec-1 series.

The source and cause of the disturbance would be

difficult to trace. Part of the problem is that the differences which cause the large χ^2 values are not confined to any one class of flanking markers. The complexity of the crossing program used to obtain the various combinations of the his-1 alleles, flanking markers and rec-1 factors compounds the problem. The differences are also not very large. This means that extensive replication and large populations would be required to give trustworthy answers to any questions asked about the control of the disturbance.

The difference in distribution of the flanking markers appears attributable soley to the substitution of rec-1$^+$ for rec-1. The variants responsible for most of the heterogeneity in the rec-1 x rec-1 crosses tend away from the means of the rec-1 x rec-1$^+$ results while the variants responsible for most of the heterogeneity in the rec-1 x rec-1$^+$ crosses tend away from the means of the rec-1 x rec-1 results.

The prototroph frequencies given in table XVI are the result of pooling the data from the crosses involved, and serve to emphasize the differences between

the various K83 x K625 crosses. The actual range of values for this series of crosses was from 37.2 ± 1.44 to 54.0 ± 2.53 prototrophs per 10^5 viable ascospores for the rec-1 x rec-1 crosses and from 2.32 ± 0.065 to 3.89 ± 0.134 per 10^5 viable ascospores for the replicated rec-1 x rec-1^+ crosses (table XVII). The differences within each class of crosses, although significant, are essentially without effect upon the distribution of flanking markers amongst the prototrophs.

b) Behaviour of progeny from a rec-1 x rec-1^+ cross.

The results in part (a) above indicated that the distributions of flanking markers among his-1 prototrophs were different depending on the rec-1 factors involved in the crosses. The observed totals for the K83 rec-1 x K625 rec-1 crosses (PD = 2,392, pd = 1,585, pD = 2,150 and Pd = 1,138) can be compared to the observed totals for the rec-1 x rec-1^+ crosses (PD = 998, pd = 1,827, pD = 1,331 and Pd = 719). The ratio of pD to Pd is found to be the same in the presence or absence of rec-1^+ (1.88), the contingency

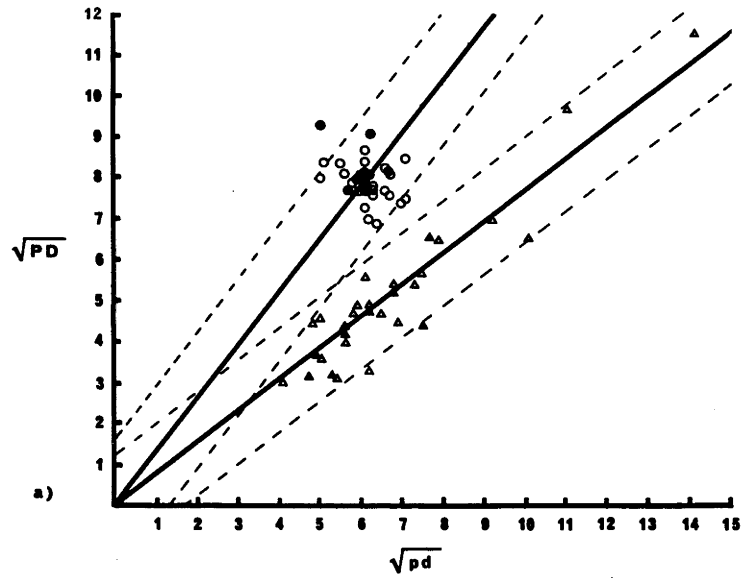
χ^2, for one degree of freedom, being 0.119. The ratios of the two parental classes are obviously significantly different, being 1.51 for the rec-1 x rec-1 crosses and 0.55 in the presence of rec-1$^+$. A further test of the association of this difference between the parental class ratios and the rec-1 factor involved would be to test a number of progeny from a rec-1 x rec-1$^+$ cross.

Sixty nine progeny from cross 0174 (am-1^6 K83 rec-1$^+$ a x K625 inos rec-1 A) were analysed to determine whether any factor, separable from rec-1, was causing the difference in distribution of flanking markers between rec-1 x rec-1 and rec-1 x rec-1$^+$ crosses. Table III shows the crosses which were involved in the analysis. Flanking marker segregation was analysed in samples each of sufficient size to give a clear measure of the proportions; a random sample of 192 prototrophs was analysed from each of the 38 rec-1 x rec-1 crosses, but in the case of the 31 rec-1 x rec-1$^+$ crosses the numbers in the samples ranged from 43 to 586.

The difference in proportion of the PD and pd classes between the rec-1 x rec-1 and rec-1 x rec-1^{+} crosses is preserved in all of the progeny of this cross. The statistical consistency of the difference can be represented graphically (Fisher and Mather, 1942; Mostellar and Tukey, 1949) by plotting the square roots of the numbers of PD against the square roots of the numbers of pd for each cross. The 95% confidence limits are defined by a band two units wide, the unit being the distance between two successive whole number roots on the ordinate or abscissa. When the observed numbers of PD and pd are plotted as a square root diagram for each of the progeny tested, there is a nearly perfect grouping into separate populations (fig. 5,a). All of the rec-1 progeny agree closely with one another, whether parental (K625 rec-1) or recombinant (K83 rec-1).

The relative proportions of the two prototrophic classes recombinant for flanking markers (pD and Pd) are the same in the presence of either rec-1 allele (fig. 5,b).

Figure 5. Square root charts demonstrating the relative frequencies of the two prototrophic classes parental for flanking markers (a) and recombinant for flanking markers (b). The data are from the crosses shown in table III. The broken lines indicate the 95% confidence limits. O = K625 *inos rec-1* x S6 and S7; ● = *am-1*^{6} K83 *rec-1* x S79 and S84; Δ = *am-1*^{6} K83 *rec-1*$^{+}$ x S79 and S84; ▲ = K625 *inos rec-1*$^{+}$ x S6 and S7.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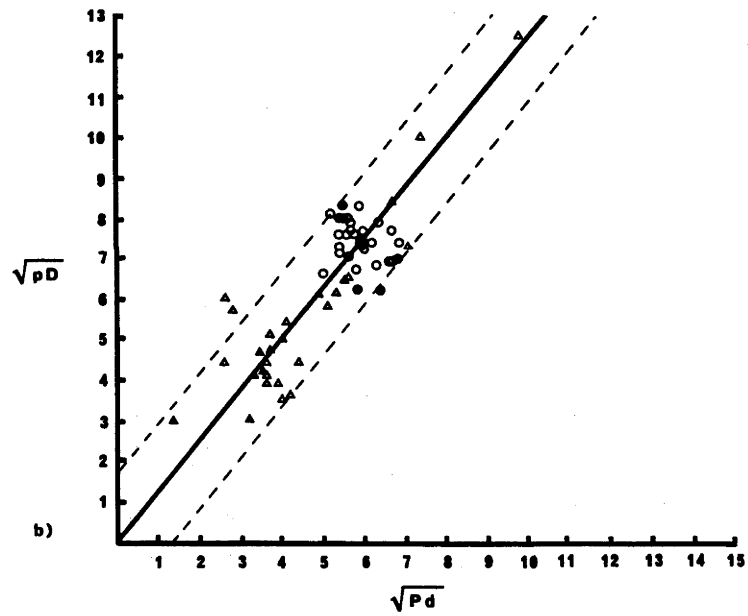
√PD
a)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
√pd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b)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
√Pd

It was previously shown that the rec-1 x rec-1 crosses in table III exhibited a wide range of prototroph frequency, i.e. from 32.3 to 94.3 prototrophs per 10^5 viable ascospores in replicated crosses (fig. 2,b). The flanking marker analysis of the prototrophs from these crosses (fig. 5,a and b) reveals that only one of the crosses which gave a relatively high prototroph frequency (72.7) is outside the 95% confidence limits. It can therefore be concluded that there is no direct association between variation of prototroph frequency and variation in flanking marker segregation in rec-1 x rec-1 crosses of the K83, K625 allele pair.

c) Results of progeny tests.

Other crosses which could help correlate the characteristics of the segregation of flanking markers with rec-1 constitution are those which provide a number of progeny with a specific his-1 allele. When these progeny are crossed to stocks with another his-1 allele the prototrophic progeny should have a characteristic distribution of flanking markers according to the rec-1 constitution. An example of a

study of this nature was found in the analysis of a number of offspring from a cross of K624 inos rec-1 a x rec-1 A, made to improve the fertility of the stocks carrying K624. Nine K624 inos rec-1 isolates from this cross were crossed to am-1^{6} K83 rec-1 stocks and the prototroph progeny were analysed for flanking markers (table XX, part a). One of the crosses (0227) appears to differ significantly from the others, i.e. it is responsible for almost one third of the contingency χ^2 upon which heterogeneity is based. It was decided to repeat this cross and to examine a larger population, and also to repeat the cross which added the smallest χ^2 to the test (cross 0223). The two replicates gave flanking marker distributions which were acceptably homogeneous within the crosses and which were not significantly different from the original nine crosses (table XX, part b). The result means that the heterogeneity shown originally must have been due to chance.

A number of other crosses,made primarily for the purpose of stock building, gave progeny which could be analysed in a similar manner (table XXI). Each of the

TABLE XX

The distribution of flanking markers among prototrophs from crosses of K624 *inos rec-1* sibs X *am-1*[6] K83 *rec-1*

	Cross Number	Distribution of flanking markers PD	pd	pD	Pd	Totals	Contingency χ^2	Degrees of Freedom	P
a)	0219	33	25	33	19	110			
	0221	25	14	13	6	58			
	0223	23	14	19	9	65			
	0225	16	15	5	11	47			
	0227	31	22	31	31	115			
	0229	37	14	29	14	94			
	0231	26	18	27	12	83			
	0233	37	22	20	7	86			
	0235	29	11	17	9	66			
	Totals	257	155	194	118	724	36.728	24	<0.05
	remove 0227	226	133	163	87	609	24.533	21	0.30-0.25
b)	Total from (a)	257	155	194	118	724			
	Repeat of 0223	130	105	91	55	381	16.194	15	0.50-0.30
	Repeat of 0227	200	128	129	107	564	13.755	12	0.50-0.30
	Totals	587	388	414	280	1,669	9.428	6	0.20-0.10

TABLE XXI

Distribution of flanking markers among prototrophs from crosses of *his-1* alleles. Several stocks have been used for each allele pair.

Alleles Crossed	PD	pd	pD	Pd	T	χ^2	D.F.	Probability
rec-1 x *rec-1*								
K83 x K627	308	322	296	143	1,069	7.869	6	0.25-0.20
K83 x Y175-M460	260	132	255	121	768	4.917	9	0.90-0.80
K626 x K625	455	125	196	184	960	21.65	12	0.05-0.025
K617 x K625	240	82	163	91	576	8.144	6	0.25-0.20
C84 x K625	572	157	358	176	1,263	14.04	18	0.75-0.70
rec-1 x *rec-1*$^+$								
K83 x Y175-M460	252	207	361	126	946	35.51	33	0.50-0.30
K626 x K625	154	212	205	113	684	35.68	30	0.25-0.20

allele combinations in the table is represented by at least three crosses and therefore give at least six degrees of freedom. These crosses, and the K83 x K624 and K83 x K625 crosses discussed above, are all of the crosses in this study in which several different stocks with the same alleles were examined to determine flanking marker distribution among their prototrophic progeny. Additional crosses involving the same allele combination in only two different crosses have not been included in the table due to their limited value in this study. The results from all such crosses were, however, homogeneous at least at the 1% probability level.

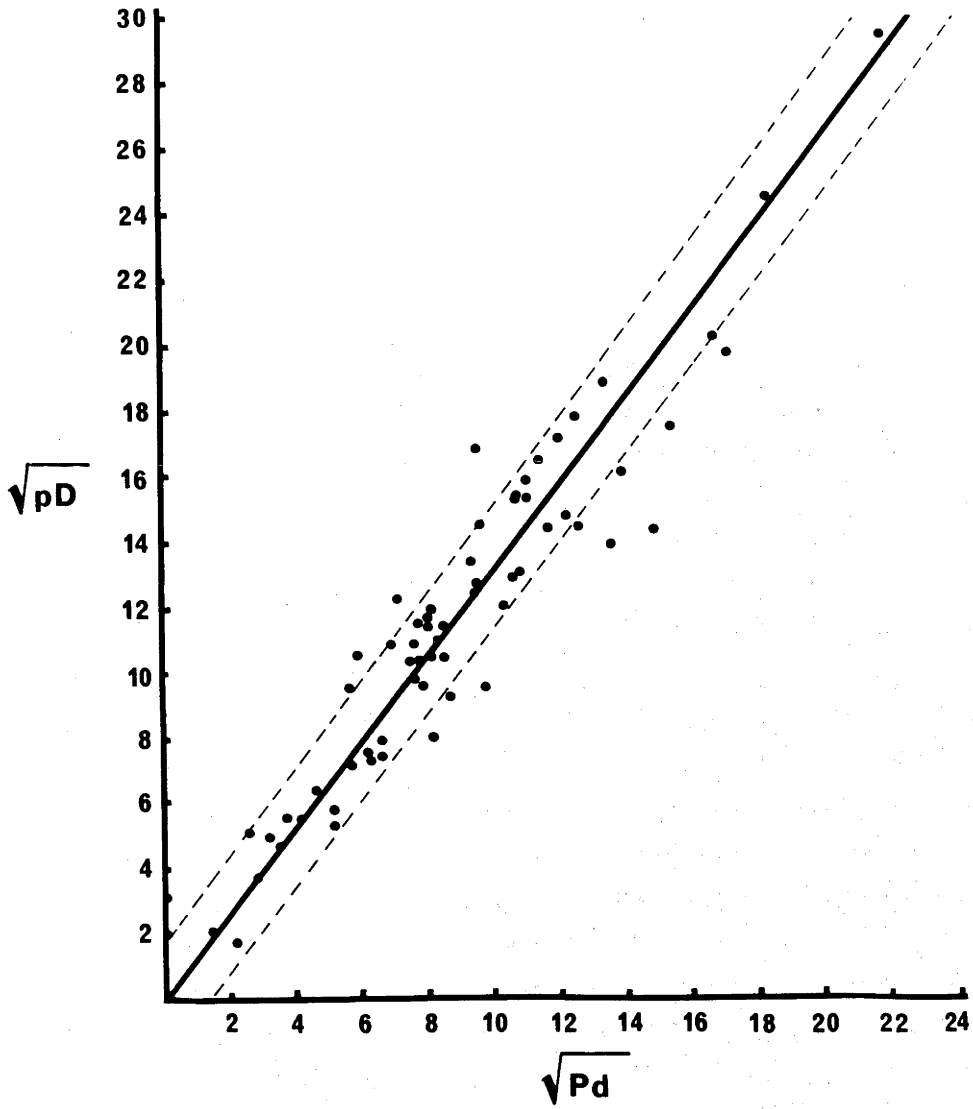
The above results all indicate that in all the stocks being studied, no factor, other than rec-1, was segregating which caused a difference in the distribution of flanking markers among his-1 prototrophs.

3. The relative proportions of the two classes of prototrophs recombinant for flanking markers.

The ratio of the two prototrophic classes recombinant for flanking markers remained unchanged when a rec-1 x rec-1 background was changed to rec-1 x rec-1^{+} in K83 x K625 crosses (fig. 5,b). Square root charts in which $\sqrt{pD}$ is plotted against $\sqrt{Pd}$ for both rec-1 x rec-1 crosses (fig. 6) and rec-1$^{(+)}$ x rec-1^{+} crosses (fig. 7) were used to examine these data from all the crosses in tables XIV and XV. The sloped lines are derived from the square roots of the sums of all of the pD and Pd data in each chart. The ratios of $\sqrt{PD}$ to $\sqrt{pd}$ associated with these lines are slightly different in figs. 5b, 6 and 7, being 1.249, 1.307 and 1.360, respectively, for the three figures. The differences in ratio only become significant with $\sqrt{pD}$ populations of about 18.

The rec-1 x rec-1 data in fig. 6 exhibit variations which cannot be accounted for on the basis of chance. Seventeen of the 69 points lie outside the 95% confidence limits. Many of the crosses which vary from the mean have been replicated several times and show

Figure 6. A square root chart, demonstrating the relative frequencies of the two prototrophic classes recombinant for flanking markers, for all the relevant data from the rec-1 x rec-1 crosses in table XIV.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24
√Pd

Figure 7. A square root chart, demonstrating the relative frequencies of the two prototrophic classes recombinant for flanking markers, for all the relevant data from the rec-1$^{(+)}$ x rec-1^{+} crosses in table XV. The symbol o denotes crosses involving alleles whose numbers are prefixed by Y. The solid dots represent the data from crosses of alleles whose numbers are only prefixed by K.

acceptable agreement between replicates. The data from one of the allele combinations which is quite far from the mean (K83 x K647), for example, are the product of two crosses of different stocks which were made on different occasions with fresh single conidal isolates each time. These crosses were each plated in four replicates, giving a total of eight replicates. The contingency χ^2 between these replicates was $\chi^2_{21} = 30.35$, $P = 0.10 - 0.05$. The data from the allele combination the furthest from the mean (K90 x K629) are from three crosses, two of which involved the same lines but were made with fresh conidial isolates on different occasions. The third cross was made using different stocks of the same alleles. The homogeneity between the 12 replicated platings from these three crosses was acceptable. There is no detectable association between the crosses varying from the means and any common factor. For example, the 10 allele combinations which are outside the 99% confidence limits were K83 x K627, K651 x K634, K83 x K620, K617 x C84, K626 x K625, K90 x K647, K744 x K629, K83 x K90, K83 x K651 and K90 x K629. Only three of these crosses are represented in the <u>rec-1</u>$^{(+)}$ x <u>rec-1</u>$^{+}$ graph (fig. 7).

Of these three, only K83 x K90 is outside the 99% confidence limits in both fig. 6 and fig. 7. Crosses of K83 by alleles with sites of difference very near the K90 site, e.g. Y189-M89 and K93, do not give the relatively depressed pD:Pd ratios shown by K83 x K90. The proximity and relative locations of the two sites of difference are therefore probably not responsible for the low ratios.

The rec-1$^{(+)}$ x rec-1^{+} data plotted in fig. 7 are comparable to the rec-1 x rec-1 data. In this case, however, three of the four points above the upper 95% confidence limit are from crosses involving alleles with numbers prefixed by Y.

In summary, the pD:Pd data indicate that, with the possible exception of some of the crosses involving Y alleles, there is no association between variability of the ratio and the two rec-1 alleles involved. In this matter the two recombinant classes of prototrophs differ from the two parental classes since the distribution of the parental classes is associated with the rec-1 constitution of the parents, at least

in K83 x K625 crosses. The variability exhibited in the recombinant classes seems to be specific to the allele pairs involved, not being related to separation of allelic differences as defined by prototroph frequency, or to any common factor, with the possible exception of certain K x Y rec-1 x rec-1^{+} crosses. The $\sqrt{pD}:\sqrt{Pd}$ ratios for the different allele combinations, despite the heterogeneity encountered, are neither dispersed as much as the ratios formed by the two parental classes, nor do they cover as wide a range (the $\sqrt{PD}:\sqrt{pd}$ ratios are given in figs. 10, 11 and 12 and discussed in section 5).

4. his-1 alleles from different backgrounds.

Early in the study an aberrant result was noted when K83 was crossed to Y189-M89. The change in the proportions of the two parental classes among the prototrophs in rec-1 x rec-1 crosses compared to rec-1 x rec-1^{+} crosses was different from that noted for crosses involving only the K alleles, especially the well studied K83 x K625 combination. The reason for the difference could be the different origin of the Y189-M89 stock (table I) or the particular mutational

change resulting in the allelic difference. Therefore two other alleles from the same source were added to the study (Y155-M302 and Y175-M460) as well as an allele from another different source (C84). The prototroph frequencies and flanking marker segregation data for all crosses involving the three Y mutants are summarized in table XXII. The Y alleles could be intercrossed, but could not be conveniently analysed due to complementation producing pseudo-wild types. The linear order and position of the Y alleles in relation to the other his-1 alleles has been established from the two prototrophic classes recombinant for flanking markers (tables XIV and XV). The distribution of the flanking markers among the prototrophs shows trends which can be summarized as follows:-

i) Like the K83 x K625 results (fig. 5a), the presence of rec-1^{+} in a cross significantly reduced the PD/pd ratio. That is, the flanking marker genotype associated with the more distal his-1 parental allele (pd) increased in proportion to the other parental class (PD) when

TABLE XXII

Prototroph distributions and frequencies from crosses involving alleles with numbers prefixed by Y.

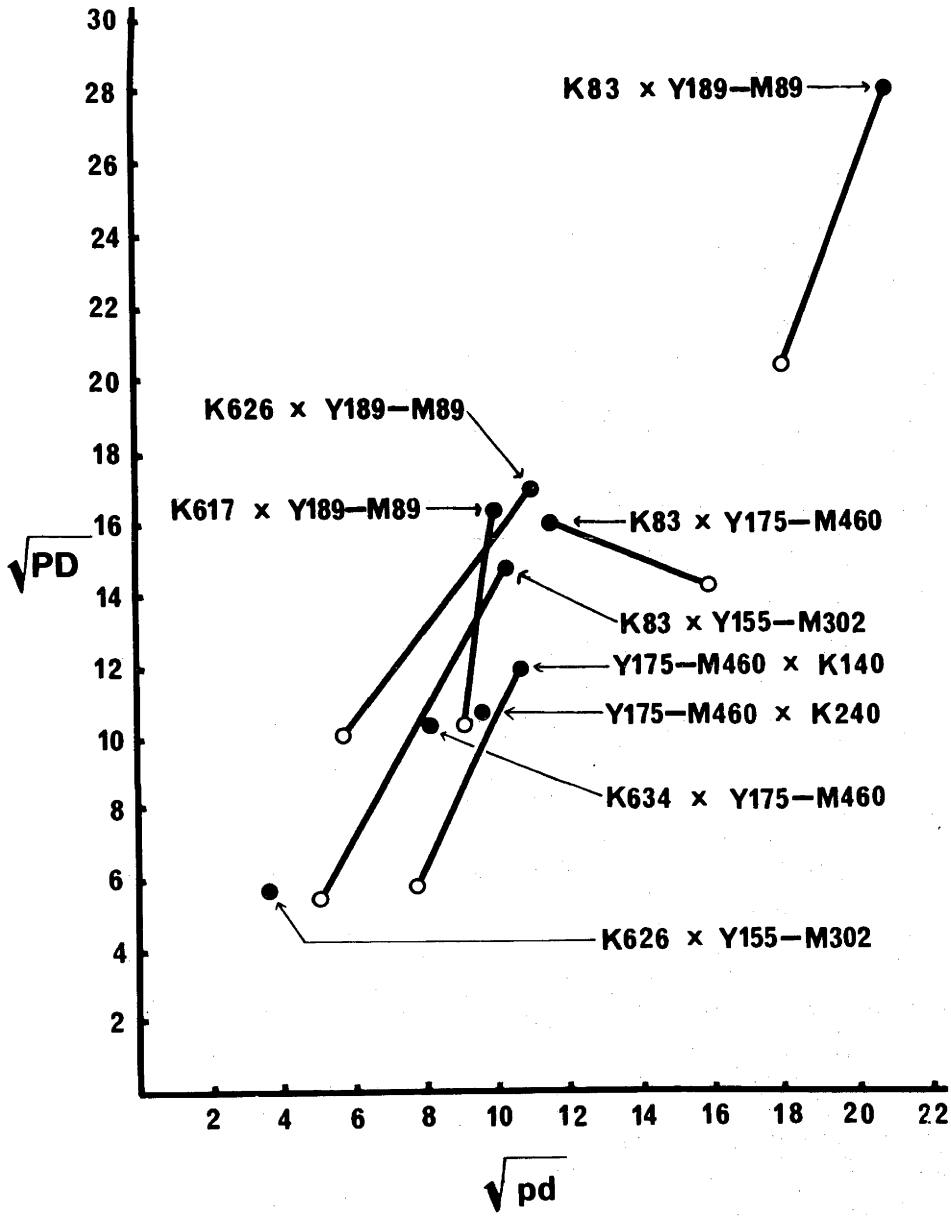
Cross Number	Alleles	Prototrophs /10^5	PD	pd	pD	Pd	Total
rec-1 x *rec-1*							
0609	K83 x Y155-M302	11.1 ± 0.435	223	105	157	89	574
0251	K83 x Y189-M89	17.5 ± 0.696	303	200	213	125	841
0252	K83 x Y189-M89	23.2 ± 0.722	488	232	388	210	1318
0740, 7, 8, 9	K83 x Y175-M460	61.2 ± 2.512	260	132	255	121	768
0954	K626 x Y155-M302	8.37 ± 1.051	33	13	26	7	79
0751	K626 x Y189-M89	20.4 ± 0.616	290	118	238	122	768
0482	K617 x Y189-M89	21.5 ± 0.947	271	99	235	115	720
0927	K634 x Y175-M460	2.96 ± 0.154	108	65	144	65	382
0898	Y175-M460 x K140	9.41 ± 0.364	145	113	113	68	439
0915	Y175-M460 x K240	6.80 ± 0.297	115	92	119	58	384
rec-1 x *rec-1*$^+$							
0610	K83 x Y155-M302	0.415 ± 0.0359	29	25	53	11	118
0250	K83 x Y189-M89	0.810 ± 0.0310	215	153	173	89	630
0825	K83 x Y189-M89	1.057 ± 0.0396	200	169	207	90	666
0734-0746*	K83 x Y175-M460		252	207	361	126	946
0823	K626 x Y189-M89	0.904 ± 0.0501	103	33	107	63	306
0481	K617 x Y189-M89	0.876 ± 0.0420	104	83	175	55	417
0899	Y175-M460 x K140	1.000 ± 0.0692	34	60	63	39	196

* Less cross number 0740. The prototrophs from these crosses are grouped on the basis of analyses done elsewhere. The prototroph frequencies could not be grouped but could be divided into two groups with values of 3.88 ± 0.180 and 6.04 ± 0.318.

rec-1$^+$ was in the cross. The K626 x Y189-M89 crosses are the only exception to this, the rec-1 x rec-1$^+$ PD/pd ratio not being significantly different from the rec-1 x rec-1 ratio. However, the K626 x Y189-M89 rec-1 x rec-1$^+$ prototroph data were heterogeneous (the contingency χ^2 test gives a value of $\chi^2_6 = 21.604$, P = 0.005 - 0.001). A graphic presentation of the data from all the crosses involving alleles whose members are prefixed by Y can be found in fig. 8. The square roots of the values PD and pd are plotted so that the 95% confidence limits of the data can easily be determined (± 1 unit from the plotted points). The rec-1 x rec-1 and rec-1 x rec-1$^+$ crosses between the same alleles are joined by solid lines to facilitate comparison.

ii) Unlike the K83 x K625 results, and all other K x K crosses studied, the rec-1 x rec-1$^+$ $\sqrt{PD}:\sqrt{pd}$ ratios were generally above 1. The one exception in fig. 8 is Y175-M760 x K140, which gives data not significantly different from the K83 x K625 results. The exceptional cross

Figure 8. A square root chart demonstrating the relative frequencies of the two prototrophic classes parental for flanking markers from the crosses involving the alleles whose numbers are prefixed by Y. Data from table XXI. ● = rec-1 x rec-1 crosses, o = rec-1 x rec-1^{+} crosses. The lines join the data from rec-1 x rec-1 and rec-1 x rec-1^{+} crosses of the same alleles.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√pd
K83 x Y189—M89
K626 x Y189—M89
K617 x Y189—M89
K83 x Y175—M460
K83 x Y155—M302
Y175—M460 x K140
Y175—M460 x K240
K634 x Y175—M460
K626 x Y155—M302

differs from the other K x Y crosses in two ways. The sites of allelic difference involved are apparently both located in the distal region of the his-1 locus, and the site of difference of the allele is proximal, rather than distal, to the K allele. The rec-1 x rec-1 ratios for this and for the other cross of the same type (Y175-M460 x K240) are well below the rec-1 x rec-1 $\sqrt{PD}:\sqrt{pd}$ ratios for the other K x Y crosses.

iii) The K83 x K625 crosses used to study the effect of different associations of flanking markers yielded classes of prototrophs with an unexpected asymmetry. The recombinant classes of flanking markers (pD and Pd) were together significantly less frequent than the combined parental classes (PD and pd) the proportion in rec-1 x rec-1^{+} crosses being less (42.1%) than in rec-1 x rec-1 crosses (45.3%). The effect of rec-1^{+} in this respect was highly significant, the contingency χ^2, for one degree of freedom, being 12.177. K83 x Y189-M89 is the only K x Y cross in which the recombinants were less frequent

in rec-1 x rec-1$^+$ crosses than in rec-1 x rec-1 crosses (table XXIII). The effect of rec-1$^+$, in this case, is not significant, the contingency χ^2, for one degree of freedom being 0.016. The remainder of the K x Y crosses show that the recombinants change, in proportion, from less than 1/2 to more than 1/2 of the total prototrophs when the genetic background is changed from rec-1 to rec-1$^+$. The crosses containing Y alleles therefore generally have a different asymmetry between parental and recombinant classes among their prototrophs from that found for the K83 x K625 crosses previously analysed. Stated another way, the production of prototrophs seems to be associated with crossing-over between the flanking markers more often when rec-1$^+$ is present in a K x Y cross than when it is absent, a trend which is opposite to that shown by the K83 x K625 data.

iv) The $\sqrt{pD}:\sqrt{Pd}$ ratios (fig. 7) for three of the six rec-1$^+$ allele combinations were very high compared to the other crosses in table XV and to the K83 x K625 value (fig. 5b). However, the

TABLE XXIII

Recombinant (pD and Pd) prototrophs from K x Y crosses, expressed as percent of the total number of prototrophs

Alleles crossed	rec-1 x rec-1	rec-1 x rec-1$^+$
K83 x Y155-M302	44.1%	54.2%
K83 x Y189-M89	43.4	43.1
K83 x Y175-M460	49.0	52.0
K626 x Y155-M302	41.8	-
K626 x Y189-M89	46.9	55.6
K617 x Y189-M89	48.6	55.2
K634 x Y175-M460	45.3	-
Y175-M460 x K140	41.2	52.0
Y175-M460 x K240	46.1	-

results are not consistent and form no recognisable pattern associated with specific alleles or pairs of alleles.

The prototroph frequencies from crosses involving the C84 his-1 allele and the distribution of flanking markers among prototrophs analysed from these crosses are shown in table XXIV. Certain similarities to the data obtained from K x K crosses can be found in a square root chart comparing the two parental classes (PD and pd) of prototrophs resulting from crosses of C84 x K alleles (fig. 9):

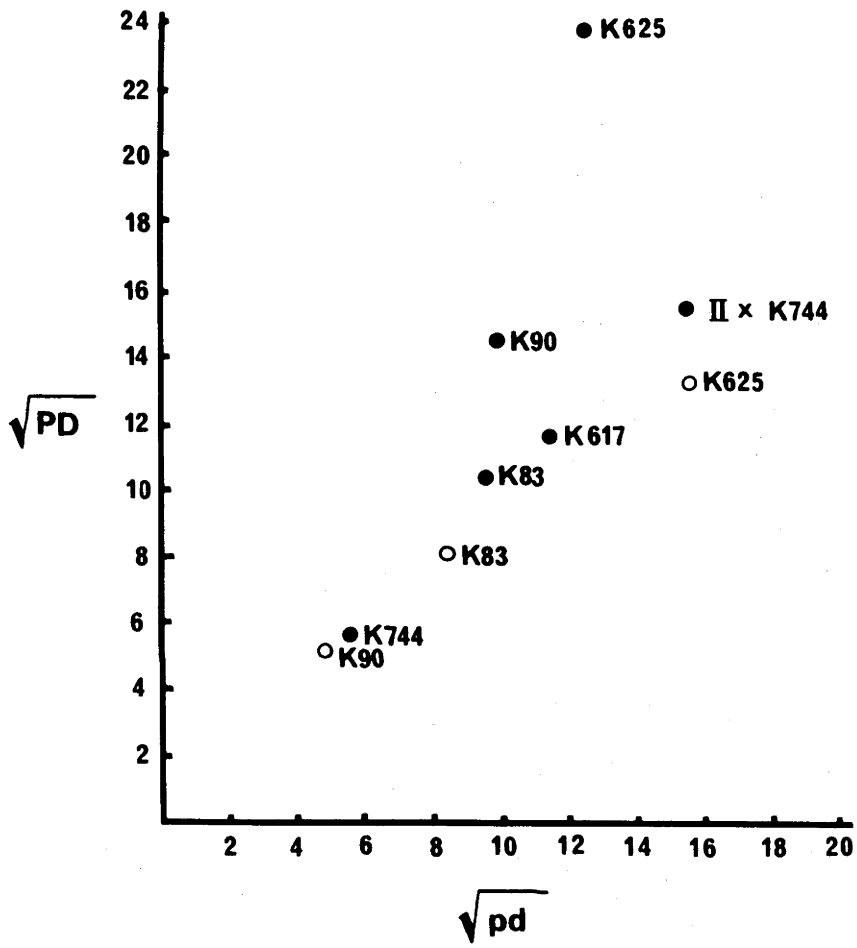
i) The crosses of C84 by K83, K617 and K744 gave $\sqrt{PD}:\sqrt{pd}$ ratios which were not significantly different from 1 for both rec-1 x rec-1 and rec-1 x rec-1^{+} crosses. The K x K crosses involving sites of allelic difference in the proximal region of the his-1 locus (group I x group II, fig. 11 and group II x K744, fig. 9) also yielded $\sqrt{PD}:\sqrt{pd}$ ratios not significantly different from 1.

TABLE XXIV

Prototroph distributions and frequencies from crosses involving the C84 *his-1* allele

Cross Number	Alleles	Prototrophs /10^5 viable Ascospores	PD	pd	pD	Pd	Total
rec-1 x *rec-1*							
0653	K83 x C84	3.189 ± 0.171	109	90	98	61	358
0766, 0822	K617 x C84	1.178 ± 0.051	137	130	93	97	457
0767	C84 x K744	1.136 ± 0.107	31	30	21	12	94
0768	C84 x K90	8.711 ± 0.360	213	98	181	87	579
14 crosses	C84 x K625	53.0 ± 4.67 to 84.3 ± 6.97	572	157	358	176	1,263
rec-1 x *rec-1*$^+$							
0771	K83 x C84	0.140 ± 0.008	65	73	61	26	225
0824	C84 x K90	0.361 ± 0.021	26	23	40	12	101
0654	C84 x K625	2.90 ± 0.107	176	242	249	117	784

Figure 9. A square root chart illustrating the relative frequencies of the two prototrophic classes parental for flanking markers from the crosses involving the C84 allele. ● = progeny from rec-1 x rec-1 crosses while o = progeny from rec-1 x rec-1^{+} crosses. The point representing the group II alleles crossed to K744 is added for comparative purposes.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√PD
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8
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12
14
16
18
20
√pd
● K625
● Ⅱ × K744
● K90
○ K625
● K617
● K83
○ K83
● K744
○ K90

ii) The crosses of C84 with K90 and K625, two alleles with sites of difference which are located distal to the proximal region mentioned in (i) above, gave $\sqrt{PD}:\sqrt{pd}$ ratios which were not significantly different from those of K617 by these distal sites of allelic difference (table XXV). The ratio tends to increase with distance from the C84 site of difference in rec-1 x rec-1 crosses. The ratio remains the same in rec-1 x rec-1$^+$ crosses except for the cross with the allele (K625) which apparently has its site of difference furthest from that of C84.

The C84 x K625 rec-1 x rec-1 crosses exhibit a range of prototroph frequencies, probably due to modifiers similar to those affecting K83 x K625. These modifiers do not seem to affect the PD:pd relationship. Therefore the relationship is probably not a precise function of the separation of mutant sites as measured by prototroph frequency.

TABLE XXV

Similarities between the $\sqrt{PD}$:$\sqrt{pd}$ ratios from C84 x K90 and K625 and K617 x K90 and K625 crosses

rec-1 x rec-1	$\sqrt{PD}$	$\sqrt{pd}$	Slope ($\sqrt{PD}/\sqrt{pd}$)
C84 x K625	23.9	12.5	1.912
K617 x K625	15.5	9.0	1.722
C84 x K90	14.6	9.9	1.474
K617 x K90	9.3	7.0	1.329
rec-1 x rec-1$^+$			
C84 x K625	13.3	15.6	0.853
K617 x K625	15.6	18.1	0.862
C84 x K90	5.1	4.8	1.062
K617 x K90	no data		

The rec-1 x rec-1 crosses of C84 x K617 and K744 are also of interest because of the very low frequencies of prototrophs which were obtained (less than 2 per 10^5 viable ascospores). The only other cross, for which sufficient flanking marker data is available and which had as low a prototroph frequency, was K625 x K140 with a frequency of 0.920 ± 0.075. The attempt to order these allelic differences with respect to each other (table XIV) proved fruitless because of insignificant differences between the numbers in the various classes of prototrophs used to order the sites of allelic difference. The order of these sites of allelic difference, used in their discussion, is derived from a combination of the insignificant differences in the three criteria and prototroph frequency and can not, therefore, be considered reliable. However, since the order does not seriously affect the analysis of the data the uncertainty is not serious.

In summary, the C84 allele shows no evidence of behaving unlike the K alleles. The Y alleles do exhibit differences from the K alleles in the proportions of the various classes of prototrophs obtained from crosses to other his-1 alleles. The

differences are probably not due to the relative positions of the sites of allelic difference (defined by prototroph frequencies) within the locus. This is illustrated by the fact that the two sites of Y allelic difference which map at the same site as various K sites of allelic difference give different results from them when crossed to a common allele. This is best shown by a comparison of the progeny in the PD and pd classes, in both rec-1 x rec-1 and rec-1 x rec-1^{+} backgrounds, from crosses of K83 x group III alleles and K83 x group IV alleles (table XXVI). There are few possible combinations within which comparison may be made, i.e. K83 crossed to each of the two groups in each rec-1 background. Contingency χ^2 tests give χ^2 values which are below the 5% probability level for each combination. The K83 x Y155-M302 and Y189-M89 crosses in all cases give higher PD:pd ratios than K83 x K alleles in the same groups. This applies to both rec-1 x rec-1 and rec-1 x rec-1^{+} comparisons. It is therefore very probable that allelic differences which are apparently at the same site can yield differing results when crossed to a common allele.

TABLE XXVI

Flanking marker distribution among prototrophs of crosses of K83 by alleles whose sites of differences map together.

Alleles Crossed	Relationships of the two parental classes of flanking markers among the prototrophs.					
	rec-1 x *rec-1* data			*rec-1* x *rec-1*$^+$ data		
	PD	pd	$\frac{PD}{pd}$	PD	pd	$\frac{PD}{pd}$
K83 x K624	587	388	1.49	95	150	0.63
K83 x K629	366	289	1.27			
K83 x Y155-M302	287	141	2.04	29	25	1.16
	$\chi^2_2 = 13.506$; P = 0.005-0.001			$\chi^2_1 = 4.062$; P = 0.05-0.025		
K83 x K90	343	280	1.23	55	81	0.68
K83 x Y189-M89	791	432	1.83	415	322	1.29
	$\chi^2_1 = 16.124$; P<0.001			$\chi^2_1 = 13.078$; P<0.001		

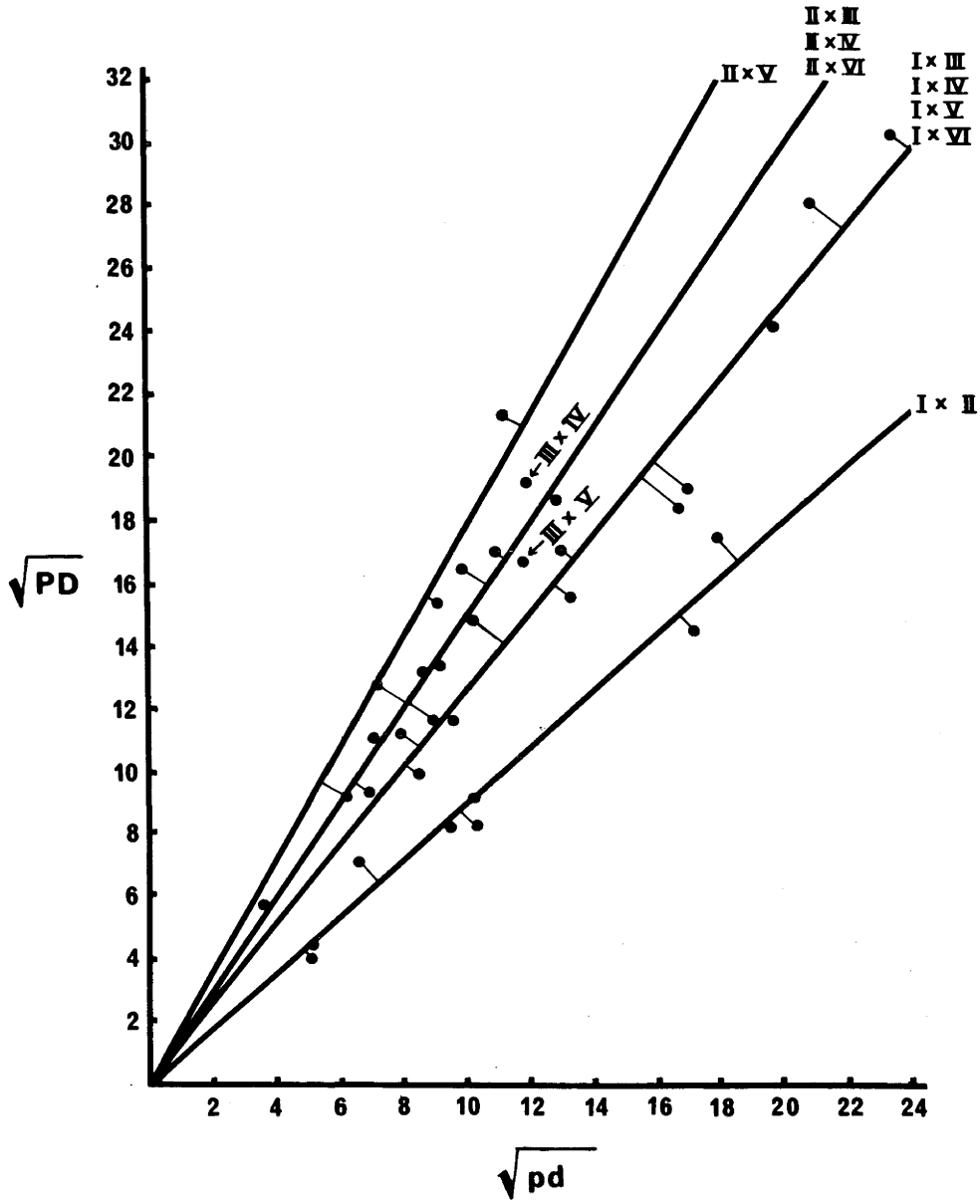
5. The effect of relative separation of sites of allelic difference on flanking marker distribution.

a) Differences between groups of alleles.

The matings of the C84 allele with alleles, whose sites of difference varied in their distance (in terms of prototroph frequency) from the C84 site of difference, gave results which indicated the possibility of relative separation of allelic difference being associated with differences in the distribution of the two parental classes of flanking markers among prototrophs. An initial test of this supposition would be to examine the prototrophic progeny from crosses involving the allelic groups described in section A-2,because the relative positions of these groups are fairly well defined.

The arrangement of the flanking markers among the prototrophs of the inter-group crosses shows interesting features which can be illustrated with a square root chart comparing the two classes having parental associations of flanking markers (fig. 10). Since alleles within the same group can yield different results when crossed to other alleles, each cross of

Figure 10. A square root chart demonstrating the relative frequencies of the two prototrophic classes parental for flanking markers for all the rec-1 x rec-1 inter-group crosses. The relevant inter-group crosses are related to their mean ratios by perpendicular lines.


II × III
II × IV
II × VI
II × V
I × III
I × IV
I × V
I × VI
I × II
←III × IV
←III × V
√PD
√pd
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
2
4
6
8
10
12
14
16
18
20
22
24

a different allele pair is represented by a point on the graph. The perpendicular lines joining the points to the sloped lines are used to relate the crosses of individual allele pairs to the mean value for all crosses involving the same groups. The slope of the $\sqrt{PD}:\sqrt{pd}$ line for the K83 x K625 crosses analysed elsewhere (fig. 5a) was the same as that shown in fig. 10 for the other group I x V crosses.

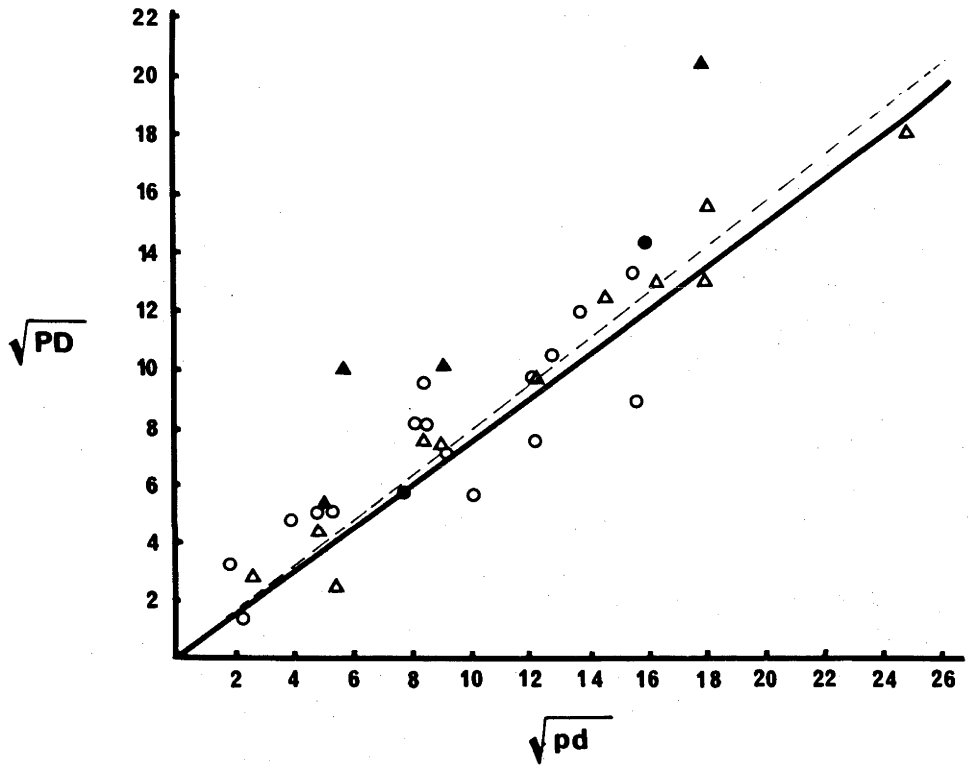
Different inter-group crosses are joined to the same sloping line (i.e. are given common means) in two instances because the individual means for each of the inter-group crosses were not significantly different for the population sizes involved. Only four crosses are significantly different from the means of the inter-group crosses to which they belong. The four deviants are I x III and I x IV crosses of the alleles K83 x Y155-M302, K83 x K629 and K83 x Y189-M89, K83 x K90. These have been cited previously as supporting the assumption that there can be variation within inter-group crosses. However, the graphical presentation of the data (fig. 10) shows that these variations are not great enough to affect the interpretation of the data seriously.

The main feature of fig. 10 is that the I x II $\sqrt{PD}:\sqrt{pd}$ ratio (0.90) is significantly lower than the ratios for I x III, I x IV, I x V and I x VI (an average of 1.25) which are, in turn, significantly lower than the ratios from crosses of members of group II by the other groups. Group II x V crosses give the highest ratio (1.83). This simply means that the flanking marker arrangement which goes into a cross in the same parent as a group II allele is relatively more frequent among the prototrophic progeny than the other parental arrangement.

The data indicate that the proximal group I site in the his-1 locus behaves in a different manner from the other sites, i.e. it depresses the ratio of PD:pd among the prototrophs. However, as will be described later, the crosses of K83 by the other, ungrouped, alleles indicate that a relatively very high $\sqrt{PD}:\sqrt{pd}$ ratio is possible. This would suggest that the proximal location of the K83 site of allelic difference is not always associated with a depressed $\sqrt{PD}:\sqrt{pd}$ ratio (compared to the higher ratio obtained from group II and group III crossed to the remaining distal groups).

The limited $\sqrt{PD}:\sqrt{pd}$ data available from rec-1 x rec-1^{+} crosses between the alleles in the various groups is graphed in fig. 11. The formerly noted rec-1 x rec-1^{+} effect of significantly reducing the $\sqrt{PD}:\sqrt{pd}$ ratio from that found with rec-1 x rec-1 holds true for all but the crosses of members of group I crossed to members of group II and the more ambiguous crosses involving Y alleles (fig. 8 and its discussion examined the Y alleles in more detail). The sloping line placed on the graph is the one calculated for the K83 x K625 rec-1^{+} crosses in fig. 5a. The points from inter-group crosses significantly different (as defined by the 95% confidence limits) from the line involve all the crosses containing Y alleles, the small population from K649 x K625, and the crosses K617 x K625 and K626 x K625. In brief, the I x V and I x VI crosses give the lowest $\sqrt{PD}:\sqrt{pd}$ ratio, contrary to the trend in fig. 10, while, as in fig. 10, the II x V crosses give a relatively high ratio. It should be noted, however, that all but the Y crosses and K649 x K625 would fall within the 95% confidence limits of a line drawn with a slightly steeper slope, indicated by the dotted line in fig. 11.

Figure 11. A square root chart of the data on the two parental classes of flanking markers among the prototrophs of the rec-1$^{(+)}$ x rec-1^{+} crosses in table XV. Δ and ▲ = the inter-group cross results, the ▲ indicates those involving alleles whose numbers are prefixed by Y. o and ● = the remainder of the crosses, the ● indicating the crosses involving the Y alleles. The solid sloping line is that derived from the K83 x K625 rec-1 x rec-1^{+} cross data in fig. 5a. The dotted line is explained in the text.

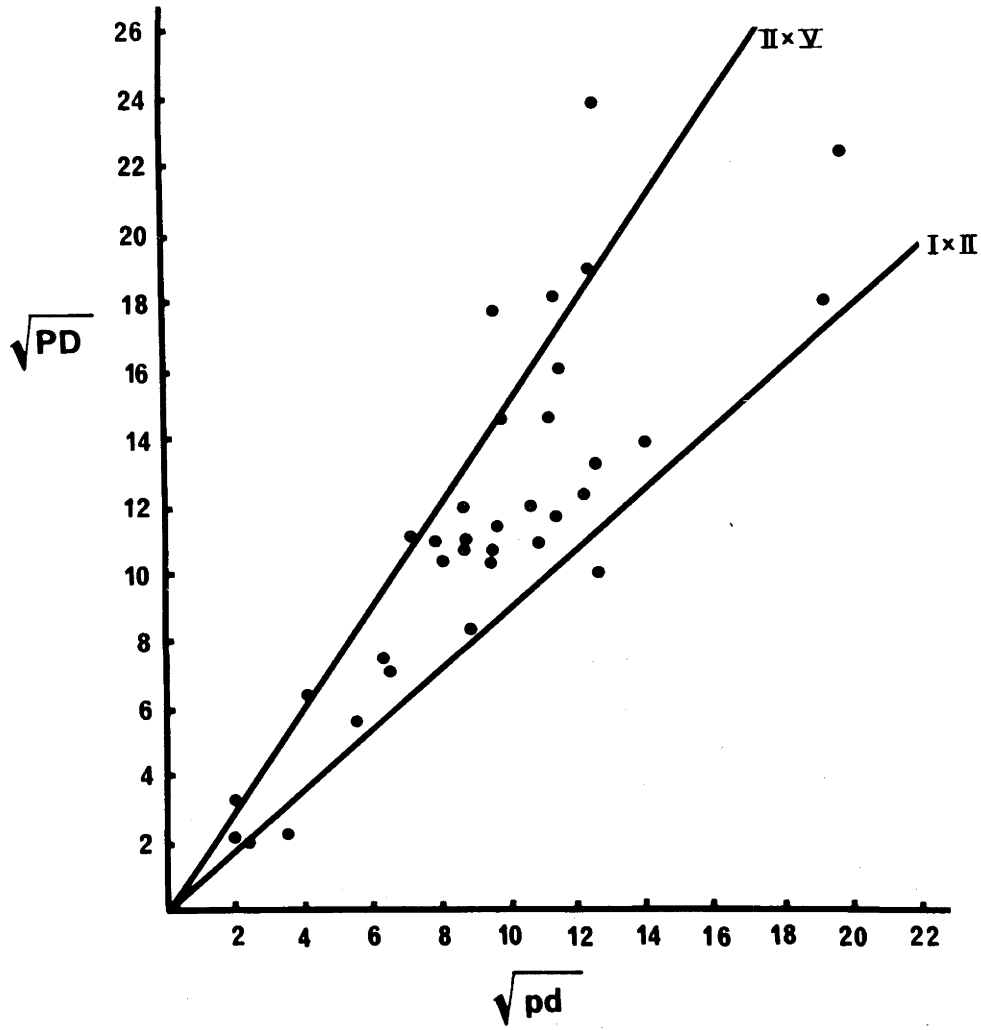

22
20
18
16
14
12
10
8
6
4
2
√PD
2
4
6
8
10
12
14
16
18
20
22
24
26
√pd

b) Comparison of all allele pairs.

The remainder of the data from the rec-1$^{(+)}$ x rec-1^{+} crosses in table XV are added to fig. 11, using distinguishing symbols. The $\sqrt{PD}:\sqrt{pd}$ ratios do not cover as large a range as the ratios from the rec-1 x rec-1 crosses diagrammed in figs. 10 and 12. The crosses involving the Y alleles have a consistently high ratio, as was discussed previously. Differences between the results from the different allele pairs are not associated with any evident common factor.

The data for all of the allele combinations in table XIV, not plotted in fig. 10, are plotted in fig. 12. The sloping lines from the I x II and II x V data are added for comparative purposes. Examination of the data from all crosses of alleles whose sites of difference are in the proximal end of the locus, ranging from the site of the group I difference to that of K744, reveals that all of the $\sqrt{PD}:\sqrt{pd}$ ratios are within or very close to the 95% confidence limits of the sloping line for the I x II data. To state this in another way, the crosses of the alleles whose sites of difference are in the proximal end of the

Figure 12. A square root chart demonstrating the relative frequencies of the two classes of prototrophs parental for flanking markers for all but the inter-group rec-1 x rec-1 crosses in table XIV. The sloping lines derived from the data from two of the inter-group crosses (I x II and II x V) were added for comparative purposes.


26
24
22
20
18
16
14
12
10
8
6
4
2
√PD
II×V
I×II
2
4
6
8
10
12
14
16
18
20
22
√pd

his-1 locus give the lowest observed $\sqrt{PD}:\sqrt{pd}$ ratios. There is, however, one other cross which gives a $\sqrt{PD}:\sqrt{pd}$ ratio of this nature. K83 x K240, apparently the allele pair with the most widely separated sites of difference, also plots within the 95% confidence limits of the I x II mean slope. This fact alone would seem to rule out any simple relationship between proximity of sites of allelic difference and distribution of flanking markers among prototrophs.

Another argument against association between proximity of sites of allelic difference and flanking marker distribution is that one of the two highest $\sqrt{PD}:\sqrt{pd}$ ratios in fig. 12 comes from K83 x K647 (the other is C84 x K625). These K83 x K647 data establish that crosses of K83 by other alleles can yield very high ratios, contrary to the trend in fig. 10. The data for the K83 x K647 allele combination are from two crosses, each of which had different parents. Each cross was plated in four replicates. The flanking marker distribution, both within and between the crosses, was homogeneous as determined by contingency χ^2 tests.

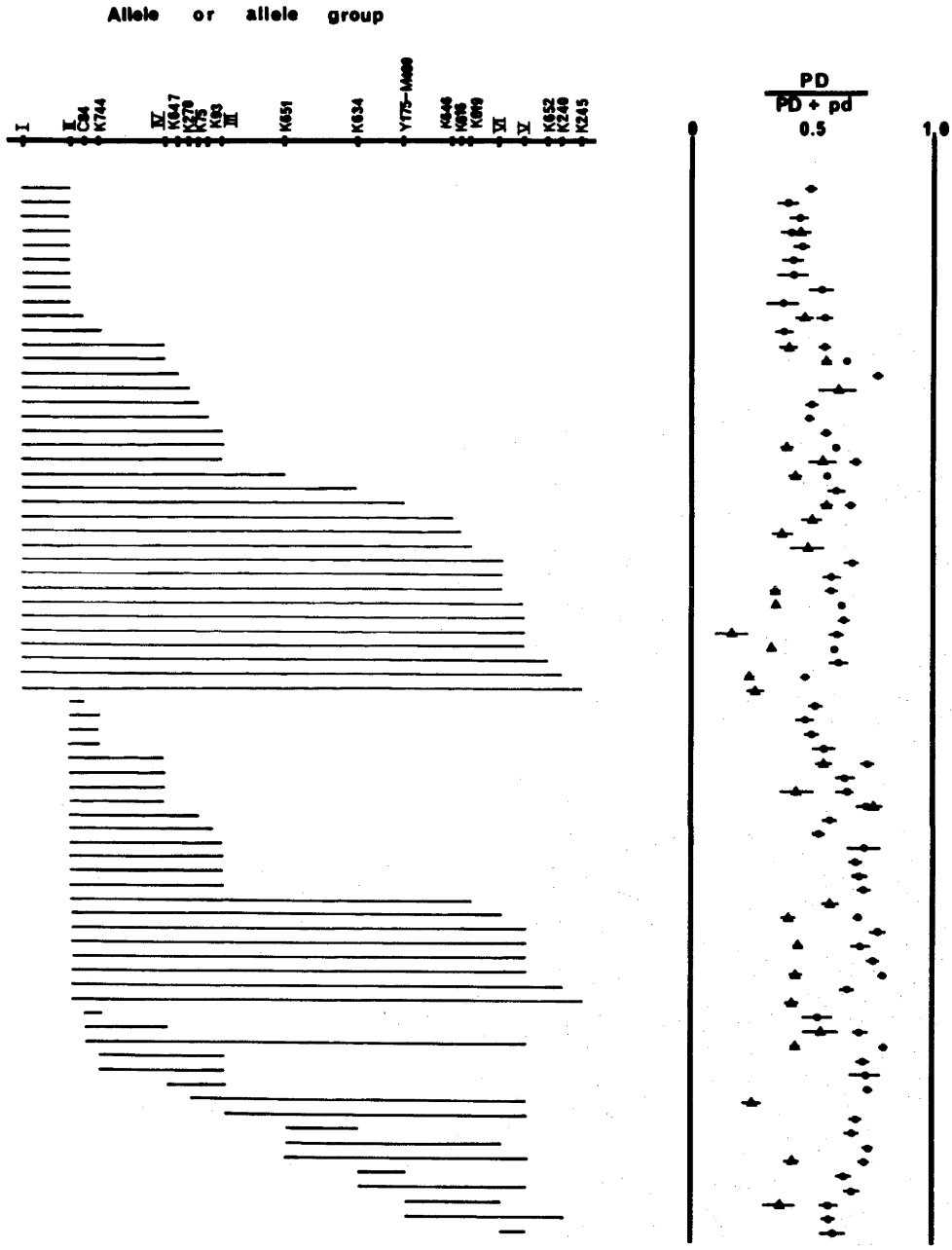
C. GENERAL RELATIONSHIPS ESTABLISHED BY THE DATA.

Whitehouse (1966) used a diagram in which the value $\frac{PD}{pd + PD}$ could be related to a fine structure map. A similar diagram is given (fig. 13) to relate the values of PD and pd visually with the relative proximity and location of the sites of allelic difference involved in the crosses and the rec-1 factor used. The $\frac{PD}{pd + PD}$ points are dervied from the data in tables XIV, XV and XVI. Crosses with small populations, i.e. 37 or less prototrophs analysed, are not included.

A number of points may be established, from the above data, which describe the relationships of mutant site position, relative separation, rec-1$^+$ allele presence and variation of the PD:pd ratio:

i) The lowest rec-1 x rec-1 PD:pd ratios come from crosses of alleles located in the proximal region of the his-1 locus and from the two alleles whose sites of difference are apparently among the most widely separated.

Figure 13. Graph showing the ratio PD/(PD + pd) for each allele pair crossed. ● = rec-1 x rec-1 data from table XIV while ▲ = $\underline{\text{rec-1}}^{(+)}$ x $\underline{\text{rec-1}}^{+}$ data from table XV. The horizontal lines extending from the points represent the standard errors of the ratios. The standard errors were calculated using the formula $\sqrt{pq/n}$ where p = the proportion $\frac{PD}{PD + pd}$ while q = 1-p and n = PD + pd.


Allele or allele group
I
II
CB4
K744
IV
K647
K276
K75
K53
III
K651
K634
Y175–M699
K446
K86
K99
VI
V
K652
K246
K245
PD
PD + pd
0
0.5
1.0

ii) Alleles with a common site of difference from the wild strain may show different ratios when crossed to a common allele.

iii) The rec-1 x rec-1^{+} PD/(PD + pd) proportion is never significantly higher than the rec-1 x rec-1 proportion for any given pair of alleles. A particular rec-1 x rec-1^{+} cross may, however, give a higher PD/(PD + pd) proportion than a rec-1 x rec-1 cross of another allele pair.

iv) The ratio of the rec-1 x rec-1 PD:pd proportion to the rec-1 x rec-1^{+} PD:pd proportion does vary considerably for different allele pairs. This variation is not directly related to the distance between the sites of allele difference but may possibly be related to the specific background of the alleles as was shown for the K x Y crosses.

The above points suggest that the only factors which can be directly linked to the variation in the PD:pd ratio are the particular his-1 allele pair being crossed and the rec-1 alleles present in the cross.

CHAPTER IV

DISCUSSION

A. INTRODUCTION.

The principal purpose of this study was to define the nature of the action of *rec-1* and *rec-1*$^+$ on recombination at the *his-1* locus. This was in the belief that examination of such genetic variants may be expected to cast light on the mechanism of recombination.

The primary manifestation of *rec-1* action is a several fold difference between the prototroph frequencies of *rec-1* x *rec-1* and *rec-1* x *rec-1*$^+$ crosses (Jessop and Catcheside, 1965). Originally it was presumed, as suggested by the early data, that the proportionate difference between the two levels of prototroph frequencies would be constant, at least for specific allele pairs. However, prototroph frequencies from K83 x K625 crosses showed that the difference between the *rec-1* and *rec-1*$^+$ frequencies from this particular allele pair was highly variable, i.e. ranged from 8.84 times to 53.5 times, due to the

action of other genetic factors.

An estimate of the rec-1/rec-1^{+} ratio, relieved of the variation caused by modifiers, would be useful when comparing the flanking marker distributions of rec-1 x rec-1 and rec-1$^{(+)}$ x rec-1^{+} crosses. In particular, prototrophs with the characteristics, in respect of flanking marker distribution, of rec-1^{+} crosses may be present among the prototrophs of rec-1 crosses, in a proportion like the rec-1/rec-1^{+} ratio. Erasing the effects of the modifiers requires the following assumptions:

i) the modifiers are correspondingly distributed among the rec-1 and rec-1^{+} stocks.

ii) the modifiers have correspondingly multiplicative effects on rec-1 crosses as on rec-1^{+} crosses.

Then, taking a given allelic cross, the weighted means for rec-1 crosses divided by the weighted means for rec-1^{+} crosses should give an estimate of the ratio

relieved of modifier effect. Some indication of the applicability of the assumptions may be had by examining the K83 x K625 crosses. These crosses are the only ones of a single allele pair which involve a large number of stocks in both rec-1 and rec-1$^+$ backgrounds. It appears that obtaining truly representative weighted means from these data would be exceedingly difficult due to the various frequencies with which different parents participate in the crosses. For example, only six stocks form one half the parents of the 102 different crosses of K83 and K625 giving the prototroph frequency data in fig. 2. To compound the problem, four of these six parental stocks apparently allow the expression of relatively high prototroph frequency while the remaining two do not.

The K83 x K625 data also provide evidence that assumption (ii) is unsound. The range of prototroph frequencies, from replicated crosses of the same stocks, was more than twice as great in the rec-1 crosses as in the rec-1$^+$ crosses. This may have been due to the use of too limited a number of stocks to

detect a larger range in rec-1^{+} crosses. In any event, the differences in ranges would suggest that any attempt to calculate a rec-1/rec-1^{+} ratio for the K83 x K625 crosses would not give a unique answer.

Other less intensively studied allele pairs also showed that variation could exist within either rec-1 x rec-1 or rec-1$^{(+)}$ x rec-1^{+} crosses but, in no case, did the variation cause confusion between the rec-1 x rec-1 and rec-1$^{(+)}$ x rec-1^{+} prototrophy frequencies. The main point to evolve from these observations is that the rec-1/rec-1^{+} ratio of prototroph frequencies cannot be used as a device to study variation between prototroph frequencies from different allele pairs because, by choosing the appropriate stocks, the ratio can be manipulated. Breeding to a standard would help, but, depending on the nature of the genetic control of the variation, would probably require a very large expenditure of effort.

The variation of prototroph frequencies also means that fine structure mapping becomes exceedingly difficult if it is based only on the prototroph

frequencies. Whether map expansion (Holliday, 1964) could be judged to be present or absent, depends on the particular stocks chosen for a series of allelic crosses.

A revised fine structure map of the his-1 locus was deduced after a consideration of the prototroph frequencies and the distribution of flanking markers among the prototrophs of crosses between different his-1 alleles (fig. 4). The prototroph frequency data alone are affected by the minor variations caused by factors other than rec-1. There is also the problem of whether a map constructed on this basis represents the true linear spacing of allelic differences, or if distortion is caused by the recombinational event giving non-additive results for differing distances between the mutant sites. Therefore, prototroph frequency data were supplemented by three different but mutually consistent ordering criteria applied to the flanking marker segregation data. Criterion I used only the two recombinant classes while criteria II and III each used all four classes. The rec-1 induced shift in the distribution

of flanking markers among prototrophs affects the use of the criteria, rendering II ambiguous in rec-1 x rec-1 crosses and III ambiguous in rec-1$^{(+)}$ x rec-1^{+} crosses. The ambiguity can be found when sites are relatively close and when sites are widely separated.

Cases of disagreement between the three criteria, or between any of the three criteria and prototroph frequency, could usually be clearly settled by considering the data from crosses involving neighbouring allelic differences. For example, alleles which gave ambiguous results could be mapped by considering close proximity of their sites of allelic difference to sites whose relative positions were known.

The order of the sites of allelic difference finally arrived at does not contain anomalies which cannot be explained. Further resolution of the map could be obtained by using the results from more extensive his-1 allelic crosses and, more certainly, by the use of short genetic deletions involving portions of the his-1 locus.

The finding of numbers of mutants mapping at the same sites is similar to that found by Case and Giles (1960) for the pan-2 locus of N. crassa. The most probable explanations to consider are that the allelic differences are too close together to give prototrophs in the population sizes studied and that the sites were particularly vulnerable to mutation. Finding different complementation groups at the same site indicates that the base pairs are different for at least some of the sites of allelic difference which are apparently located together.

Differences in flanking marker distribution among prototrophs definitely occurred between rec-1 x rec-1 and rec-$1^{(+)}$ x rec-1^{+} crosses in all but the combinations involving allele pairs whose sites of difference are relatively close together. These differences in distribution varied according to the particular allele pair involved or, in the case of the Y alleles, the particular allele or its background. The backgrounds of the K and Y alleles differ by two important factors. The K alleles were isolated from Emerson a using ultra-violet radiation while the Y

alleles (or at least two of them) were isolated from 74A, or closely related strains, using X-ray radiation. Differences between the K and Y strains could therefore be reflections either of differences in their his-1$^+$ allele or of different types of mutational changes.

The factor causing the difference in flanking marker segregation between the rec-1 x rec-1 and the rec-1 x rec-1$^+$ crosses of K83 x K625 was not separable from the rec-1 locus (section B of the results). The difference in segregation was manifested most strongly by the two classes of prototrophs which had the parental associations of the flanking markers. The change in K83 x K625 crosses was from 1.51 PD : 1 pd from rec-1 crosses to 0.55 Pd : 1 pd from rec-1$^+$ crosses. Thus rec-1$^+$ reduces the frequency of prototrophs to somewhere between about one eigth and one fiftieth of that in rec-1 x rec-1 crosses and also alters the proportions of the prototrophs which exhibit parental combinations of flanking markers.

Variation in prototroph frequency within either

rec-1 x *rec-1* or *rec-1*$^{(+)}$ x *rec-1*$^{+}$ crosses did not cause an associated, detectable variation in the flanking marker distribution. This was demonstrated by:

i) Square root charts of the four classes of flanking markers among prototrophs from K83 *rec-1* x K625 *rec-1* crosses with significantly different prototroph frequencies (fig. 5, a and b; discussed on page 60).

ii) Contingency χ^2 tests using the data on flanking marker distributions among prototrophs from two *rec-1* crosses of K83 x K140 which had significantly different prototroph frequencies (table IX, discussed on page 30).

iii) Contingency χ^2 tests using the data on flanking marker distributions among prototrophs from K83 *rec-1* x Y175-M460 *rec-1*$^{+}$ crosses which had significantly different prototroph frequencies (table X, discussed on page 31).

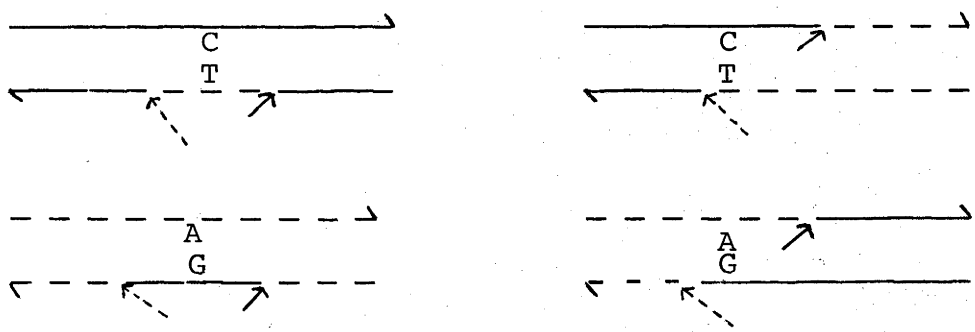
Therefore, the distribution of flanking markers among the prototrophs does not seem to be changed by any factors which modify either the rec-1 or rec-1^{+} prototroph frequencies.

B. ANALYSIS ON THE BASIS OF A HYPOTHESIS USING THE HYBRID DNA THEORY.

1. The Hypothesis.

The discussion in the introduction concluded that a hybrid DNA hypothesis of the type proposed by Holliday (1964, 1968) provides predictions which can be conveniently subjected to analysis. The first assumption inherent in the hypothesis is that the primary breaks occur in DNA chains of like polarity. Secondly, it is supposed that the single strands unravel from their continuous complementary strands, in each chromatid, and anneal with their complementary counter-parts in the non-sister chromatids. The chromatids are reconstituted following secondary breaks in the DNA strands originally broken, or in their sister strands, giving rise to a section of hybrid DNA which may or may not be associated with crossing-over between non-sister chromatids (fig. 14).

Figure 14. Diagrams showing the results of the process of hybrid DNA formation. The solid lines indicate a single chromatid (two DNA chains) contributed by one parent while the broken lines indicate the homologous, non-sister chromatid from the other parent. The original single strand breaks occurred at the broken arrows (↖). The single strands then unravelled and annealed as shown. Second single strand breaks at the points indicated by the solid arrows (↗) resulted in a short hybrid section in (a), and a short hybrid section associated with crossing-over in (b). An A T base pair from one parent at the same site as a C G base pair from the other would give rise to mis-matched base pairs as shown.



(a) (b)

The length and location of the hybrid DNA section, relevant to cases of hybridity for two auxotrophic alleles at a single locus, should reveal itself in the proportions of the various classes of flanking markers found among any prototrophic progeny.

The length and location of the hybridity is also important in the determination of prototroph frequency and thus in the construction of fine structure maps. The hybrid DNA theory originally did not explain why such maps were relatively additive, because the closer sites of difference become,the greater the probability of their inclusion in the same region of hybrid DNA. The frequency of prototrophic recombinants produced due to both mismatched base pairs being repaired to wild type would therefore not be related to distance between the sites. However, if excision involving a mismatched base pair leads to degradation of the same single strand over a distance sufficient to affect the second mismatched base pair, a prototroph would not be produced. Holliday (1968) suggested that this would provide the means by which prototroph frequency would

decline with increasing proximity of mutant sites. The data of Fogel and Mortimer (1969) support this by showing an increasing amount of symmetrical double site conversion with decreasing separation between the sites of allelic difference. The effect of these events on flanking marker distribution among prototrophs is difficult to predict. However, the distribution would not necessarily be expected to change with increasing proximity of the sites of allelic difference if the decreasing number of prototrophs, due to increasing symmetrical double site conversion in single regions of hybridity covering both sites, was directly proportional to the number of prototrophs produced by the decreasing amount of hybridity covering only single sites. An additional condition would be that the single site hybridity could be maintained with a proportion covering the proximal and/or distal site(s) even when the sites of allelic difference are apparently close to each other. If dual site hybridity becomes the rule when two sites are in very close proximity and if the sites involved can occasionally escape symmetrical double site conversion, the four different classes of flanking markers among

the prototrophs would be expected to be of equal frequency.

2. K83 x K625 Results.

Interpretation of the results from the well studied K83 x K625 allele combination, on the basis of a hybrid DNA theory, indicates that the change in frequency of the prototrophs in the two parental classes is explicable in terms of a change in the distribution of the hybridity, depending on the rec-1 constitution. Relatively more distal hybrid DNA, covering the K625 allelic difference, would explain the increase in the pd class at the expense of the PD class in the presence of rec-1^{+}. This change apparently accompanies a great reduction in a condition (hybrid DNA) necessary for prototroph formation. It may be noted that rec-1^{+} does not remove all allelic recombination. The residue may represent a "leak" due to incomplete inhibition or repression by rec-1^{+} or may represent a small amount of recombination initiated from a different site, insensitive to rec-1^{+}. It may be noted that

different rec$^+$ genes differ considerably in the proportion of the total recombination which they repress, perhaps due to differences in the loci which they affect. Moreover, a given sensitive locus may be affected by more than one rec$^+$ gene (Jha, 1969). However, if the small effects being attributed to rec genes are actually the result of modifiers acting on a basic rec factor, as was shown for K83 x K625, the variation in prototroph frequency need not be accompanied by changes in flanking marker distribution.

The data for K83 x K625, summarised in table XVI, establish a number of relationships between the proportions of the four classes of flanking marker combination among his-1$^+$ prototrophs in relation to the effect of rec-1$^+$ and lead to a number of interferences. Considering only the first four sets of data in each rec-1 class, because they are more homogeneous than those constituting the last set:

i) The recombinant classes of prototrophs (pD and Pd) changed from 42.1% to 45.3% of the total prototrophs when the genetic constitution was

changed from rec-1$^+$ to rec-1. Taken together with the relationship noted in (iii) below, the difference between the frequencies of parental and non-parental classes shows that the recombination system is not fully symmetrical as was assumed in the theoretical analysis discussed by Catcheside (1966b, 1968). Evidently, also, the departure from symmetry is different according to the presence or absence of rec-1$^+$.

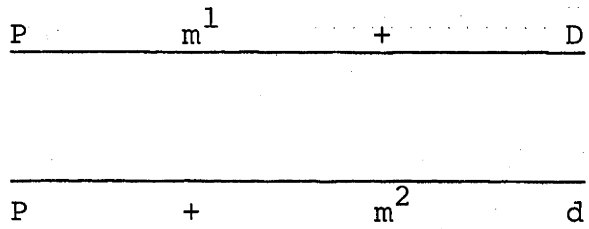
ii) The ratio of pD to Pd is the same (1.875) in the presence or absence of rec-1$^+$. The contingency χ^2, for one degree of freedom, is 0.119.

iii) The ratio of PD to pd (0.546) is not significantly different from the ratio of Pd to pD (0.540) in the presence of rec-1$^+$. This would be expected if, in these crosses, sections of hybrid duplex DNA covered only the distal site (K625) or both sites, with a minority of the prototrophs (about 30%) originating from the hybrid DNA covering only the distal site. The

ratio of PD to pd (1.509) is similar to, but not the same as, the ratio of pD to Pd (1.889) in the rec-1 x rec-1 crosses, the contingency χ^2, for one degree of freedom, being 21.118. This would be expected if sections of hybrid duplex DNA covered, respectively, the proximal site, both sites and the distal site, with the great majority covering the proximal and both sites. The expectations are derived from a mathematical consideration of the proportional sizes of the four classes of flanking markers amongst the his-1^+ prototrophs being analysed.

If we assume that the hybrid DNA can enter from either or both sides of the his-1 locus, and cover either or both sites of allelic difference (fig. 15), depending on the rec-1 constitution, the expected relative sizes of the various prototroph classes are as shown in table XXVII . The rec-1^+ crosses are assumed to have only hybrid DNA entering from the distal side of the locus and covering only the distal site or both sites, which would yield prototrophs in the relative proportions of y and

Figure 15. Location and origin of DNA hybridity with respect to a pair of allelic differences flanked by two closely linked markers.



Type of hybrid DNA

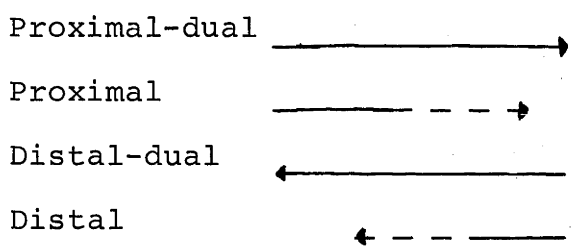


TABLE XXVII

Contributions of different classes of hybrid DNA to prototrophs with varying combinations of flanking markers

Hybrid DNA		Distribution of flanking markers among prototrophs				Proportion of prototrophs from this source	
Source	Mutant sites covered	PD	pd	pD	Pd	rec-1 x rec-1$^+$	rec-1 x rec-1
Proximal	Both	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	-	1-x } 1-z
Proximal	Proximal only	$\frac{1-s}{2}$	$\frac{s}{2}$	$\frac{1-s}{2}$	$\frac{s}{2}$	-	x }
Distal	Both	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	1-y	1-y } z
Distal	Distal only	$\frac{r}{2}$	$\frac{1-r}{2}$	$\frac{1-r}{2}$	$\frac{r}{2}$	y	y }

(1 - y), respectively. The rec-1 x rec-1 crosses are assumed to have the DNA hybridity of the rec-1$^+$ crosses, yielding prototrophs in the proportion z, as well as hybridity which is proximal in origin, yielding prototrophs in the proportion (1 - z). This hybrid DNA entering proximally may cover only the proximal site, giving x prototrophs, or both sites, giving (1 - x) prototrophs. Corrections for additional recombination (r = 0.04) between his-1 and am-1 and recombination (s = 0.06) between his-1 and inos are also introduced. The adjustments due to asymmetry will be considered later.

Since rec-1 x rec-1$^+$ crosses are assumed to possess only hybrid DNA covering the distal or both sites, we can easily calculate the relative proportions of prototrophs derived from these two types of hybridity. The expected proportions are:

PD = ¼ (1 - y + 2ry)	(observed 998)
pd = ¼ (1 + y - 2ry)	(" 1,827)
pD = ¼ (1 + y - 2ry)	(" 1,331)
Pd = ¼ (1 - y + 2ry)	(" 719)

Thus $\frac{p}{P} = \frac{1 + y(1 - 2r)}{1 - y(1 - 2r)}$ or $\frac{1 + 0.92y}{1 - 0.92y} = \frac{p}{P}$,

therefore

$$0.92y = \frac{p - P}{p + P}$$

By substituting the observed values for P and p we get:

$$0.92y = \frac{1441}{4875} \text{ hence } y = \frac{0.2955}{0.92}$$

Therefore y = 0.3213 or 32.13% of the prototrophs from these rec-1$^+$ crosses were the result of hybrid DNA covering only the distal site while (1 - y) or 67.87% of the prototrophs were from hybridity covering both sites.

Calculations of the proportions of prototrophs from the various types of hybrid DNA involved in rec-1 x rec-1 crosses is more complex since we have assumed that the hybridity of the rec-1 x rec-1$^+$ situation is present as well as a larger amount of hybridity from a proximal source which covers either the proximal (K83) site or both sites. The expected proportions are:

$PD = \frac{1}{4}[(1 + m)(1 - z) + (1 - n)z]$ (observed 2392)

$pd = \frac{1}{4}[(1 - m)(1 - z) + (1 + n)z]$ (observed 1585)

$pD = \frac{1}{4}[(1 + m)(1 - z) + (1 + n)z]$ (observed 2150)

$Pd = \frac{1}{4}[(1 - m)(1 - z) + (1 - n)z]$ (observed 1138)

where $m = x(1 - 2s)$ and $n = y(1 - 2r)$.

Thus $\frac{P}{p} = \frac{1 - zn}{1 + zn}$ whence $zn = \frac{p - P}{P + p} = \frac{205}{7265}$; so $0.3213z = 0.02821$ and $z = 0.09543$. This value is within the range of the ratio of the prototroph frequency for rec-1$^+$ crosses to the prototroph frequency for rec-1 crosses. The weighted mean for the prototroph frequencies of the rec-1 x rec-1 crosses analysed is $42.8/10^5$ while the weighted mean for the rec-1 x rec-1$^+$ prototroph frequencies is $3.14/10^5$. Since z is the proportion of prototrophs in rec-1 x rec-1 crosses from hybrid DNA originating distally and since it is assumed to be the only kind of hybrid DNA in rec-1 x rec-1$^+$ crosses, the ratio $\frac{3.14}{42.8}$ should be an independent estimate of z. This value (0.073) is comparable to, but smaller than that (0.095) obtained from the classes of flanking markers. Further: $\frac{D}{d} = \frac{1 + m(1 - z)}{1 - m(1 - z)}$ whence $m(1 - z) = \frac{D - d}{D + d} = \frac{1819}{7265}$, therefore $m = \frac{0.2541}{0.9046}$. Since $x = \frac{m}{1 - 2s}$, $x = \frac{0.2768}{0.88}$ and $x = 0.3146$.

This means that 31.46 x 0.9046 or 28.46% of the prototrophs in the rec-1 x rec-1 crosses were the result of DNA hybridity covering only the proximal site (K83). The majority of the prototrophs [(68.54 x 0.9046) + (67.87 x 0.09543) = 68.48%] are thus apparently the result of hybridity covering both sites, even though the sites of difference are relatively widely separated.

If the additional recombination between his-1 and am-1 and between his-1 and inos are not taken into account, the value of y becomes 0.2956, z is unchanged and x becomes 0.2768.

3. Application of the calculations to allele combinations other than K83 and K625.

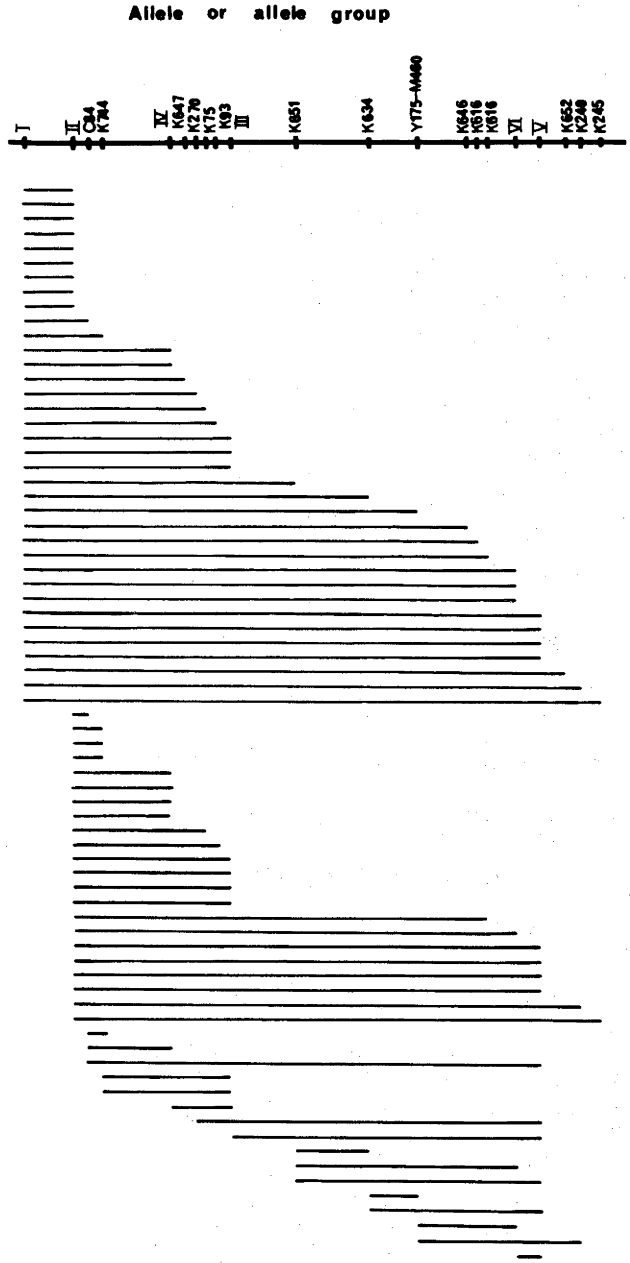
Any negative values of the parameters y, z and x would be meaningless and would probably indicate a flaw in either the basic theory or in the data. Therefore, it seems worthwhile to examine the data from crosses of allele pairs other than K83 x K625 to determine if the hypothesis applies to other allele combinations equally well.

Since, in rec-1$^{(+)}$ x rec-1^{+} crosses, $(1 - 2r)y = \frac{p - P}{p + P}$, negative values of y would arise whenever $p < P$. An examination of the values of p and P among the prototrophic progeny shown in table XV reveals that the populations of p are larger than those of P in all but three of the 33 allele combinations. However, these three allele combinations are all in the group of nine in which, on the basis of χ^2 tests, p is not significantly different from P, i.e. the nine crosses which give an insignificant difference for ordering using criterion II. Presumably minor variations due to sampling errors, viability differences, or modifiers having slight effects, could shift the ratio of p : P enough to give y a slight negative value in the three instances. The calculated value of y, for the 33 allele combinations in table XV, is found to vary from a slight negative value (not significantly different from zero) to one as high as 0.467 (K270 x K625) or even 0.618 (the small population from K649 x K625). The value of y, for each allele pair crossed, is given in fig. 16. There is no obvious, direct association between changes in y and the proximity or location of the sites of allelic difference involved.

Figure 16. Graph showing the contributions of the different classes of hybrid DNA to prototrophs from crosses of his-1 alleles. The values were found using the formulae explained in the text:

$$x = \frac{D - d}{(D + d)(1 - 2s)} \text{ and } y = \frac{p - P}{(p + P)(1 - 2r)}$$

where x = the proportion of prototrophs due to hybrid DNA covering the proximal site of allelic difference and y = the proportion of prototrophs due to hybrid DNA covering the distal site. The proportion of prototrophs due to dual site hybridity can be found by calculating (1-x) and (1-y). Instances of x and y equalling zero (i.e. all prototrophs are from dual site hybridity) are from crosses in which either D and d or p and P were insignificantly different. The distributions of flanking markers among the prototrophs were such that the prototrophs from all but eight of the rec-1 x rec-1 crosses could be explained by DNA hybridity covering both sites or the proximal site and both sites. The rec-1$^{(+)}$ x rec-1^{+} crosses gave prototrophs which could be explained by DNA hybridity covering both sites or the distal site and both sites.



Percentage of prototrophs resulting from DNA hybridity covering: proximal site rec-1	distal site rec-1+	distal site rec-1
X	Y	Y
15		
0		21
0		
0	0	20
0		
0		20
0		
24		
0		34
18	21	
0		24
13	0	
26	0	
61		
	0	
0		7
7		
24		
23	26	
37	35	
15	18	
31	22	
39		
	31	
	40	
	0	
40		
26		
21	26	
30	27	
36	62	
24		
28		
36		
0	41	11
	42	
0		
0		13
0		
0		
46	26	
28		
31	0	
43	0	
23		
16		
59		
42		
39		
37		
	12	
31	24	
48		
38	27	
45		
58	24	
26	26	
0		
41	27	
54	27	
52		
47		
30		
	47	
39		
47		
42		
44	27	
30		
41		
20	28	
25		
0		

Indeed, there could be no differences except for the very low values found when the sites of allelic difference are very close.

Examination of the nine allele combinations in which p is not significantly different from zero reveals that only four have relatively large populations (ranging from 227 to 306) while the remaining five have populations of from 13 to 81 prototrophs. Three of the four allele combinations represented by relatively large populations have their four prototrophic classes distributed in an equally divided fashion. χ^2 tests of equality between the four classes give $\chi^2_3 = 6.498$ for K83 x K617, $\chi^2_3 = 7.632$ (P = 0.10 - 0.05) for K83 x K90 and $\chi^2_3 = 1.891$ for K83 x K619. This type of distribution is the one which is expected to result from hybrid DNA covering both sites. The remaining cross (K626 x Y189-M89) is represented by a relatively large population but has data which are relatively heterogeneous between replicates, $\chi^2_6 = 21.604$, P = 0.005 - 0.001. This cross is the only rec-1 x rec-1$^+$ cross in table XV which could conceivably possess prototrophs due to

hybrid DNA covering the proximal site as well as both sites.

It can therefore be concluded that these crosses do not yield data which seriously disagree with the assumption that only hybrid DNA entering distally is present in rec-1 x rec-1^{+} crosses.

The next consideration is whether or not the rec-1 x rec-1 crosses in table XIV give data which would result in negative values of z. In these the values of z are calculated from the formula $zy = \frac{p - P}{P + p}$, modified by corrections involving r and s.

The values of y, calculated for the rec-1$^{(+)}$ x rec-1^{+} data for any allele pair, have been shown to be positive. Therefore, any rec-1 x rec-1 crosses which show values of $p<P$ will have negative values of z. Since p and P are used in the determination of criterion II, all instances of criterion II not being applied mean that p is not significantly different from P. By the same reasoning, all crosses which give data which result in criterion II giving a presumed

faulty order will have a negative value for (p - P) and therefore for z. A very significant number of the allele combinations fall into this category. Positive values for z (significantly different from zero) can be obtained from the data derived from only 11 of the 69 allele combinations in table XIV.

The factor z was included in the calculations because the ratio of PD to pd was similar to, but not the same as, the ratio of pD to Pd for the K83 x K625 rec-1 x rec-1 crosses in table XVII. On the other hand, the K83 rec-1 x K625 rec-1 crosses which produced the data plotted in fig. 5 had a PD to pd ratio of 1.680 and a pD to Pd ratio of 1.570. The χ^2 for one degree of freedom equals 1.976, P = 0.20 - 0.10. Therefore the two ratios, for this series of crosses, are probably not different. This fact, along with the large number of negative values of z and of values of z not significantly different from zero which were found in the data in table XIV, casts doubts on the validity of using calculations containing the factor z.

If z is removed from the calculations, i.e. if we assume that only hybrid DNA entering proximally is present in rec-1 x rec-1 crosses, calculating the contribution of the DNA hybridity is simplified. The proportion of prototrophs due to hybridity entering proximally and covering only the proximal site becomes:

$$x = \frac{D - d}{D + d}$$ ($x = 0.2504$ for the K83 x K625 crosses previously analysed), or

$$(1-2s)x = \frac{D - d}{D + d}$$ if corrected for additional crossing over

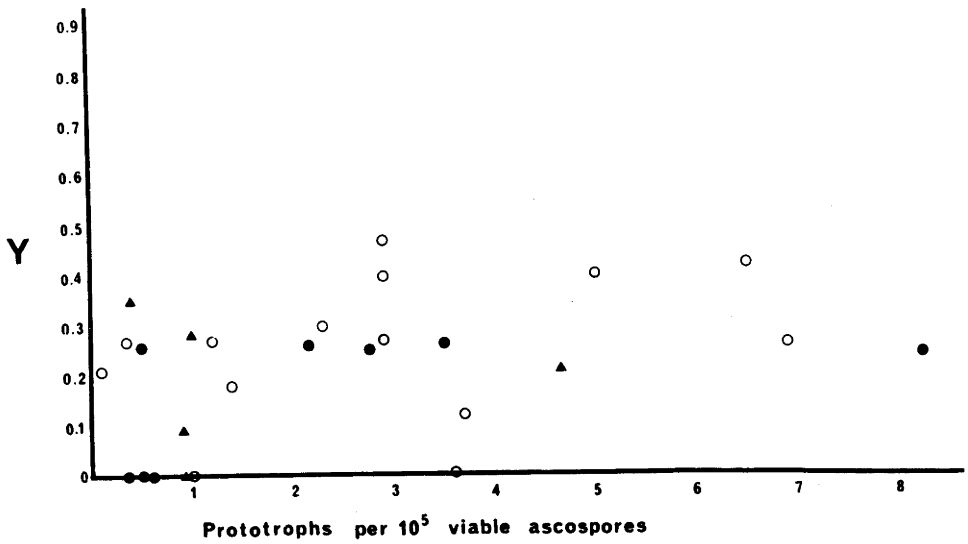
($x = 0.2845$ for the K83 x K625 crosses previously analysed).

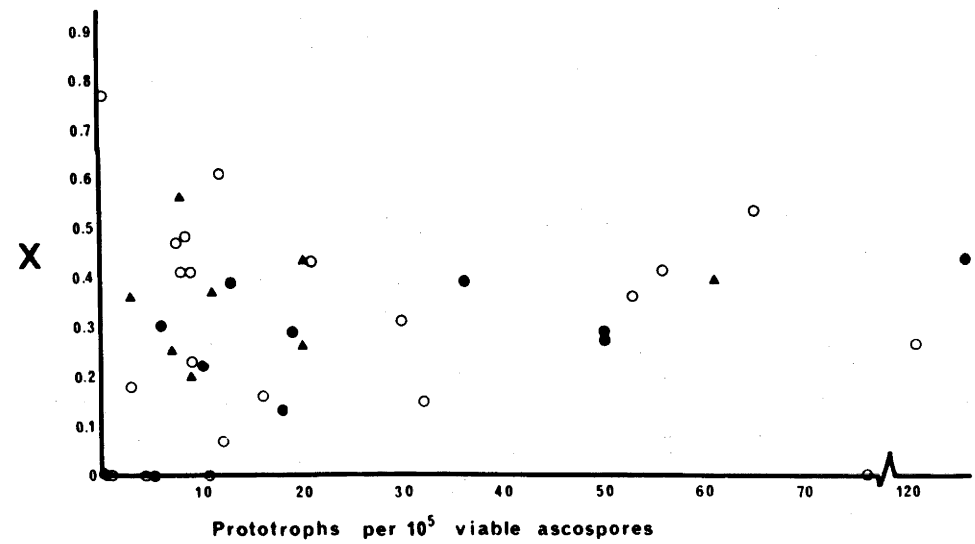
None of the allele combinations in table XIV gave values of x which can be considered to be negative since the d class was never significantly larger than the D class. The calculated values of x are tabulated in fig. 16. The data indicate a possible association between low values of x and close proximity of the sites of allelic difference. The values x and y (proportions of prototrophs due to single site hybridity) have therefore been plotted

against approximate prototroph frequency (fig. 17, a and b) to determine if any relationship exists. The inter-group results have been pooled, with the exception of the crosses involving the Y alleles. As was noted in fig. 16, the alleles whose sites of difference are close give a number of values of x and y which are not significantly different from zero. The sites in close proximity also give some comparatively large values of x and y. There are only two crosses with relatively large prototroph frequencies which apparently lack prototrophs from single site hybridity, one cross being rec-1 x rec-1, the other being rec-1 x rec-1^{+}.

The graphs indicate that there is a tendency for allele pairs whose sites of difference are close to give prototrophs only from dual site hybridity. The large number of crosses involving mutant sites which are close and which have a proportion of prototrophs due to single site hybridity indicate that the individual allele pairs involved in the crosses probably have an effect on the type of hybridity formed.

Figure 17. Graphs which relate the proportions of single site hybridity (y in rec-1^{+} crosses, part a; and x in rec-1 crosses, part b) to prototroph frequency. The results from the inter-group crosses, with the exception of crosses involving Y alleles, have been pooled and are denoted by solid dots. The crosses involving the Y alleles are shown by triangles while the open dots are for the data from all other non-inter group crosses. The prototroph frequencies are only approximate, being weighted means from all the data available.


Y
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
Prototrophs per 10^5 viable ascospores


X
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
70
120
Prototrophs per 10^5 viable ascospores

There is definitely no direct correlation between the relative closeness of the sites of allelic difference and the number of prototrophs due to the different extents of hybrid DNA. This supports the assumption made earlier, that the distribution of flanking markers need not change with separation of the sites. This constancy requires that the proportions of prototrophs from dual and single site hybridity remain similar through equivalent reductions in both classes with increasing closeness. The proportion of dual hybrid DNA is expected to increase with decreasing separation of the sites. However, fewer prototrophs are assumed to arise from this source because, as the distance between the sites within the dual hybrid DNA decreases, symmetrical double site conversion increases. The reduction in the number of prototrophs from hybrid DNA covering single sites is, however, assumed to be directly proportional to the decreasing amounts of hybridity covering either the proximal or the distal site.

Nineteen of the 69 crosses in table XIV (those with no notation in the criterion III column) gave x

not significantly different from zero, implying that only hybrid DNA covering both sites is present in the crosses. If this is indeed the case, the four classes of prototrophs should be in equal frequency. Examination of the data reveals that the four classes are nearly always significantly different. There are two possible explanations based on a hybrid DNA theory. If the two parental classes are equal and the two recombinant classes are equal but the combined parental classes do not equal the combined recombinant classes (e.g. as for K617 x C84), dual hybridity (hybrid DNA covering both sites) could still be responsible for the prototrophs. Eleven of the 19 allele combinations in which x approximates zero gave prototrophic classes in which the two parental classes were not significantly different and the two recombinant classes were not significantly different. The remaining 8 allele combinations (K83 x K626, K83 x K617, K83 x K518, K644 x K617, K83 x K744, K83 x K75, K83 x K240, and K620 x K744) require the presence of hybridity covering the distal site only as well as both sites. There does not seem to be any simple relationship between either the position of the sites or the relative separation

of the sites and the proportion of prototrophs due to hybrid DNA over the distal mutant site (fig. 16). The proportion of prototrophs due to DNA hybridity covering the distal allele difference ranges from 7% for K83 x K75, to 34% for K644 x K617 (calculated by using the formula $\frac{p - P}{(P + p)(1 - 2r)}$.

4. Inequality between the frequencies of prototrophs with parental and recombinant classes of flanking markers.

The K83 x K625 data exhibited a feature whose effect on the previous calculations should be examined. The frequency of the class of prototrophs parental for flanking markers does not equal the frequency of the recombinant class. Such inequality could arise through the secondary breaks, ending the hybrid DNA section, occurring with unequal frequency in the strand which carries the primary break and in its sister.

The assumptions that rec-1$^{(+)}$ x rec-1^{+} crosses possess hybrid DNA covering the distal site and both sites and that rec-1 x rec-1 crosses possess hybrid DNA covering either the proximal site and both sites

and, in a minority of cases, the distal site, simplify the addition of the effect of asymmetry to the calculations. A significant amount of inequality between the frequency of the two parental classes and the frequency of the two recombinant classes has been previously noted. This asymmetry has been demonstrated to be different for different allele pairs and different rec-1 backgrounds, but without noticeable pattern.

The necessary assumption required in the calculation of the effects of asymmetry on the contributions of the various classes of DNA hybridity to prototrophs is that the probability of the occurrence of full non-sister chromatid exchange does not equal the probability of no exchange. In other words, the DNA hybridity does not terminate in chromatid exchange with the same frequency that it terminates in no chromatid exchange. If we let (a) equal the frequency with which the chromatids involved in hybrid DNA formation remain parental and (1 - a) equal the frequency with which non-sister chromatid exchange occurs, the expectations for the contribution of the

various classes of hybrid DNA to the prototrophs become those shown in table XXVIII.

Considering the data from the first four K83 x K625 rec-1 x rec-1$^+$ crosses in table XVI, we can calculate the effect of asymmetry on the values of y and (1 - y). The factor N = the total number of prototrophs analysed for flanking marker constitution.

$$\frac{PD}{N} = r(1-a)y + \tfrac{1}{4}[2a(1-r-a) + (r+a)](1-y)$$

$$\frac{pd}{N} = (1-r)ay + \tfrac{1}{4}[2a(1-r-a) + (r+a)](1-y)$$

$$\frac{pD}{N} = (1-r)(1-a)y + \tfrac{1}{4}[2a(1+r+s) + (2-r-s)](1-y)$$

$$\frac{Pd}{N} = ray + \tfrac{1}{4}[2a(1+r+s) + (2-r-s)](1-y)$$

The observed values are PD = 998, pd = 1,827, pD = 1331 and Pd = 719.

$$\frac{PD}{N} - ry(1-a) = \frac{pd}{N} - (1-r)ya, \text{ whence } Ny = \frac{pd - PD}{(a-r)}$$

$$\text{and } \frac{pD}{N} - (1-r)(1-a)y = \frac{Pd}{N} - ray, \text{ whence } Ny = \frac{Pd - pD}{(a+r-1)}$$

Therefore $(pd - PD)(a + r - 1) = (Pd - pD)(a - r)$

$$a = \frac{r(-pd + PD - Pd + pD) + pd - PD}{pd - PD - Pd + pD}$$

$$a = \frac{0.04\ (-1827+998-719+1331) + 1827-998}{1827-998-719+1331}$$

$$a = 0.5693$$

$$\text{and } 4875\ y = \frac{1827 - 998}{0.5693 - 0.04} \text{ therefore } y = 0.3213$$

TABLE XXVIII

Contributions of different classes of hybrid DNA to prototrophs, assuming inequality between the proportions of full chromatid exchanges and no exchanges.

Hybrid DNA		Distribution of flanking markers among prototrophs				Proportion of prototrophs from this source
		PD	pd	pD	Pd	
Source	Mutant sites covered					
rec-1$^{(+)}$ x rec-1^{+}						
Distal	Distal only	r(1-a)	(1-r)a	(1-r)(1-a)	ra	y
Distal	Both	2a(1-r-s) + (r+s)*		2a(1+r+s) + (s-r-s)#		1-y
rec-1 x rec-1						
Proximal	Proximal only	(1-s)a	s(1-a)	(1-s)(1-a)	sa	x
Proximal	Both	2a(1-r-s) + (r+s)*		2a(1+r+s) + (2-r-s)#		1-x

* The same formula applies to both PD and pd.
\# The same formula applies to both pD and Pd.

This is exactly the same value of y determined from the formula $(1 - 2r)y = \frac{p - P}{p + P}$. We may therefore safely assume that the asymmetry, in this case, has absolutely no effect on the proportions of prototrophs from DNA hybridity covering the distal site and both sites.

We may likewise calculate the effect of the asymmetry on the values of x and (1 - x) for the data from the first four K83 rec-1 x K625 rec-1 crosses in table XVI:

$$\frac{PD}{N} = (1-s)ax + \tfrac{1}{4}\,[2a(1 - r - s) + (r + s)]\,(1-x)$$

$$\frac{pd}{N} = s(1 - a)x + \tfrac{1}{4}\,[2a(1 - r - s) + (r + s)]\,(1-x)$$

$$\frac{pD}{N} = (1-s)(1-a)x + \tfrac{1}{4}\,[2a(1 + r + a) + (2 - r - a)]\,(1-x)$$

$$\frac{Pd}{N} = sax + \tfrac{1}{4}\,[2a(1 + r + a) + (2 - r - a)]\,(1-x)$$

The observed values are PD = 2,392, pd = 1,585, pD = 2,150 and Pd = 1,138.

$\frac{PD}{N} - (1-s)ax = \frac{pd}{N} - s(1-a)x$, whence $xN = \frac{pd - PD}{s - a}$

and $\frac{pD}{N} - (1-s)(1-a)x = \frac{Pd}{N} - sax$, whence $xN = \frac{Pd - pD}{a + s - 1}$

Therefore $(pd - PD)(a + s - 1) = (Pd - pD)(s - a)$

$$a = \frac{s(-pd + PD + Pd - pD) + pd - PD}{pd - PD + Pd - pD}$$

$$a = \frac{0.06\ (-1585 + 2392 + 1138 - 2150) + 1585 - 2392}{1585 - 2392 + 1138 - 2150}$$

$$a = 0.4504$$

and $7265x = \frac{1585 - 2392}{0.06 - 0.4504}$, therefore $x = 0.2845$

This is exactly the same value of x determined for these data by the formula $(1 - 2s)x = \frac{D - d}{D + d}$. In fact, deriving equalities for a from the formulae

$$xN = \frac{pd - PD}{s - a} \text{ and } xN = \frac{Pd - pD}{a + s - 1}$$

gives $x = \frac{PD + pD - pd - Pd}{N\ (1 - 2s)}$ which is the same formula as $(1 - 2s)\ x = \frac{D - d}{D + d}$. The asymmetry therefore has no effect on the proportions of prototrophs due to hybridity covering the proximal and both sites.

The above calculations show that corrections for asymmetry do not need to be added to the formulae used to obtain the proportions x and y.

5. Further calculations on the effect of additional crossing-over between his-1 and its flanking markers.

Whitehouse and Hastings (1965) have also suggested a mathematical treatment of prototroph data which can be used to calculate the effects of additional recombination between the locus being studied and its flanking markers. The calculations analyse both a situation in which the recombinational event at the locus producing the prototrophs causes positive interference on crossing-over in the neighbouring regions and a situation in which the event contributes no interference. The latter calculations are equivalent to those which were used in sections 2, 3 and 4. Their formulae can be used to obtain corrected values of the observed PD, pd, pD and Pd classes in the previously analysed K83 x K625 crosses in table XVI. The symbols r and s are the same as those used in the preceeding discussion while a represents the observed number of prototrophs in the Pd class and A represents the expected number with no additional recombination in the r and s regions. The respective letters for the pd, pD and Pd classes are b + B, c + C and d + D.

With positive interference:

$$A = \frac{a}{1 - (r + s)}$$

$$B = \frac{b}{1 - (r + s)}$$

$$C = c - \frac{ra + sb}{1 - (r + s)}$$

$$D = d - \frac{sa + rb}{1 - (r + s)}$$

With no interference:

$$A = \frac{E(Ga + Fb) - (Jc + Hd)}{G^2 - F^2}$$

$$B = \frac{E(Fa + Gb) - (Hc + Jd)}{G^2 - F^2}$$

$$C = \frac{E(Gc + Fd) - (Ja + Hb)}{G^2 - F^2}$$

$$D = \frac{E(Fc + Gd) - (Ha + Jb)}{G^2 - F^2}$$

where $E = 1 - (r + s) = 0.9$

$F = 2rs = 0.0048$

$G = 2E + F - 1 = 0.8048$

$H = Fr + Gs = 0.04848$

$J = Gr + Fs = 0.64768$

For the rec-1 x rec-1 crosses, with interference:

$$A = \frac{2392}{0.9} = 2657.8$$

$$B = \frac{1585}{0.9} = 1761.1$$

$$C = 2150 - \frac{0.04 \times 2392 + 0.06 \times 1585}{0.9} = 1938.0$$

$$D = 1138 - \frac{0.06 \times 2392 + 0.04 \times 1585}{0.9} = 908.1$$

For the rec-1 x rec-1^{+} crosses, with interference:

$$A = \frac{998}{0.9} = 1108.9$$

$$B = \frac{1827}{0.9} = 2030.0$$

$$C = 1331 - \frac{0.04 \times 998 + 0.06 \times 1827}{0.9} = 1164.8$$

$$D = 719 - \frac{0.06 \times 998 + 0.04 \times 1827}{0.9} = 571.3$$

If interference is not considered to be affecting the results, the rec-1 x rec-1^{+} expectations become:

A = 1007.7

B = 1914.2

C = 1306.5

D = 646.6

while the rec-1 x rec-1 expectations become:

A = 2492.6

B = 1570.5

C = 2173.4

D = 1028.5

These"corrected"values can now be used in the calculation of x and y. For the rec-1 x rec-1^{+} crosses, with interference:

$$y = \frac{p - P}{P + p} = \frac{1514.7}{4875}, \quad y = 0.3107.$$

with no interference:

$$y = \frac{1566.3}{4875}, \quad y = 0.3213.$$

The values for the rec-1 x rec-1 crosses are, with interference:

$$x = \frac{D - d}{D + d} = \frac{1926.6}{7265}, \quad x = 0.2652.$$

with no interference;

$$x = \frac{2067.0}{7265}, \quad x = 0.2845.$$

The values of x and y calculated on the basis of no interference are exactly the same as those calculated previously. Interference would seem to have little effect (from 1 - 2%) on the predicted proportions of prototrophs due to hybrid DNA covering either the proximal site or the distal site.

Whitehouse and Hastings also developed formulae to calculate the proportions of the various classes of prototrophs due to hybridity covering the proximal, distal, or both mutant sites within a locus. Their

method of calculation has not been used because, in it, the dual hybrid DNA contribution is derived using only the two classes of prototrophs recombinant for flanking markers. The advantage in using the data from all of the prototrophs is therefore lost.

CHAPTER V

CONCLUSIONS

The most probable explanation for the action of the rec-1$^+$ gene has been given by Catcheside (1968). He suggested that the evidence indicates that the rec-1$^+$ gene produces a regulator which might act by reducing the amount of hybrid DNA or by reducing correction of mis-matched base pairs. The former now seems to be the more likely. The latter mode of action should result in an increase in the proportion of asci with spores distributed in a manner indicative of post-meiotic segregation, rather than a change in the distribution of flanking markers among prototrophs. This is because the different combinations of mis-matched base pairs occur equally frequently in each of the combinations of flanking markers if hybrid DNA is formed symmetrically as is assumed (see fig. 14, page 92). The experimental data, however, indicate that the reduction in prototroph frequency is accompanied by a change in the flanking marker distribution. The remaining explanation, of rec-1$^+$ reducing the amount of hybrid DNA, could account for

the change in the distribution of flanking markers if the effect of the regulator were stronger at one end of the locus.

Calculations using the his-1 data revealed that the effect of the regulator could be stronger at one end of the locus. The proportions of prototrophs due to hybrid DNA of various extents were calculated from the his-1 flanking marker data. This required the assumption that a particular type of hybrid DNA can be formed at a given locus in a minority of meiotic cells. It is assumed to start at either end of the locus and proceed to some point, within the locus or perhaps beyond it, so that the hybrid DNA may cover either or both sites of allelic difference in a cross. In crosses with one or both of the parents rec-1^{+}, the DNA hybridity formed appeared to cover either both sites or the distal site, the latter occurring less frequently and sometimes not at all. The DNA hybridity in these crosses would thus seem to be distal in origin. The data on flanking marker distribution among prototrophs therefore indicate that the regulator produced by rec-1^{+} blocks the

formation of hybrid DNA initiated proximally. Presumably the blockage allows a small amount, relative to that proportion possible from a proximal origin, of hybrid DNA to enter the his-1 locus from the distal end.

Crosses which were rec-1 x rec-1 appeared to have only, or mainly, dual or dual and proximal hybridity of the DNA. In a few cases, there appeared to be a small proportion of prototrophs due to distal site hybridity. The majority of the rec-1 x rec-1 crosses thus seem to have hybridity which is wholly proximal in origin. The conclusion is that in the absence of the regulator (i.e. in rec-1 x rec-1 crosses) hybrid DNA initiated proximally is the rule.

The exceptions, which have some hybrid DNA of distal origin, may owe this occurrence to the particular pair of alleles involved. Evidence for particular alleles having an effect on the DNA hybridity came from experiments in which alleles with sites of difference at a common location were crossed to a common allele. The flanking marker distribution in

prototrophs from such crosses were shown to be significantly different (table XXVI, discussed on page 74).

The alleles having peculiar effects in crosses could have deficiencies or duplications, rather than particular classes of base substitutions. Altered chromatid pairing structures, caused by deficiencies or duplications, might interfere with the formation of hybridity from the proximal end of the locus. This might, in turn, result in some hybridity originating distally.

We may also consider whether there may be a differential action due to the different possible mis-matched pairs of nucleotides. Several different pairs of mis-matched bases are possible. A pyrimidine may be paired with the wrong purine (T-G or C-A), a purine may be paired with a purine (A-G, A-A and G-G) and a pyrimidine may be paired with a pyrimidine (T-C, T-T and C-C). A mutation due to a base substitution at any given site can be one of three alternatives, e.g. for an A-T pair the

alternatives are the two transversions T-A and C-G and the transition G-C. Transversions account for two out of the three alternatives possible at any site. The mis-matched base pairs formed between transistions and their progenitors are A-C or G-T pairs, i.e. the wrong pyrimidine-purine combinations. The mis-matched base pairs formed between transversions and their progenitors are either heterologous or homologous purine-purine or pyrimidine-pyrimidine pairs. If the crosses giving the exceptional results involved mis-matched pairing of identical bases,or classes of bases, their scarcity could be accounted for by rarity of transversions.

The sensitivity of a given mis-matched base pair may also depend on the sequence of nucleotides in the neighbourhood. The base differences involved in the exceptions might, either directly or indirectly, result in conditions of stress between the effectively paired, interacting chromatids. This stress might cause breakage of half chromatids at the site of the stress (Fogel and Hurst, 1967) with resulting hybrid

DNA which appears to have a distal origin. However, stress conditions should then also exist in rec-1$^{(+)}$ x rec-1^{+} crosses, resulting in hybrid DNA apparently initiated proximally. There was no conclusive evidence for this class of hybrid DNA in rec-1^{+} crosses.

The suggestion that rec-1^{+} blocks hybrid DNA initiated proximally is supported quantitatively by the data from the crosses analysed in this study. It should be emphasised, however, that this suggestion is based on a number of assumptions. These involve a particular, comparatively simple mode of formation of hybrid DNA and allow a quantitative analysis of the data on that basis. It would be extremely difficult, from the his-1 data, to dismiss the possibility that a different or more complex mechanism could be correct.

The choice of a hypothesis similar to that of Holliday (1964) is supported by the work of Emerson (1966) on Ascobolus. He found that the distribution of aberrant octads from a cross of W-62 alleles was

such that 6+ : 2m > 2+ : 6m while 5+ : 3m < 3+ : 5m. These relative proportions of octads were explained by assuming that the mismatched bases were dissimilar and that they respond differently to the repair mechanism. Any hybrid DNA hypothesis, such as that of Whitehouse (1963), which predicts that the mismatched bases in the hybrid region are the same, would not give octad distributions in the proportions mentioned above. Whitehouse (1969), however, suggested that enzymic recognition of mismatched bases could also involve recognition of some other feature in the neighbouring DNA which distinguishes between the parental strands.

There are a larger number of assumptions in other hypotheses of hybrid DNA formation than in those of the general kind proposed by Holliday. The uncertainties contributed by the extra assumptions must be resolved by further experimental evidence. Analyses of the type performed on the his-1 data may then be possible. These may help determine the actual events in the process of recombination.

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